FISV - Federazione Italiana Scienze della Vita

Program and Abstracts of the XIV FISV CONGRESS

Sapienza University of Rome, Italy

September 20 - 23, 2016

CONTENTS

WELCOME LETTER

The XIV Congress of the Italian Federation of Life Sciences (FISV) will take place from 20th to 23rd September 2016 at Sapienza, University of Rome.

The positive experience of the past 2012 Congress with almost 800 participants encourages us to believe that also the 2016 Congress will have a great success and attract many participants, especially young scientists.

Fifteen Scientific Societies AAI, ABCD, AGI, SIB, SIBBM, SIBE, SIBV, SIC, SICA, SIF, SIGA, SIMA, SIMGBM, SIP, SIPaV and more than 10,000 researchers are active members of the FISV. All these Societies are contributing to organize an exciting Congress in Rome, which especially highlights the topics of common interest dealing with forefront and stimulating aspects of biology. Internationally emerging issues and contributions from some of the best Italian and foreign laboratories will be presented.

The meeting will also offer a forum to young Italian and foreign scientists engaged in research at the highest level. Specific topics will be chosen among the emerging subjects in life sciences.

Five plenary lectures, 5 plenary symposia and 6 parallel symposia will organized together two poster sessions of 2,5 hours. Poster contributions will be chosen for oral presentations in both parallel Symposia and 24 additional Short Talks.

Finally, an evening event will be organized as a round table on inter-disciplinary aspects of Biology and Physics.

We are sure that you will enjoy very much this exciting Congress and anxiously look forward to meeting you in Rome.

With best wishes,
Il Presidente della FISV
Prof. Felice Cervone

MEMBER SOCIETIES

FISV - Federazione Italiana Scienze della Vita

Italian Federation of Life Sciences

AAI	**Associazione Antropologia Italiana**
ABCD	**Associazione di Biologia Cellulare e del differenziamento**
AGI	**Associazione Genetica Italiana**
SIB	**Società Italiana di Biochimica e Biologia Molecolare**
SIBBM	**Società Italiana di Biofisica e Biologia Molecolare**
SIBE	**Società Italiana di Biologia Evoluzionistica**
SIBV	**Società Italiana di Biologia Vegetale**
SIC	**Società Italiana di Cancerologia**
SICA	**Società Italiana di Chimica Agraria**
SIF	**Società Italiana di Farmacologia**
SIGA	**Società Italiana di Genetica Agraria**
SIMA	**Società Italiana di Mutagenesi Ambientale**
SIMGBM	**Società Italiana di Microbiologia Generale**
SIP	**Società Italiana di Patologia**
SIPaV	**Società Italiana di Patologia Vegetale**

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Rodolfo Negri (*FISV Secretary*, Rome)

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SESSION ORGANISERS

Plenary Lecture

Gennaro Ciliberto
Rodolfo Costa
Bruno Giardina
Rodolfo Negri
Eugenia Schininà
Paolo Trost

Plenary Symposia

Gennaro Ciliberto
Bianca Colonna
Rodolfo Costa
Bruno Giardina
Michele Morgante
Ruggero Pardi
Eugenia Schininà
Paolo Trost

Parallel Symposia

Margherita Bignami
Giovanni Destro Bisol
Bruno Giardina
Michele Morgante
Roberto Pinton
Paolo Trost
Eugenia Schininà

Mini Symposia

Lilia Alberghina
Roberto Amendola
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Stefania Astolfi
Maurizio Badiani
Alma Balestrazzi
Matteo Barberis
Guido Barbujani
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Antonio Torroni
Claudio Tuniz
Daniela Uccelletti
Aldo Venuti

SAPIENZA
UNIVERSITÀ DI ROMA

University Map

LEGEND

① Aula Magna - Rettorato
② Hall A - Building CUO10
③ Giacomini Hall - Botany building
④ Montalenti Hall - Genetic building
⑤ Blu Halls building (All five halls are in the same building)

PROGRAMME OVERVIEW

Tuesday, September 20

9:00	**Registration**
11:00	**Opening & Welcome** ①
11:30	**Plenary Lecture** ① Gerry Melino *"The P53 family in cancer biology"*
12:30	Free time for lunch
14:00	**Plenary Lecture** ① Ernesto Di Mauro *"On the spontaneous generation of ribozymes and of Life"*
15:00	**Plenary Symposium** ① *"Cancer Stem Cells"*
17:00	Coffee Break
17:30	**Plenary Symposium** ① *"CRISPR/CAS: from a prokaryotic immune system to a powerful tool for biomedical and agricultural research"*
19:30	**Round Table** ④

Wednesday, September 21

9:00	**Plenary Lecture** ① Roland Foisner *"Nuclear architecture and chromatin dynamics"*
10:00	Coffee Break
10:30	**Plenary Symposium** ① *"Oxygen Sensing and Redox Signalling: common themes of aerobic life"*
12:30	Free time for lunch **Posters viewing (Session I)**
14:30	**Short talks by participants (Session I)** **Topics** PSI.1 ④ PSI.2 ③ PSI.3 ⑤ PSI.4 ⑤ PSI.5 ⑤ PSI.6 ⑤
17:30	Coffee Break
18:00	**Societies' time** **AGI** ③ **SIB** ② **SIBV** ⑤ **SIMA** ⑤ **SIMGBM** ④
21:00	**Social Dinner**

Thursday, September 22

9:00	**Plenary Symposium** ① *"Systems Biology: from Genetic Networks to Organismal Functions"*
11:00	Coffee Break
11:30	**Parallel Symposia** *1 - The (recent) evolution of human evolution* ④ *2 - From Reverse- to Structural-Vaccinology and beyond. Current challenges against infectious diseases* ① *3 - Nitrogen: Nutritious and Noxious* ⑤
13:30	MERCK Short Talk ①
13:45	Free time for lunch **Posters viewing (Session II)**
15:30	**Short talks by participants (Session II)** **Topics** PSII.1 ⑤ PSII.2 ⑤ PSII.3 ④ PSII.4 ③ PSII.5 ⑤ PSII.6 ⑤
18:30	**Plenary Lecture** ① Anna Moroni *"Plant photoreceptors as a tool in neuroscience"*

Friday, September 23

9:00	**Parallel Symposia** *1 - Shaping the Cancer Genome: from pathways to mutational signatures* ① *2 - Unfolding truth: making sense of intrinsically disordered proteins* ③ *3 - Plant adaptation and phenotypic plasticity to climate change* ④
11:00	Coffe Break
11:30	**Plenary Symposium** ① *"New roles and molecular pathophysiology of mitochondria"*
13:30	Free time for lunch
15:00	**The EMBO Keynote Lecture** ① Titia Sixma *"Trapping transient states in DNA mismatch repair"*
16:00	**Congress Closure**

PROGRAMME

Tuesday, September 20

9:00 - 11:00 **Registration**

11:00 - 11:30 **Opening & Welcome** (Aula Magna)

Eugenio Gaudio *(Rettore – Sapienza, University of Rome)*
Felice Cervone *(FISV President)*
Commemoration of Paolo Bianco by Giulio Cossu and Guido Tarone by Ruggero Pardi

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11:30 - 12:30 **Plenary Lecture** (Aula Magna)

Chair: Felice Cervone *(FISV President)*

Gerry Melino *(Rome and Leicester, UK)*
The P53 Family in cancer biology

12:30 - 14:00 **Free time for lunch**

14:00 - 15:00 **Plenary Lecture** (Aula Magna)

Chair: Rodolfo Negri *(Rome)*

Ernesto Di Mauro *(Viterbo)*
On the spontaneous generation of ribozymes and of Life

15:00 - 17:00 **Plenary Symposium** (Aula Magna)

Cancer Stem Cells

Chairs: Gennaro Ciliberto *(Naples)*, Ruggero Pardi *(Milan)*

Dominique Bonnet *(London, UK)*
Remodelling the Hematopoietic Stem Cell Niche by Acute Myeloid Leukemic Cells

Riccardo Fodde *(Rotterdam, Netherlands)*
Intestinal Stem Cells in Homeostasis, Inflammation and Cancer: a Matter of Nich

Pier Paolo Di Fiore *(Milan)*
The Numb: p53 axis connect asymmetric cell division and tumor suppression in mammary stem cells

Stefano Piccolo *(Padua)*
YAP/TAZ in stem cell and tissue regeneration

17:00 - 17:30 **Coffee Break**

17:30 - 19:30 **Plenary Symposium** (Aula Magna)

CRISPR/CAS: from a prokaryotic immune system to a powerful tool for biomedical and agricultural research

Chairs: Bianca Colonna *(Rome)*, Michele Morgante *(Udine)*

Rotem Sorek *(Rehovot, Israel)*
The immune system of bacteria: CRISPR and beyond

Wolfgang Wurst *(Neuherberg, Germany)*
CRISPR/Cas9 Technology: Universal Tool for Functional Genome

Holger Puchta *(Karlsruhe, Germany)*
Double-strand break induced genome engineering in plants

19:30 - 21:00 **Round table** (Montalenti Hall)

The odd couple: Biology and Physics working together

Chair: Silvia Bencivelli *(RadioRaiTre, Rome)*

Speakers:

Giulio Giorello *(Milan)*

Riccardo Cortese *(Okairòs, Rome)*

Massimiliano Viale *(Rome)*

Wednesday, September 21

9:00 - 10:00 **Plenary Lecture** (Aula Magna)

Chair: Isabella Saggio *(Rome)*

Roland Foisner *(Vienna)*
Nuclear architecture and chromatin dynamics

10:00 - 10:30 **Coffee Break**

10:30 - 12:30 **Plenary Symposium** (Aula Magna)

Oxygen Sensing and Redox Signalling: common themes of aerobic life

Chairs: Pierdomenico Perata *(Pisa)*, Giulia De Lorenzo *(Rome)*

Peter J. Ratcliffe *(Oxford, UK)*
Sensing oxygen levels in animal cells by HIF hydroxylases

Francesco Licausi *(Pisa)*
When plants face oxygen limitations

Stéphane D. Lemaire *(Paris, France)*
Proteomics unravel an intricate redox network of 1000 proteins regulated by thioredoxin, glutathione and nitric oxide

Paolo Trost *(Bologna)*
How ROS-mediated glutathionylation may induce amyloid-like aggregation of plant glycolytic GAPDH

12:30 - 14:30 **Poster viewing (Session I) and free time for lunch**

Short talks by participants (Session I)

PSI.1 (Montalenti Hall)

14:30 - 16:00 **Environmental Microbiology and Biotechnology**

Chairs: Daniela Uccelletti, Michele Maria Bianchi, Annamaria Bevivino

Andrea Franzetti *(Milan)*
Light promotes the growth of heterotrophic bacteria and pollutant biodegradation on glacier surface

Alessandro Giuffrè *(Rome)*
How bacteria can respire O2 in sulfide-rich environments: a new role for bd-type terminal oxidases

Nicoletta La Rocca *(Padua)*
Photosynthesis on extrasolar planets: state of art and preliminary results of a pioneering experiment

Rachele Isticato *(Naples)*
Localization of a red fluorescence protein adsorbed on wild type and mutant spores of Bacillus subtilis

Francesco Restivo (*Parma)*
In vitro evaluation of the activity of thiosemicarbazone derivatives against mycotoxigenic fungi affecting cereals

Elisa Scioscia *(Benevento)*
Antimicrobial activity of unifloral honeys extracts from Campania against pathogenic bacteria and fungi

16:00 - 17:30 **Genetics of Microorganisms**

Chairs: Alessandra Polissi, Gianni Prosseda

Federica Briani *(Milan)*
Temperature-dependent regulation of the *lpxT* gene in *Escherichia coli* and *Pseudomonas aeruginosa*

Giulia Giallonardi *(Rome)*
Alkyl-quinolone-dependent quorum sensing controls prophage activation, autolysis and antibiotic resistance in *Pseudomonas aeruginosa* biofilm

Sarah Hijazi *(Rome)*
Gallium-protoporphyrin IX uptake pathways in *Pseudomonas aeruginosa* and growth inhibition by cytochromes targeting

Beatrice Silvia Orena (Pavia)
Multitargeting antitubercular compounds: a new precious tool in multidrug resistance age

Anna Maria Puglia *(Palermo)*
The small protein SCO2038 modulates tryptophan biosynthesis and morpho-physiological differentiation in *Streptomyces coelicolor*

PSI.2 (Giacomini Hall)

14:30 - 16:00 Genomics, Proteomics and Systems Biology

Chairs: Lilia Alberghina, Diego Di Bernardo

Emanuele Bosi *(Florence)*
Comparative genome-scale modelling of *Staphylococcus aureus* strains identifies strain-specific metabolic capabilities linked to pathogenicity

Alexey Kolodkin *(Belvaux, Luxembourg)*
Dynamic networks dealing with oxidative stress: from design principles to personalised therapies for Parkinson's disease

Marco Montini *(Florence)*
Using CRISPR/Cas9 to determine the order of specific events in a cellular system

Marco Vanoni *(Milan)*
Multi-level modeling of Metabolism, Growth and Cycle in *Saccharomyces cerevisiae*

Domenico Raimondo *(Rome)*
Computational design of short linear D-tripeptides as binding moieties for protein pockets

16:00 - 17:30 Transcription Mechanisms and Networks

Chairs: Paolo Vincenzo Pedone, Giorgio Dieci

Flavia Bernardi *(Rome)*
Inhibition of Hedgehog-dependent tumors and cancer stem cells by a newly identified naturally occurring chemotype

Leonardo Gatticchi *(Perugia)*
Intracellular trafficking of labelled BODIPY-FF-MAS reveals nuclear lipid droplets localization

Ilaria Baglivo *(Caserta)*
A new DNA target site for transciption factors from *M. loti*

Susanna Ambrosio *(Naples)*
LSD1 mediates MYCN control of epithelial-mesenchymal transition through silencing of metastatic suppressor NDRG1 gene

Annapina Russo *(Naples)*
5-FU targets rpL3 to induce mitochondrial apoptosis via cystathionine-b-synthase in colon cancer cells lacking p53

Stefano Amente *(Naples)*
High-resolution genome profiles of 8-oxodeoxyguanine, gH2AX and NBS1 reveals their co-association at transcribed long genes

PSI.3 (Blue Hall 2)

14:30 - 16:00 **Chromosome Biology, Cell Division and Cell Cycle**

Chairs: Matteo Barberis, Patrizia Lavia

Deborah Pajalunga *(Rome)*
A dysregulated dNTP pool hinders DNA replication in cell cycle-reactivated terminally differentiated cells

Marco De Vitis *(Rome)*
Alternative lengthening of telomere (ALT) implicated in telomere length modulation induced by x-rays in human primary fibroblasts

Giusj Monia Pugliese *(Rome)*
CK2 phosphorylation of MUS81 regulates its activation for proper resolution of dna intermediates in mitosis

Veronica Ferrucci *(Naples)*
Microcephaly with neurodevelopmental impairment shows microtubule assembly defects with h-prune mutations during mitosis

Elena Paccosi *(Viterbo)*
CSA and CSB proteins localize to the midbody during cytokinesis and regulate abscission through PRC1 degradation

16:00 - 17:30 **DNA replication, Repair and Recombination**

Chairs: Marco Muzi Falconi, Antonio Antoccia

Ennio Prosperi *(Pavia)*
DNA repair defects in Rubinstein-Taybi syndrome caused by low acetylation levels of base excision repair factors

Cinzia Caggiano *(Rome)*
Balancing DNA double strand break repair pathway choice as trigger for testicular germ cell tumors acquired-resistance to cysplatin

Francesco Cucco *(Pisa)*
Separase prevents genome instability by controlling fork replication speed

Eva Malacaria *(Rome)*
Inaccurate GEN1 DSBs formation promote genome instability in FA-P cells

Francesca Ripanti *(Rome)*
Measuring oxidized DNA and RNA precursors by Micro-Raman spectroscopy

Elisa Coluzzi *(Rome)*
Effects of oxidative stress on telomere structure and telomeric epigenetic modifications

PSI.4 (Blue Hall 3)

14:30 - 16:00 **Epigenetics and Epigenetic Therapies**

Chairs: Carlo Presutti, Michele Milella

Andrea Iannello *(Orbassano, TO)*
Genomic regulatory regions role in Th17 and Treg cells balance during pregnancy of multiple sclerosis patients

Alice Mazzagatti *(Pavia)*
The epigenetic landscape of equid centromeres: a cytogenetic approach

Alberto Passi *(Varese)*
Epigenetic control of hyaluronan synthases

Simone Pippa *(Rome)*
Use of JARID histone demethylases inhibitors to enlighten the biological role of these enzymes in yeast and mammalian cells with focus on transcriptional regulation

Angela Sparago *(Caserta)*
Unraveling the role of NAP1L1 into the chromatin of mammals

16:00 - 17:30 **Non-coding RNA**

Chairs: Alessandro Fatica, Francesco Fazi

Cecilia Battistelli *(Rome)*
The lncRNA HOTAIR links the repressor Snail to epigenetic modifications of specific genomic sites in Epithelial-to-Mesenchymal Transition

Pietro Laneve *(Rome)*
Linc-NeD125 establishes a ceRNA network in Group 4 Medulloblastoma

Mariangela Morlando *(Rome)*
Newly identified long non-coding RNAs promote proliferation and differentiation of murine myoblasts

Paola Infante *(Rome)*
Serum miRNAs as novel biomarkers in spinal muscle atrophy

PSI.5 (Blue Hall 4)

14:30 - 16:00 **Oncogenes and Tumor Suppressors**

Chairs: Silvia Soddu, Giulia Piaggio

Manuela Caputo *(Viterbo)*
CSB ablation induced apoptosis is mediated by increased endoplasmic reticulum stress response

Francesca Bufalieri *(Rome)*
ERAP1 is a novel drug target in the oncogenic Hedgehog signaling pathway

Andrea Camperi *(Turin)*
Role of STAT3 in the cross-talk between cancer associated fibroblasts and cancer cells

Simone Di Giacomo *(Bologna)*
MYC-mediated cell competition as an evolutionary trait of cancer

Fabiana Ferrara *(Rome)*
Human lung adenocarcinoma cell cultures derived from malignant pleural effusions as model system to predict patients chemosensitivity

PSI.6 (Blue Hall 1)

14:30 - 16:00 **Plant Metabolism and Environmental Stress**

Chairs: Concetta De Pinto, Maurizio Badiani

Olivia Demurtas *(Rome)*
Crocin biosynthesis in saffron stigmas: a tale of three compartments

Michela Zottini *(Padua)*
The mitochondrial nucleoid associated WHIRLY2 affects morphology and dynamics of mitochondria

Francesca Sparla *(Bologna)*
β-amylase-1-dependent starch degradation in mesophyll cells releases carbon skeletons required for the production of proline

Francesca Secchi *(Grugliasco, TO)*
Acidification of xylem sap pH observed in woody plants provides apoplastic environment for facilitating recovery from water stress

Namrata Mancuso *(Rome)*
Comparative analysis of the role of the different *Arabidopsis* polyamine oxidases in plant defense responses to environmental stresses

16:00 - 17:30 **Plant Nutrition**

Chairs: Irene Murgia, Stefania Astolfi

Gianpiero Vigani *(Milan)*
Keynote Lecture: Plant adaptation mechanism to Fe deficiency: the role of metabolism

Laura Zanin *(Udine)*
The urease inhibitor N-(N-butyl) thiophosphoric triamide (NBPT) affects urea acquisition and metabolism in maize seedlings

Silvia Perotto *(Turin)*
Fungal and plant gene expression in the *Tulasnella calospora - Serapias vomeracea* association provides cues on the N pathways in orchid mycorrhiza

Davide Sega *(Verona)*
FePO4 nanoparticles ad fertilizers: synthesis and evaluation of their effect on plants

Eleonora Cominelli *(Milan)*
Isolation and characterization of a new *low phytic* acid mutant in the common bean *PvMRP1* gene and study of *PvMRPs* promoters in two different plant systems

17:30 - 18:00 **Coffee Break**

18:00 - 20:00 **Societies' time**

AGI (Giacomini Hall)

18:00 - Premio Premio AGI "Ferruccio Ritossa" 2016 e presentazione del lavoro premiato
18:20 - Premio Dottorato AGI/Zanichelli 2016 e presentazione del lavoro premiato
18:40 - Assemblea dei Soci AGI

SIB (Hall A - Building CUO10)

Assemblea dei Soci
Consegna Premio SIB
Consegna Premio Heritage
Votazioni per il parziale rinnovo del CD

SIBV (Blue Hall 2)

Premio Assunta Baccarini Melandri
Assemblea dei Soci

SIMA (Blue Hall 3)

Assemblea dei Soci

SIMGBM (Montalenti Hall)

Premi 2016:
- Franco Tatò
- Mario Campa
- NAICONS

Assemblea dei Soci SIMGBM

21:00 **Social Dinner**

Thursday, September 22

9:00 - 11:00 **Plenary Symposium** (Aula Magna)

Systems Biology: from Genetic Networks to Organismal Functions

Chairs: Duccio Cavalieri *(Florence)*, Matteo Barberis *(Amsterdam, Netherlands)*

Brenda Andrews *(Toronto, Canada)*
From phenotypes to pathways: global exploration of cellular networks and pathways using systematic yeast genetics and cell biology

Jens Nielsen *(Göteborg, Sweden)*
Systems Biology of Yeast Metabolism

Diego Di Bernardo *(Naples)*
Robustness and evolvability of gene networks, role of miRNAs and protein stability in rewiring gene expression

Matteo Barberis *(Amsterdam, Netherlands)*
The Multiplex Phase Interlocker– A novel and robust molecular design synchronizing transcriptional regulation of cell cycle dynamics

11:00 - 11:30 **Coffee Break**

Parallel Symposia

11:30 - 13:30 **1. The (recent) evolution of human evolution** (Montalenti Hall)

Chairs: Giovanni Destro Bisol *(Rome)*, Luigi Capasso *(Chieti)*

Andrea Manica *(Cambridge, UK)*
The role of climate in shaping human genetic variation

Guido Barbujani *(Ferrara)*
Human evolutionary genomics: Out of Africa and beyond

Claudio Tuniz *(Trieste)*
Methodological and conceptual advancements in human evolution studies

Antonio Rosas González *(Madrid, Spain)*
Recent discoveries and classic models: Neandertals and *Outs of Africa*

11:30 - 13:30 **2. From Reverse- to Structural-Vaccinology and beyond. Current challenges against infectious diseases** (Aula Magna)

Chairs: Martino Bolognesi *(Milan)*, Guido Grandi *(Trento)*

Steve Michell *(Devon, UK)*
New vaccines against Clostridium difficile based on surface proteins

Giorgio Colombo *(Milan)*
Antigen and epitope design: what can computational biology tell us?

Louise Gourlay *(Milan)*
A structural vaccinology approach to melioidosis vaccine component development

Guido Grandi *(Trento)*
Vaccinology: the art of putting together the right ingredients

11:30 - 13:30 **3. Nitrogen: Nutritious and Noxious** (Blue Hall 1)

Chairs: Francesca Sparvoli *(Milan)*, Roberto Pinton *(Udine)*

Christoph Müller *(Giessen, Germany)*
Understanding the nitrogen cycle in a changing world

Zeno Varanini *(Verona)*
The regulation of nitrogen uptake by plants in the context of root-soil relationships: which form and which control?

Steven Spoel *(Edinburgh, UK)*
Signalling with nitrogen: reversible S-nitrosothiol protein modifications

Roberto Tuberosa *(Bologna)*
Leveraging the root QTLome for enhancing nitrogen-use efficiency in cereals

13.30 - 13.45 **MERCK - Short Talk** (Aula Magna)

Silvia Di Meglio *(Milan)*
Genome Editing with CRISPR Technology

13:45 - 15:30 **Posters viewing (Session II) and free time for lunch**

Short talks by participants (Session II)

PSII.1 (Blue Hall 2)

15:30 - 17:00 **Cellular Stress, Apoptosis and Autophagy**

Chairs: Gerry Melino, Roberto Amendola

Federico Bianchi *(Turin)*
Citron Kinase deficiency leads to p53-dependent microcephaly and DNA damage accumulation indipendently of its cytokinetic function

Sara Beji *(Rome)*
The nucleolar protein Nucleophosmin is rapidly secreted by human cardiac stromal cells in response to genotoxic stress: implications in miRNA mediated cell/cell communication

Cristina Mazzoni *(Rome)*
RNA oxidation and ageing in mRNA degradation mutants of *S. cerevisiae*

Annapina Russo *(Naples)*
rpL3 mediates the cell response to nucleolar stress induced by Act D in p53 null cancer cells

Marialuisa Piccolo *(Naples)*
In vitro bioactivity of ruthenium-based multifunctional nucleolipidic liposomes: new promising agents for cancer therapy

17.00 - 18.30 **Protein Synthesis, Degradation and Homeostasis**

Chairs: Antonio Feliciello, Marzia Perluigi

Loredano Pollegioni *(Varese)*
Schizophrenia susceptibility genes: on the characterization of two variants of human pLG72

Vittorio Bellotti *(Pavia)*
A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis

Giulia Tedeschi *(Milan)*
The double life of a disordered protein between solubility and aggregation propensity

Antonio Feliciello *(Naples)*
Integration of cAMP and the ubiquitin system at primary cilium

PSII.2 (Blue Hall 3)

15:30 - 17:00 **Development, Differentiation and Aging**

Chairs: Giorgio Camilloni, Graziella Messina

Chiara Gabellini *(Pisa)*
Modeling human intellectual disability and autism: role of the chromatin regulator setd5 during zebrafish brain development

Catherine Labbaye *(Rome)*
miR-143/β-DB/synapsin I, new players in Early Stages of Neural Differentiation

Mattia la Torre *(Rome)*
AKTIP (Ft1), a protein that interacts with lamins, is required for telomere maintenance and mouse development

Duccio Cavalieri *(Sesto Fiorentino, FI)*
The human fungal mycobiome composition reflects age, gender, immune function and diet

Valentina Mularoni *(Rome)*
Regulation of human testicular steroidogenesis during aging

17.00 - 18.30 **Cells, iPS, Cancer Stem Cells**

Chairs: Caterina Missero, Alessandro Rosa

Alessia Noto *(Rome)*
Stearoyl-CoA-Desaturase (SCD1) regulates lung cancer stemness via stabilization and nuclear localization of YAP/TAZ

Ilio Vitale *(Rome)*
CHK1 as a target to kill colorectal cancer stem cells

Bruna Corradetti *(Ancona)*
Biomimetic coatings to improve MSC homing toward the site of inflammation

Riccardo De Santis *(Rome)*
Induced Pluripotent Stem Cells (iPSCs) as a disease model system to identify altered pathways in Amyotrophic Lateral Sclerosis

Eris Bidollari *(Rome)*
Induced Pluripotent Stem Cells (iPSC) as a model of Huntington disease

PSII.3 (Montalenti Hall)

15:30 - 17:00 **Metabolism and its Regulation in Health and Diseases**

Chairs: Mauro Magnani, Francesco Paolo Mancini

Simona Barbato *(Bologna)*
Modulation of bioenergetics by the F1F0-ATPase inhibitor protein in cancer cells

Sara Biagiotti *(Urbino)*
Dexamethasone effects in Ataxia Telangiectasia cell metabolism

Chiara Damiani *(Milan)*
Unraveling the design principles of cancer metabolic rewiring with constraint-based modeling

Alessio Paone *(Rome)*
Toll like receptors: linking inflammation to carcinogenesis

Matteo Audano *(Milan)*
Zc3h10 controls mitochondriogenesis and differentiation in skeletal muscle

Alberto Zullo *(Benevento)*
DNA methylation of sirtuin genes in nutrient-deprived cultured cells

17.00 - 18.30 **Nutrition Biochemistry**

Chairs: Silvana Hrelia, Francesco Bonomi

Emanuela Leoncini *(Rimini)*
Evaluation of the potential protective effect on vascular endothelium exerted by the peptide nsLTP2 identified in wheat

Emma De Fabiani *(Milan)*
Strawberry tannins exhibit anti-inflammatory activities *in vitro* by inhibiting the NF-κB pathway and by other mechanisms

Paola Coccetti *(Milan)*
Methionine metabolism imbalance in AMPK-deficient yeast models

Alberto Barbiroli *(Milan)*
Polystyrene nanoparticles to mimic a complex matrix: functional and structural features of a hypoglycaemic lupin protein

Laura De Gara *(Rome)*
Protective effect of inulin on LPS-induced intestinal smooth muscle impairment: a redox and proteomic approach

15:30 - 17:00 **PSII.4** (Giacomini Hall)

Human Genetics and Genomic Diversity

Chairs: Davide Pettener, Antonio Torroni

Vincenza Battaglia *(Pavia)*
The worldwide spread of the tiger mosquito as revealed by mitogenome diversity

Andrea Brunelli *(Ferrara)*
Who likes to travel alone? Grammars and genes in the history of Old World migrations

Nicole Grandi *(Monserrato, CA)*
HERV-W presence and evolution within the primates lineage: characterization of the group in non-human primates and identification of highly related elements in New World Monkeys

Viola Grugni *(Pavia)*
The phylogeny of Y-chromosome haplogroup Q-L54: new insights on the first peopling of South America

Ugo Perego *(Pavia)*
Reconstructing the autosomal profile of Joseph Smith Jr, founder of Mormonism: a paternity application

Marco Sazzini *(Bologna)*
Evolutionary medicine insights from the depiction of the Italian genomic landscape

17:00 - 18:30 **Evolution**

Chairs: Guido Barbujani, Claudio Tuniz

Marco Barucca *(Ancona)*
Evolution of vitellogenin gene family in basal sarcopterygians

Giorgio Binelli *(Varese)*
Tropical rainforests that persisted: histories from the Quaternary in the Guiana shield

Cristina Giuliani *(Bologna)*
DNA methylation diversity in human populations

René Marsano *(Bari)*
The role of promoter in horizontal transposon transfer: the case of Bari transposons

Francesca Tassi *(Ferrara)*
Complete mitochondrial sequences from Mesolithic Sardinians suggest genetic discontinuity within the island

15:30 - 17:00 **PSII.5** (Blue Hall 4)

Neurobiology

Chairs: Andrea Mele, Francesco Lacquaniti, Arianna Rinaldi

Marzia Perluigi *(Rome)*
Crosstalk between insulin and mTOR signaling in Down syndrome and Alzheimer disease

Roberta Piovesana *(Rome)*
Effects mediated by M2 muscarinic receptors activation in Schwann-like cells induced from adipose mesenchymal stem cells: implication in nerve regeneration

Alessandra Maria Adelaide Chiotto *(Turin)*
Neuronal cell autonomous defects in a mouse model of Down Syndrome: contribution of TTC3 gene

Pamela Cappelletti *(Varese)*
Amino acid substitutions in D-amino acid oxidase related to human pathologies

Lionella Palego *(Pisa)*
Peripheral biomarkers of oxidative stress in depressed elderly patients

17:00 - 18:30 **Cell Communication, Cell Adhesion and Membrane Trafficking**

Chairs: Fulvio Della Ragione, Mauro Maccarrone

Andrea Magrì *(Catania)*
Does SOD1 mediate the cellular communication between mitochondria and nucleus in stress condition?

Simona Reina *(Catania)*
Cysteine mutagenesis in VDAC3 reveals a functional role of these residues in the intracellular communication

Lorenzo Depau *(Siena)*
Tetra-branched peptide theranostics: preclinical development for cancer targeting

Debora Bencivenga and Fulvio Della Ragione *(Naples)*
Biochemical and functional characterization of cancer-associated p27Kip1 mutants

Mauro Maccarrone *(Rome)*
S-Palmitoylation modulates type 1 cannabinoid receptor localization, trafficking and biological activity

15:30 - 17:00 **PSII.6** (Blue Hall 1)

Immunology and Host-Pathogen Interaction

Chairs: Anna Teresa Palamara, Aldo Venuti

Giovanni Bacci *(Sesto Fiorentino, FI)*
Shotgun metagenomic analysis of sputum samples from cystic fibrosis patients revealed distinct metabolic modules and antibiotic resistance genes along with severe lung disease

Carlotta De Filippo *(Florence)*
Alteration of gut microbiota profiles in juvenile idiopathic arthritis. Associations with HLA-B27 status and disease activity

Angela Arciello *(Naples)*
Novel human antimicrobial peptides from ApoB are endowed with promising anti-inflammatory properties

Alessandra di Masi *(Rome)*
Human serum albumin acts as a self-defense protein towards *Clostridium difficile* infection

Moira Giovannoni *(Rome)*
Identification and analysis of candidate genes involved in the oligogalacturonide-induced responses in *Arabidopsis thaliana*

17:00 - 18:30 **Plant Development and Disease**

Chairs: Alma Balestrazzi, Franco Faoro

Ilaria Fraudentali *(Rome)*
The apoplastic copper amine oxidase AtCuAOβ plays a role in stomatal closure induced by wounding, jasmonate or Microbe Associated Molecular Patterns (MAMPS)

Damiano Lironi *(Rome)*
Role of lysin motif-containing receptor-like Kinase2 in *Arabidopsis* immunity

Giovanni Mele *(Monterotondo, RM)*
Meristem activity is controlled via extensive regulation of brassinosteroid pathway by class 1 homeobox transcription factors family

Maurizio Trovato *(Rome)*
Proline modulates root meristem size and root growth in *Arabidopsis*

Ilaria Verrascina *(Rome)*
Homeostasis of oligogalacturonides and their activity as Damage-Associated Molecular Patterns (DAMPs)

Matthias Weiland *(Bonn, Germany)*
The glutamate receptor AtGLR3.7 as a regulator of growth and development in *Arabidopsis thaliana*

18:30 - 19:30 **Plenary Lecture** (Aula Magna)

Chair: Tomas Morosinotto *(Padua)*

Anna Moroni *(Milan)*
Plant photoreceptors as a tool in neuroscience

Friday, September 23

Parallel Symposia

9:00 - 11:00 **1. Shaping the Cancer Genome: from pathways to mutational signatures** (Aula Magna)

Chairs: Margherita Bignami *(Rome)*, Silvo Conticello *(Firenze)*

Ludmil Alexandrov *(Los Alamos, NM, USA)*
Signatures of mutational processes in human cancer

Angela Gallo *(Rome)*
RNA Editing dynamically rewrites the cancer code

Pietro Pichierri *(Rome)*
Pathological replication fork recovery mechanisms and signature of genome instability in human cell

Svend Petersen-Mahrt *(Milan)*
Targeted genomic lesions - to ignore, to repair, to repair badly, or to mutate. Understanding DNA deaminase induced lesions and DNA repair choice

9:00 - 11:00 **2. Unfolding truth: making sense of intrinsically disordered proteins** (Giacomini Hall)

Chairs: Rita Casadio *(Bologna)*, Andrea Mozzarelli *(Parma)*

Peter Tompa *(Brussel, Belgium)*
The tripartite degron model: the role of structural disorder in protein quality control

Sonia Longhi *(Marseille, France)*
Protein complementation and kinetics illuminate mechanisms of partner recognition by IDPs

Stefano Gianni *(Rome)*
Understanding the mechanisms of folding and binding of intrinsically disordered proteins

Silvio Tosatto *(Padua)*
Cataloguing and annotating flavours of intrinsic disorder in proteins

9:00 - 11:00 **3. Plant adaptation and phenotypic plasticity to climate change** (Montalenti Hall)

Chairs: Mario Pezzotti *(Verona)*, Andrea Schubert *(Turin)*

Christophe Maurel *(Montpellier, France)*
Plant aquaporins as targets and players of cell signalling

Andrea Nardini *(Trieste)*
Climate change and forest decline: the physiology of tree mortality

Maarten Koornneef *(Wageningen, Netherlands)*
Exploring natural variation in *Arabidopsis*

Silvia Dal Santo *(Verona)*
Dissecting GxE interactions in grapevine through a transcriptomic approach

11:00 - 11:30 **Coffee Break**

11:30 - 13:30 **Plenary Symposium** (Aula Magna)

New roles and molecular pathophysiology of mitochondria

Chairs: Paolo Sarti *(Roma)*, Vito De Pinto *(Catania)*

Andrea Rasola *(Padua)*
Shutting your TRAP to kill tumors? The oncogenic role of the mitochondrial chaperone TRAP1

Lorenzo Galluzzi *(Paris, France)*
Mitochondrial control of stress responses and danger signalling

Vito De Pinto *(Catania)*
Mitochondrial VDAC isoforms: a bridge (and a target) between an oxidative and a reducing compartment

Isabella Panfoli *(Genoa)*
Pathophysiology of the extra-mitochondrial oxidative phosphorylation

13:30 - 15:00 **Free time for lunch**

15:00 - 16:00 **The EMBO Keynote Lecture** (Aula Magna)

Chair: Gennaro Ciliberto *(Naples)*

Titia Sixma *(Amsterdam, Netherlands)*
Trapping transient states in DNA mismatch repair

Congress Closure

PLENARY LECTURE

PL.1
The p53 family in cancer biology

I. Amelio[1], F. Bernassola[2], E. Candi[2], G. Chillemi[3],T. Wah Mak[4] and **Gerry Melino**[1,2]
[1]MRC Toxicology Unit, LeicesterLE1 9HN, United Kingdom, [2]University of Rome Tor Vergata, Rome, Italy, [3]SuperComputing Applications & Innovation Department, CINECA, Rome, Italy, [4]The Campbell Family Cancer Research Institute, Toronto, Ontario M5G 2M9, Canada

p53, p73 and p63 are involved in female infertility maternal reproduction (Nature Rev MCB 2011;12,4:259) and in cancer (TiBS 2014;39,4:191). We studied their activation, transcriptional targets, mechanisms, degradation. On p53 we performed a molecular dynamics study, showing an induced-fit interaction of the C-terminus with DBD domain. We detect dynamic deformations by p53 tetrameric conformations; these modulate the electrostatic potential isosurfaces of the whole p53-DNA complex (Oncogene, PMID: 26477317). TAp73$^{-/-}$ mice show high tumor incidence with neurodegeneration. TAp73 opposes HIF-1 activity, affecting tumour angiogenesis. This novel mechanism of HIF-1 regulation provides additional molecular explanation for growth, progression, and invasiveness of human cancers. (PNAS-USA 2015;112,1:226) (TiBS 2015;40,8:425) P63 is a determinant of skin development. We found that ΔNp63 regulates mammary Cancer Stem Cells self-renewal and breast tumorigenesis via the direct transactivation of Sonic Hedgehog, Gli2, and Ptch1. (PNAS-USA 2015. 112,11: 3499-504.). At least in part, this seems to be exerted by regulation of the metabolism via Hexokinase II (PNAS-USA 2015. 112,37: 11577-82).

PL.2
On the spontenous generation of ribozymes and of Life

Ernesto Di Mauro
Dept SEB, Tuscia University, Viterbo, Italy

We have analyzed the reactions leading from the one-carbon atom compound NH_2COH formamide to prebiotically relevant compounds in the presence of catalysts. We observe the formation of all the extant biological nucleic bases, carboxylic acids, aminoacids and condensing agents. We also observe in the same chemical frame the formation of cyclic nucleotides and their spontaneous polymerization to oligonucleotides, their terminal ligation yielding longer polymers, a ribozyme activity causing the terminal transfer of nucleotides between in vitro abiotically generated oligomers. In vitro generated oligonucleotides thus automatically increase the chemical information of the system. Numerous hurdles remain. Anyhow: all extant nucleic bases and nucleosides can be abiotically synthesized. Phosphorylation: observed formation of cyclic nucleotides from preformed nucleosides. Polymerization: characterized for 3',5'cGMP, 3',5'cAMP, and 3',5'cCMP. These results entail that the spontaneous generation of proto-metabolic and proto-genetic systems did not require an exceedingly complex initial set-up, but was the result of the interplay between combinatorial chance and thermodynamic necessity.

PL.3
Nuclear architecture and chromatin dynamics

K. Gesson, S. Vidak, P. Rescheneder, T. Dechat, **Roland Foisner**
Max F. Perutz Laboratories, Medical University Vienna, Austria

Lamins are components of the peripheral nuclear lamina, which interacts with heterochromatic genomic regions, termed lamina-associated domains (LADs). Unlike lamin B1 that is exclusively found at the inner nuclear membrane, lamins A and C localize also in the nuclear interior in association with the chromatin-binding LEM-protein lamina-associated polypeptide (LAP) 2α. Chromatin immunoprecipitation of euchromatin- and heterochromatin-enriched samples surprisingly revealed that in contrast to lamin B1, lamin A/C associates with both types of chromatin. Euchromatic regions occupied by lamin A/C overlap with those bound by LAP2α, are gene rich, and enriched in active histone marks. Lamin A/C's chromatin interaction shifts towards heterochromatic regions in LAP2α-deficient cells affecting epigenetic histone marks in euchromatin and gene expression. Overall our data describe a novel role of nucleoplasmic lamins and LAP2α in the regulation of gene expression in a context- and cell type-dependent manner by affecting the organization of euchromatic chromatin throughout the nucleus. This novel function of lamins may also be involved in lamin-linked diseases, such as premature aging disease.

PL.4
Plant photoreceptors as a tool in neuroscience

Anna Moroni
Department of Biosciences, University of Milan, Italy

Optogenetics uses light-activated ion channel that are found in plants [1] to control action potential firing in neurons. Channelrhodopsin 2 (ChR2) is a cation aspecific light-gated ion channel first characterized in the green photosynthetic alga *Chlamydomonas*. When expressed in neurons, ChR2 can be switched on/off remotely by visible light. This in turn modulates the membrane voltage and as a consequence the firing of action potentials. The ability to control the activity of genetically specified neurons in a temporally precise fashion provides the opportunity to investigate the causal role of specific cell classes in neural computations, behaviors and pathologies. After the initial success of optogenetics, based on naturally occurring light-gated channels, there was a demand in the scientific community for channels with different ion selectivity and/or light-dependent properties. Here I report the engineering of a synthetic light-gated potassium (K^+) channel, which was built by fusing a plant photoreceptor to the viral protein Kcv [5].

[1] Boyden et al, Nat Neurosci. 2005 Sep;8(9):1263-8.
[2] Ryan et al, Science 348:6238 1007-1013 (2015)
[3] van Wyk et al, PLOS Biology (2015)
[4] Adamantidis et al, Nat Neurosci. Aug 26;18(9):1202-12
[5] Cosentino et al, Science. 2015 May 8;348(6235):707-10.

PLENARY SYMPOSIUM

PS1 - Cancer Stem Cells

PS1.1
Remodelling the Hematopoetic Stem Cell Niche by Acute Myeloid Leukemic Cells

Dominique Bonnet
Hematopoietic Stem Cell Lab, The Francis Crick Institute, London, UK

Acute myeloid leukemia (AML) is a hematologic malignancy, arising within the bone marrow, which is characterized by the uncontrolled proliferation of leukemic blasts, often in association with a disruption of normal hematopoiesis. Like their normal counterparts, AML cells depend upon both cell-intrinsic and -extrinsic regulatory signals generated by their surrounding microenvironment, for their survival and proliferation. AML has long been considered a hematopoietic-cell autonomous disorder in which disease initiation and progression is driven by hematopoietic cell intrinsic genetic events. Recent experimental findings in diverse model systems have challenged this view, implicating different stromal cells of the bone marrow in disease pathogenesis. Thus it is now accepted that leukemic hematopoiesis can turn the BM niche into a "leukemic niche" which promotes leukemic stem cell (LSC) function and impairs the maintenance of normal HSC. However, much remains to be understood about how different leukemic cells impacts the BM microenvironment and, in turn, how changes in the activity of specific BM niche cells contribute to AML pathogenesis. This talk will highlight some of the current understanding of the alterations of BM niche components and how the dialogue between leukemic and stromal cells participated in leukemogenesis.

PS1.2
Intestinal Stem Cells in Homeostasis, Inflammation and Cancer: a Matter of Niche

Riccardo Fodde
Dept of Pathology, Erasmus Univ. Medical Center, Rotterdam, The Netherlands

The intestinal stem cell niche provides cues that actively maintain gut homeostasis. Dysregulation of these cues may compromise intestinal regeneration upon tissue insult and/or promote tumor growth. Here, we identify secreted phospholipases A2 (sPLA2s) as stem cell niche factors with context-dependent functions in the digestive tract. We show that group IIA sPLA2, a known genetic modifier of mouse intestinal tumorigenesis, is expressed by Paneth cells in the small intestine, while group X sPLA2 is expressed by Paneth/goblet-like cells in the colon. During homeostasis, group IIA/X sPLA2s inhibit Wnt signaling through intracellular activation of Yap1. However, upon inflammation they are secreted into the intestinal lumen, where they promote prostaglandin synthesis and Wnt signaling. Genetic ablation of both sPLA2s improves recovery from inflammation but increases colon cancer susceptibility due to release of their homeostatic Wnt-inhibitory role. This "trade-off" effect suggests sPLA2s have important functions as genetic modifiers of inflammation and colon cancer.

PS1.3
The Numb:p53 axis connect asymmetric cell division and tumor suppression in mammary stem cells

Pier Paolo Di Fiore
University of Milan and IFOM, Milan Italy

Numb is a cell fate determinant that by asymmetrically partitioning at mitosis controls binary cell fate decisions. In human breast cancers, there is frequent loss of Numb expression, due to its exaggerated ubiquitination and ensuing degradation. This causes alterations in two major downstream pathways. On the one hand, lack of Numb allows for unchecked signaling activity of the Notch receptor. On the other, lack of Numb causes attenuation of the p53 signaling pathway. Tumors displaying loss-of-Numb expression are addicted to this event and to its molecular consequences. Our recent results point to the mammary stem cell (MaSC) compartment as the cellular "target" of Numb misregulation in breast tumors. We have developed a technology to cultivate and purify MaSC. In normal MaSC, Numb is asymmetrically partitioned at mitosis. This in turn dictates the replicative fate, in that the Numb(+) cell remains quiescent (and retains MSC capabilities), whereas the Numb(-) cell acquires a progenitor fate and undergoes rapid symmetric divisions. The control of Numb over MaSC fate is executed through the ability of Numb of silencing Notch signaling and maintaining high levels of p53 in the MSC. This latter event is due to Numb-mediated inhibition of the ubiquitinating function of the E3 ligase Mdm2 over p53. Lack of Numb in cancer MaSC causes a switch form the asymmetric to the symmetric mode of division, thus forcing both daughter cells to assume the same replicative fate. In addition, Numb has a role in the mammary gland hierarchy at the level of progenitors, by enforcing the actuation of a luminal differentiative program, and loss of Numb results into the reversion of progenitors to cancer stem-like cells, in association to EMT. In human breast cancers, loss of Numb is a frequent event and leads to expansion of the SC compartment. Interference with the Numb:p53 axis in human tumors causes tumor reversion through specific inhibition of cancer stem cells. Our understanding of how Numb is mechanistically involved in all these aspects will be discussed.

PS1.4
YAP/TAZ in stem cells and tissue regeneration

Stefano Piccolo
Department of Molecular Medicine, University of Padua School of Medicine, via Ugo Bassi 58/B 35131 Padua, Italy

We study how cells sense their environment and use this information to build and maintain tissues with specific form, size and function, and how these systems are corrupted in diseases. At the centerpiece of these events is the activity of the transcriptional coactivators YAP and TAZ. Enhanced YAP/TAZ activity is emerging as a hallmark of multiple human tumors. I will discuss the cell and tissue-level mechanisms that lead to unrestrained YAP/TAZ activity, in turn essential for tumor formation and for tissue regeneration upon injury. I will also present new evidence on the function of YAP/TAZ in regulating the biology of normal somatic stem cell explanted from adult tissues.

PS2 - CRISPR/CAS: from a prokaryotic immune system to a powerful tool for biomedical and agricultural research

PS2.1
The immune system of bacteria: CRISPR and beyond

Rotem Sorek
Department of Molecular Genetics, Weizmann Institute of Science

Phages are arguably the most genetically diverse organisms on earth. To mitigate phage infection, bacteria developed a sophisticated set of defense systems that includes CRISPR-Cas, the prokaryotic adaptive defense system. The talk will describe recent understanding in CRISPR defense against phage, as well as other strategies used by bacteria to defense from phage infection.

PS2.2
CRISPR/Cas9 Technology: Universal Tool for Functional Genome Annotation

Wolfgang Wurst

The discovery of the Cripr/Cas9 endonuclease from the bacterial adaptive immune system Crispr can be easily adapted and programmed to bind, cleave or modify eukaryotic DNA sequences using just a short guide RNA (sgRNA) as vehicle. Application of Cas9 is enabling the creation of sophisticated disease models by introducing precise point mutations, deletions, inversions and duplications. Crispr/Cas9 is broadening the genetically tractable organisms that can be used to study biological mechanism during health and disease. In addition, Crispr/Cas9 technology has the potential for somatic gene manipulation in vivo, as well. In this presentation, I will give an overview of the latest developments of Cripsr/Cas9 applications in our laboratory to increase frequency of homologous recombination (HR), to establish disease models in vivo as well as in vitro efficiently.

PS2.3
Double-strand break induced genome engineering in plants

Holger Puchta
Bontanical Institute, Karlsruhe Institute of Technology, Germany

In the last years the CRISPR/Cas system became the major tool for genome engineering. We were able to demonstrate *S. pyogenes* Cas9 nuclease induced, heritable targeted mutagenesis in *Arabidopsis* as well as homology dependent *in planta* gene targeting. A major concern is the specificity of Cas9. Off-target effects might be avoided using two adjacent sgRNA target sequences to guide a Cas9 protein that was transformed into a nickase to each of the two DNA strands, resulting in the formation of adjacent single strand breaks (SSBs). We could show that this strategy is as mutagenic as the nuclease on the target site. Interestingly; sequence duplications are a prominent outcome of this approach, hinting to the possibility that in general the repair of adjacent SSBs is a major cause of sequence duplications during genome evolution of plants. We also applied successfully the Cas9 orthologues from *S. thermophilus* and *S. aureus* for genome engineering in plants. The simultaneous use of different Cas9 orthologues will offer the opportunity to control genetic information of plant cells on more complex levels than before and will lay the basis for future synthetic approaches in plant biology.

PS3 - Oxygen Sensing and Redox Signalling: common themes of aerobic life

PS3.1
Sensing oxygen levels in animal cells by HIF hydroxylases

Peter J. Ratcliffe
Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK

Maintenance of oxygen homeostasis is a fundamental physiology challenge and hypoxia is a component of many human diseases. The transcriptional response to hypoxia is mediated by hypoxia inducible factor (HIF), the oxygen sensitive signal being generated by a series of protein hydroxylases that catalyse prolyl and asparaginyl hydroxylation at specific residues in the regulatory HIF-alpha subunits. In the presence of oxygen prolyl hydroxylation directs HIF-alpha for destruction by the pVHL-ubiquitin-proteasome pathway, whilst asparaginyl hydroxylation blocks recruitment of co-activators. The HIF hydroxylases belong to two distinct groups of Fe(II) and 2-oxoglutarate dependent dioxygenase, which split dioxygen and couple oxidation (hydroxylation) of HIF-alpha to the oxidative decarboxylation of 2-oxoglutarate to succinate and carbon dioxide. This lecture will review recent advances in understanding of the HIF hydroxylase system, including its evolution, transcriptional organization and role in the integrated physiology of oxygen homeostasis, as well as its tractability as a therapeutic target.

PS3.2
When plants face oxygen limitations

Francesco Licausi
Biology Dept, Università di Pisa, Italy

Oxygen is an indispensable substrate for many biochemical reactions in eukaryotes, among which respiration is essential to produce energy. However, plants lack an active transport mechanism to distribute oxygen to all cells. Therefore, steep oxygen gradients are often generated inside many plant tissues, which can be exacerbated by environmental perturbations that further reduce oxygen availability. Plants possess various strategies to cope with spatial and temporal variations in oxygen availability, many of which involve metabolic adaptations to deal with energy crises induced by low oxygen. There responses are induced gradually when oxygen concentrations decrease and are rapidly reversed upon reoxygenation. A direct effect of the oxygen level can be observed in the stability, and thus activity, of group-VII Ethylene Response Factors (ERFs) that control the expression of hypoxia-induced genes. This is mediated by an oxygen-dependent proteolysis that follows the N-end rule pathway.

PS3.3
Proteomics unravel an intricate redox network of 1000 proteins regulated by thioredoxin, glutathione and nitric oxide

C. H. Marchand[1], E. Pérez-Pérez[1], S. Morisse[1], M. Zaffagnini[2], P. Trost[2], **S. D. Lemaire**[1]
[1]Institut de Biologie Physico-Chimique, UMR8226 CNRS, Sorbonne Universités UPMC, Paris, France, [2]Laboratory of Plant Redox Biology, Department of Pharmacy and Biotechnology, University of Bologna, Italy

Protein redox regulation and redox signaling mainly rely on a set of post-translational modifications (PTMs) of protein cysteine thiols essentially controlled by conserved oxidoreductases named thioredoxins and glutaredoxins. In photosynthetic organisms, besides the well-established regulations mediated by disulfide/dithiol exchange reactions under the control of thioredoxins, two redox PTMs, namely nitrosylation and glutathionylation, recently emerged as mechanisms playing a major role in numerous fundamental cell processes. Emerging evidence suggests the existence of an intricate crosstalk between the different redox PTMs which remains largely unexplored. Using large scale proteomic approaches in the unicellular green alga*Chlamydomonas reinhardtii*, we have unraveled the complexity of the redox network by identifying more than 1000 proteins regulated by thioredoxins, by nitrosylation or by glutathionylation. Targeted biochemical studies allowed to confirm the regulation of several proteins, such as Calvin-Benson cycle enzymes, and to analyze the underlying molecular mechanisms.

PS3.4
How ROS-mediated glutathionylation may induce amyloid-like aggregation of plant glycolytic GAPDH

Paolo Trost[1], S. Fermani[2], M. Malferrari[1], C.H. Marchand[3], S. Murail[3], S. Bonacchi[2], G. Falini[2], M. Montalti[2], G. Venturoli[1], M. Badeen[3], S.D. Lemaire[3], M. Zaffagnini[1]
[1]Dept Pharmacy and Biotechnology (FABIT), University of Bologna, [2]Dept Chemistry "G. Ciamician", University of Bologna, [3]Sorbonne Universités, UPMC University of Paris 6, Centre National de la Recherche Scientifique, Institut de Biologie Physico-Chimique, Paris, France

Cysteine-based redox signaling pathways play a central role under both physiological and stress conditions. The extent and type of cysteine redox modifications depend upon ROS content and exposure. Specific cysteine residues that are made reactive ($-S^-$) by their protein microenvironment may be primarily oxidized by H_2O_2 to sulfenic acids (-SOH) and then recovered via a glutathionylation/deglutathionylation cycle. However, prolonged oxidant exposure may cause irreversible oxidation when the –SOH is further oxidized to $-SO_2H$ or $–SO_3H$. Glycolytic GAPDH is an abundant cytosolic enzyme with a catalytic cysteine that reacts at similar rates, either with the substrate G3P or with H_2O_2. Besides inhibiting the dehydrogenase activity, redox modification of GAPDH induce novel functions including the triggering of apoptosis upon nuclear translocation. Moreover, human oxidized GAPDH tend to form complexes and associate to amyloid aggregates. Here we show that plant glycolytic GAPDH forms amyloid-like aggregates as a consequence of persisting glutathionylation. The structural basis are investigated and the relevance for protein stability in plants under oxidative stress will be discussed.

PS4 - Systems Biology: from Genetic Networks to Organismal Functions

PS4.1
From phenotypes to pathways: global exploration of cellular networks and pathways using systematic yeast genetics and cell biology

Brenda J. Andrews
The Donnelly Centre, University of Toronto, Toronto, Canada

We exploit the budding yeast system to produce quantitative data that yield the spatio-temporal resolution of dynamic biological processes required to model complex systems. In particular, we implemented two experimental pipelines that combine array-based genetics and automated microscopy for quantitative cell biological screens. Our first pipeline enables the introduction of fluorescent markers of subcellular compartments of interest into genome-wide yeast mutant collections. We plan to produce comprehensive cell biological datasets that will reveal all genes that influence the function of fundamental cell compartments and structures in a model eukaryotic cell. Our second phenomics pipeline involves a high-content screening approach designed to rapidly survey proteome dynamics in living cells, and to define proteome flux using a computational image-based method. Our project provides a proof-of-principle for both a technical and conceptual platform that we intend to adapt to other systems.

PS4.2
Systems Biology of Yeast Metabolism

Jens Nielsen[1,2]
[1]Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, [2]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK-2800 Lyngby, Denmark

Metabolism is highly complex involving a large number of chemical reactions. These reactions are traditionally grouped into pathways with dedicated functions, but recent analysis of metabolism has shown that there is a high degree of connectivity between these pathways due to common sharing of co-factors and key metabolites. Also regulation of metabolism is complex due to the requirements for maintaining cellular homeostasis. In this talk I will give illustrations of how different parts of cellular metabolism are connected, i.e. central carbon metabolism, lipid metabolism and protein secretion. I will illustrate how metabolism can be modelled at the genome-scale and how incorporation of protein crowding can constrain model simulations and hereby allow for very accurate model predictions. I will further demonstrate how different types of quantitative omics data, e.g. transcriptome and proteomie data, can be integrated into this kind of modeling and hereby new insight into cellular metabolism can be obtained. Finally I will discuss how this modeling concept can be expanded to incorporate regulation and how we can get new insight into transcriptional regulation through ChIP-exo analysis.

PS4.3
Robustness and evolvability of gene networks, role of miRNAs and protein stability in rewiring gene expression

G. Gambardella[1], A. Carissimo[1], R. Blelloch[2,3], **Diego di Bernardo**[1,4]
[1]Telethon Institute of Genetics and Medicine, Naples, Italy, [2]The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, Center for Reproductive Sciences, [3]Department of Urology, University of California, San Francisco, San Francisco, California, 94143, USA, [4]Dept. of Chemical, Materials and Industrial Production, Univ Federico II of Naples, Italy.

MiRNAs act post-transcriptionally to suppress multiple target genes simultaneously and it has been postulated that one of their main functions is to suppress biological variability across cells. To directly assess the impact of multi-targeting in embryonic stem cells (ESCs), we used single cell sequencing combined with manipulation of individual miRNA levels. Two distinct microRNAs, miR-294 and let-7, were introduced into otherwise miRNA deficient Dgcr8 knockout ESCs. MiR-294 reduced, while let-7 increased transcriptional heterogeneity within the population of cells. Both miRNAs induced the co-regulation of their respective gene targets. However, they had opposing effects on pathways important to ESC self-renewal. Especially striking, let-7 promoted, while miR-294 suppressed the co-regulation of cell cycle genes, consistent with a role for the two miRNAs in driving the distinct cell cycle structures of embryonic versus somatic cell types. Together, these findings show that microRNAs link their targets into networks while either increasing or decreasing cellular heterogeneity depending on context.

PS4.4
The Multiplex Phase Interlocker – A novel and robust molecular design synchronizing transcriptional regulation of cell cycle dynamics

Matteo Barberis
Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

The eukaryotic cell cycle is regulated by a transcriptional oscillator enabling its temporal dynamics. Oscillator-mediated gene expression is dependent on genetic background and interactions with co-factors. Whether the former is known, the identification of mechanisms that underlie genetic interactions is still a challenge. Here we show that a Systems Biology approach integrating dynamic ODE-based modeling and biochemical experimentation allows for the identification of such mechanisms. A regulatory motif responsible for gene expression mediated by transcription factors (TF) that control cell division in budding yeast was predicted. This novel molecular design, that we coined as Multiplex Phase Interlocker, MPI, uniquely describes a molecular timer (TF) that relies on separate inputs (kinase complexes) converging on a common target (TF itself). Within the motif, a progressive TF activation may be realized by the sequential activation of kinase complexes. This prediction is validated experimentally, indicating that the kinase-TF axis is pivotal for timely transcriptional dynamics. Finally, robustness analyses highlight that MPI is a conserved design principle in cell cycle control.

PS5 - New roles and molecular pathophysiology of mitochondria

PS5.1
Shutting your TRAP to kill tumors? The oncogenic role of the mitochondrial chaperone TRAP1

Andrea Rasola
Dept Biomedical Sciences, Univ. of Padova

We have recently shown that the mitochondrial molecular chaperone TRAP1 contributes to the metabolic rewiring of tumor cells by down-regulating oxidative phosphorylation through inhibition of succinate dehydrogenase (SDH), the complex II of the respiratory chain. In this way, TRAP1 plays an important role in tumor growth, as it (i) promotes HIF1α stabilization via accumulation of the oncometabolite succinate, leading to the establishment of a pseudohypoxic state, and (ii) displays an anti-oxidant effect that protects neoplastic cells from lethal oxidative insults. We have now observed that TRAP1 induction is involved in the establishment of early neoplastic lesions during the process of hepatocarcinogenesis, and that TRAP1 takes part in a mitochondrial signalling cascade following oncogenic hyperactivation of the Ras/ERK kinase pathway. Our results indicate that TRAP1 is a major player of early bioenergetic changes that characterize tumor cells, and a possible target for novel anti-neoplastic strategies targeting metabolic changes of neoplasms.

PS5.2
Mitochondrial control of stress responses and danger signaling

Lorenzo Galluzzi
Centre de Recherche des Cordeliers, Paris

Mitochondria not only mediate key bionergetic functions by synthesizing the majority of ATP, but are also intimately involved in cellular stress responses. Moreover, mitochondria control several pathways that lead to regulated cell death, which exerts prominent homeostatic functions at the organismal level. Thus, mitochondria contribute to the maintenance of both cellular and organismal homeostasis. Recently, we focused on the mechanisms whereby cell death driven by mitochondria informs the organism on a state of danger, resulting in the activation of an adaptive immune response. This is particularly relevant for cancer therapy, as altering the mechanisms through which malignant cells die significantly affects their immunogenicity, and ultimately the efficacy of treatment.

PS5.3
Mitochondrial VDAC isoforms: a bridge (and a target) between an oxidative and a reducing compartment

S. Reina[1], V. Checchetto[2], R. Saletti[3], A. Gupta[4], D. Chaturvedi[4], C. Guardiani[5], F. Guarino[1], M. A. Scorciapino[6], A. Magrì[1], S. Foti[3], M. Ceccarelli[6], A. A. Messina[7], R. Mahalakshmi[4], I. Szabo[2] and **Vito De Pinto**[1]
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Cell requires perfectly functioning mitochondria. Neverthless the organelle is the source of redox species. The accumulation in mitochondria of oxidative agents must be signaled to the cell. VDACs (Voltage Dependent Anion selective Channel) are pore-forming mitochondrial outer membrane proteins involved in important cellular processes. There are 3 VDAC isoforms and VDAC3 has not yet been characterized. We addressed the specific structural questions related to the peculiar function of VDAC3 cysteines. These residues protrude towards the intermembrane space. In particular we find that cysteines in VDAC3 can stay in different oxidation states, ranging from the most reduced sulfhydryl to the most oxidized, sulfonic. Each VDAC3 molecule has a distinct set of cysteines with variable oxidation states, i.e. they are "redox isomers". Such VDAC3 "redox isomers" are not genetically determined but are a consequence of the environmental state. This means that their oxidation depends on the redox potential variation in the mitochondrial intermembrane space. Since this complex oxidation pattern is a consequence of the ROS level in the IMS, VDAC3 monitores the redox homeostasis. This work gives new insight in mitochondrial redox sensing and in the description of the function ot the outer membrane and the intermembrane space, two organelle compartments poorly characterized.

PS5.4
Pathophysiology of the extra-mitochondrial oxidative phosphorylation

Isabella Panfoli
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A major challenge in biomedical science is ATP supply in the nervous system (NS). The mitochondrial Electron Transfer Chain (ETC), F_oF_1-ATP synthase and Tricarboxylic Acid (TCA) Cycle enzymes are functionally expressed outside the mitochondrion, in myelin and rod outer segments (OS). These extra-mitochondrial sites of oxidative phosphorylation (OXPHOS), devoid of mitochondria, consume oxygen (O_2) and synthesize ATP in the presence of any TCA Cycle intermediate, in a manner sensitive to mitochondrial probes and inhibitors. During rat myelinogenesis expression of myelin basic protein, ETC, F_oF_1-ATP synthase and mitochondrial fusion proteins increased from day 0 to 33, while that of inner mitochondrial membrane proteins decreased. Exosomes from various sources conduct an extra-mitochondrial OXPHOS, suggesting a protein transfer from mitochondria to the endoplasmic reticulum. The OXPHOS is a major source of reactive O_2 species in the rod OS, that polyphenols can scavenge, by inhibiting the ectopic F_oF_1-ATP synthase. Oxidative stress originated by the extra-mitochondrial OXPHOS may represent a pivotal pathogenic mechanism for many neurodegenerative diseases.

The EMBO Keynote Lecture

E1.1
Trapping transient states in DNA mismatch repair

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To avoid mutations in the genome, DNA replication is followed by DNA mismatch repair (MMR). This well-conserved cascade of MutS and MutL homologs is critical for genome integrity. Mutations in MMR proteins lead to mutator phenotypes and predispisition to cancer in humans. The activation of MMR starts when a MutS homolog recognizes a mismatch and undergoes an ATP-dependent transformation to an elusive sliding clamp state. How this transient state promotes MutL homolog recruitment and activation of repair is unclear. Here we crystallography and cryo-EM to present structures of MutS and the MutS/MutL complex where we trap transient states, by making use of highly specific mutants and single-cysteine crosslinking. The suprisingly large conformational changes that we observe could be validated by FRET, binding studies and mutagenesis and interpreted in terms of the MMR cycle. The structures capture MutS in various conformations. Particularly the MutS/MutL complex traps the sliding clamp conformation, where pivoting of MutS subunits around ATP causes it to push DNA into a new channel and reorientation of a connector domain creates an interface for MutL with both MutS subunits. Our work explains how the sliding clamp promotes loading of MutL onto DNA, to activate downstream effectors. We thus elucidate a crucial mechanism that ensures that MMR is initiated only after detection of a DNA mismatch.

PARALLEL SYMPOSIA

S1 - The (recent) evolution of human evolution

S1.1
The role of climate in shaping human genetic variation

Andrea Manica
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The timing and tempo of the expansion of Anatomically Modern Humans out of Africa have been argued to be governed by changing climatic conditions that opened and closed key gateways to different regions. However, whilst genetics has played a key role in determining the timing of expansion waves, causative climatic factors are mostly invoked without any formal testing. In this talk, I will discuss the key dispersal events of Anatomically Modern Humans that have been associated with climatic changes, and discuss how genetic, archaeological and climatic information can be formally integrated in a quantitative framework to study their interaction, and better understand the processes by which our species expanded to colonise the whole globe.

S1.2
Human evolutionary genomics: Out of Africa and beyond

Guido Barbujani
Dept. Life Sciences and Biotechnology, University of Ferrara

Fossil and genetic data agree in indicating the African continent as the main place of origin for anatomically modern humans. However, it is unclear whether early modern humans left Africa through a single, major process, dispersing simultaneously over Asia and Europe, or in two main waves, first through the Arab peninsula into Southern Asia and Melanesia, and later through a Northern route crossing the Levant. An analysis of genome diversity shows that accurate estimates of the divergence times between Europeans and Africans are more recent than those between Australo-Melanesia and Africa, and statistically incompatible with the effects of a single dispersal. This difference cannot possibly be accounted for by the effects of either hybridization with archaic human forms in Australo-Melanesia, or back migration from Europe into Africa. Furthermore, in several populations of Asia there is evidence for relatively recent admixture events, which could have obscured the signatures of the earliest processes. The hypothesis of a single major human dispersal from Africa appears hardly compatible with the observed patterns of genome diversity.

S1.3
Methodological and conceptual advancements in human evolution studies

Claudio Tuniz
The Abdus Salam International Centre for Theoretical Physics, Trieste, Italy, Centro Fermi, Rome, Italy, Centre for Archaeological Science, Univ. of Wollongong, Australia

Advanced tools developed by physicists contribute to methodological and conceptual progress in human evolution studies by in-depth characterisation of hominin fossils and archaeological materials. A number of advanced geo-chronometers - based on radioactivity - provide high precision dating, which are critical to study hominin philogenesis and to synchronise anatomical, cultural, environmental and genetic variations. Non-destructive x-ray and neutron imaging techniques allow us to reconstruct the internal microstructure of fossilised bones and teeth and perform virtual 3D histological analyses which provide detailed information on the life history and diet of hominins. X-ray microtomography of their skulls can also be used to reconstruct the external structure of the brain. In addition, digital microstructural images provide useful information on the biomechanical stress of specific skeletal components via finite element analyses. This method has been recently applied to hyoids, mandibles, knee patellae and other bones. Finally, concepts originated in physics - such as complex system analyses and network theory - are recently proposed to study the evolution of societies.

S1.4
Recent discoveries and classic models: Neandertals and *Outs of Africa*

Antonio Rosas González
Dept Paleobiology, Museo N. Ciencias Naturales, CSIC, Madrid, Spain

Paleoanthropological research has considerably evolved in the last decade, both methodologically and conceptually. A unique and fertile intertwining between new molecular sciences (e.g. paleogenetics) and the classic natural history disciplines such as paleontology and prehistory has become established. Advances can be organized around both the development of Paleogenomics (obtaining high coverage complete fossil genomes) and the discovery of new fossil samples, and improved fieldwork methodologies (e.g. excavation protocols to avoid modern human DNA contamination). Perhaps, the most conspicuous recent achievement has been the finding in Southern Siberia of an unknown hominin lineage - the so-called *Denisovans*-, identified exclusively from ancient DNA material. Besides, the detection of low frequency though recurrent interbreeding among human species is altering our models of recent human evolution. Of especial interest is the issue of *H. sapiens* and Neandertal genetic interactions. Very recently, it has been proposed that there were at least two cases of cross-breeding between both species: one which is already dated at 50 ky ago and an earlier one which date at 100 ky, possibly in the Near East. Currently, processes related to Neandertal population movements across western Eurasia and its interaction with modern human *Outs of Africa* events are at the core of the discussion.

S2 - From Reverse- to Structural-Vaccinology and beyond. Current challenges against infectious diseases

S2.1
New vaccines against *Clostridium difficile* based on surface proteins

Steve Michell
Biosciences, College of Life and Environmental Sciences, University of Exeter, UK

Clostridium difficile is a Gram positive bacteria that is currently the cause of 2,000 deaths per annum in the United Kingdom alone. Globally the disease is a major cause of health care acquired infections. Most diseases caused by clostridia are the result of the action of the potent toxins that these bacteria produce. As such, many of these diseases including tetanus and botulism, are preventable through the availability of corresponding toxoid vaccines. Major pharma have been developing toxoid vaccines for *C. difficile* based on the exotoxins produced by this bacterium in addition to monoclonal antibodies against these exotoxins. To date, none of these vaccine candidates have passed investigational status. Given the variability of the toxins between different strains of *C. difficile* we sought to commence understanding the mechanisms of adherence of this pathogen with an aim to developing alternative vaccination strategies. This presentation will describe the work of our laboratory, and others, in characterising the role of surface components of *C. difficile* involved in adherence that have a potential role as novel vaccine candidates.

S2.2
Antigen and epitope design: what can computational biology tell us?

Giorgio Colombo
Istituto di Chimica del Riconoscimento Molecolare, CNR

Here, I will present novel computational strategies to investigate the role of sequence and structures in determining the interaction properties of antigenic proteins with antibodies, with the final goal to predict specific antibody-binding sites, a task that has proven particularly challenging so far. The antibody binding properties of an antigen depend on its structural dynamics. We have thus developed a method that is based on the idea that recognition sites may correspond to localized regions with low-intensity energetic couplings with the rest of the protein allowing them to undergo conformational changes, to be recognized by a binding partner and to tolerate mutations with minimal energetic expense. Analyzing the results on isolated proteins and benchmarking against antibody-complexes, the method successfully identifies antibody binding sites. We test our predictions on antigens from *B. pseudomallei*, the etiological agent of melioidosis, a serious and often fatal infectious disease that is poorly controlled by existing treatments. Predicted epitopes are engineered as synthetic peptides and shown to be selectively immunorecognized to the same extent as recombinant proteins in sera from melioidosis-affected subjects. Moreover, antibodies raised against designed sequences prove to be bactericidal. We will discuss the implication of these methods in vaccine discovery.

S2.3
A structural vaccinology approach to melioidosis vaccine component development

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Structure-based antigen engineering is frequently used in the vaccine development process to specifically modify protein antigens of a pathogen, to enhance their immunogenic properties, with the aim of improving their protective efficacy. Such approaches may entail engineering epitope-containing regions of the protein, or simply the epitope sequences themselves in the form of synthetic peptides. We are applying a structural vaccinology approach to design protective vaccine components (peptides and immunogenic domains) targeting melioidosis, a disease endemic in Southeast Asia and N. Australia, caused by the bacterium *Burkholderia pseudomallei.* Treatment of melioidosis with antibiotics is ineffective, calling on new therapeutic solutions, such as a vaccine. We illustrate the application of our SV approach for the design of epitope peptides from known, seroreactive *B. pseudomallei* antigens. Based on their crystal structures, two computational-based epitope prediction methods, developed by our network, were applied and used to successfully identify and design peptide epitopes that possessed interesting immunological properties, in comparison with their recombinant protein counterparts. Our methods are fully applicable to any protein antigen from any pathogen, bacterial or otherwise, and our results reinforce the use of our methods for the design of new therapeutic/diagnostic molecules.

S2.4
Vaccinology: the art of putting together the right ingredients

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Historically vaccines were produced using whole attenuated or killed pathogens and still a large proportion of current vaccines utilized such procedure. However, the development of novel vaccines is preferentially based on the selection of specific components which alone are capable of eliciting protective immune responses. This is particularly true for cancer vaccines, which must include tumor-specific and tumor associated antigens. Therefore, the big challenge for vaccinologists is how to select the right antigens and to combine them with proper immune stimulatory components (adjuvants) in order to induce protective immunity. In this lecture strategies for the efficient identification of protective antigens against infectious diseases and cancer will be presented. Furthermore, since subunit-based vaccines against recalcitrant pathogens and cancer require more than one antigen and/or immune stimulator, this poses the problem of how to make such vaccines not only efficacious but also economically acceptable. To address this issue data will be presented showing how bacterial Outer Membrane Vesicles (OMVs) could become a promising platform for the development of future vaccines.

S3 - Nitrogen: Nutritious and Noxious

S3.1
Understanding the nitrogen cycle in a changing world

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The availability of reactive nitrogen (N) is key to understand ecosystem productivity but also provides one of the greatest environmental threats. Under a changing climate (e.g. elevated CO_2), more C is available for plant growth but this is only effective if also N is available. Thus, long-term adjustments of the C-N cycles may lead to progressive N limitation with a negative impact on plant growth. Furthermore, the feedback effect of climate change on the internal N cycle may stimulate climate relevant trace gas emissions such as nitrous oxide (N_2O) and may promote a switch from a CO_2-equivalent C sink to a C source system. What are the underlying processes that drive such a change? With respect to the N cycle we are dealing with complex interactions of aerobic and anaerobic processes that occur in suitable soil microsites which may change with climate. Mitigation options such as the use of nitrification inhibitors or the supply of stable C rich additives can only be effective if we understand the prevailing N transformation processes. This highlights the importance of basic research for the development of effective agronomic options in response to global climate change.

S3.2
The regulation of nitrogen uptake by plants in the context of root-soil relationships: which form and which control?

Zeno Varanini
Dept Biotechnology, Verona Univ. Verona, Italy

Unique among the other mineral nutrients, nitrogen (N) is present in soil solution in different inorganic and organic forms having peculiar behaviour with respect to soil matrix. Therefore, plant roots face at the same time with variable concentrations of nitrate, ammonium and very often in cultivated soils, urea, that is the most used N-fertilizer worldwide. A huge quantity of literature deals with the description of the complex biochemical and molecular aspects underlying the mechanisms of N uptake by roots. However, these studies have been often carried out using *Arabidopsis* as model organism and do not take into account the possible differences displayed by crops in N uptake. In addition, they are preferentially performed considering the presence of only one of the above-mentioned N-forms, a situation that is far from the real conditions. In the presentation after a brief description of the behaviour of N-form in soil and of the main features of N-form uptake by maize we will show how these mechanism can be greatly modified when N-form are present together in the uptake solution.

S3.3
Signalling with nitrogen: reversible S-nitrosothiol protein modifications

Steven H. Spoel
School of Biological Sciences, University of Edinburgh, United Kingdom

Nitrogen assimilation plays a vital role in plant metabolism. Assimilation of nitrate, the primary source of nitrogen in soil, is linked to the generation of cellular redox signals, including nitric oxide (NO). Cellular redox changes mediate signalling events and responses to the environment in eukaryotic cells. Responses to environmental stress are frequently associated with bursts of NO, which can modify cysteine thiols of signalling proteins. Thiol reactivity towards NO leads to formation of S-nitrosothiols (SNO), which may alter the function, localization, or activity of signalling proteins that harbour them. Because SNO modifications occur spontaneously, we questioned how this process is employed by the cell as a specific, reversible signalling cue. Here we show that the plant immune system utilizes multiple members of the Thioredoxin family, a class of oxidoreductases, to directly engineer the thiol redox states of immune signalling proteins. We will discuss how several Thioredoxins exhibit novel molecular modes of action and introduce previously unrecognized specificity into protein-SNO signalling networks that lead to immune gene expression.

S3.4
Leveraging the root QTLome to enhance nitrogen-use efficiency in cereals

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Enhancing nitrogen-use efficiency (NUE) in crops is a challenging undertaking due to the quantitative nature of its main components, such as N-uptake efficiency (NupE), the ability of roots to remove N from the soil. In rice and maize, variation in root system architecture (RSA) has been shown to influence NupE under both high-N and low-N conditions. Therefore, identifying the quantitative trait loci (QTL) governing RSA and its plasticity to N availability would allow for the selection via marker-assisted selection (MAS) of plants with the root ideotype expected to enhance NUE. At UNIBO, both biparental and association mapping have uncovered a number of major QTL for RSA in maize and durum wheat that are being tested for their effects on NUE and water-use efficiency in field trials. The QTL for root growth angle (RGA), an important trait for N capture, appear particularly promising. In durum wheat, the field evaluation revealed six major QTL for NUE-related traits, namely vegetation index (NDVI), grain protein content, grain yield and proteins ha-1. Two RGA QTL on chr. 2BL and 4AL with large effects (up to 10%) on the NUE-related traits are suitable for the introgression via MAS of the beneficial allele in unrelated cultivars to evaluate their effects on NUE and grain yield.

S4 - Shaping the Cancer Genome: from pathways to mutational signatures

S4.1
Signatures of mutational processes in human cancer

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All cancers are caused by somatic mutations. These mutations may bethe consequence of the intrinsic slight infidelity of the DNA replication machinery, exogenous or endogenous mutagen exposures, enzymatic modification of DNA, or defective DNA repair. In some cancer types, a substantial proportion of somatic mutations are known to be generated by exposures, for example, tobacco smoking in lung cancers and ultraviolet light in skin cancers, or by abnormalities of DNA maintenance, for example, defective DNA mismatch repair in some colorectal cancers. In this talk, I will present analysis encompassing 12,023 samples across 40 distinct types of human cancer revealing more than 30 different signatures of mutational processes. Certain signatures are associated with known mutagenic exposures or defects in DNA maintenance, but many are of cryptic origin. Importantly, two mutational signatures play the roles of endogenous mutational clocks that are operative in normal somatic cells. This talk reveals thediversity of mutational processes underlying the development of cancer, with potential implications for understanding of canceretiology, prevention and therapy.

S4.2
RNA Editing dynamically rewrites the cancer code

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A-to-I/G RNA editing modifies the nucleotide sequence of RNA target genes. The ADARs (Adenosine Deaminases that Act on RNA) are the enzymes responsible for this conversion acting on both pre-mRNAs or non-coding RNAs (microRNA). In mammals, there are three different ADAR enzymes (ADAR1-3). The deregulation of ADAR2 activity has been connected with several neurological human diseases and Adar2 knockout mice show a lethal neurological phenotype. We demonstrated that there is a decrease of ADAR2 editing activity in glioblastoma compared to control tissues. Rescue of ADAR2 in glioblastoma cells significantly inhibits glioblastoma tumor growth while its silencing boost several cancers cell features such as cell proliferation, migration and colony formation. By integrating NGS/bioinformatics and molecular studies, we show that ADAR2 is essential for the editing of mature miRNAs and modulation of about 90 miRNAs essential for cancer progression. Our findings disclose an additional layer of complexity in miRNome regulation and provide information to better dissect the impact of ADAR2 editing enzyme in glioblastoma.

S4.3
Pathological replication fork recovery mechanisms and signature of genome instability in human cell

Pietro Pichierri
Dept. of Environment and Health, Istituto Superiore di Sanità, Rome, Italy

DNA replication is a fundamental process that is often altered in cancer or in several genetic diseases. Aberrant DNA replication can trigger replication fork stalling and collapse leading to formation of DNA damage and genome instability. Thus, cells evolved several redundant, yet not-equivalent in terms of genome integrity, replication fork recovery pathways. The detailed knowledge of these pathways is of outmost importance as a subset of them may be mutated or dysfunctional in cancer, resulting in over-usage of back-up mechanisms that, if inhibited, may represent good candidates for targeted therapies. Similarly, the association of a specific pattern of chromosome or genomic instability to the inactivation of a given replication recovery mechanism may be relevant for developing genetic biomarkers for therapy. Over the last years, our group contributed to identify a prominent function of a pathway involving RAD52 and the MUS81 complex in ensuring replication recovery under pathological conditions, such as oncogene activation. Now, additional data from functional analysis, and both chromosome and deep-sequencing using cell models are expected to define if RAD52 alone or in collaboration with the MUS81 complex may be involved in the generation of specific patterns of genome instability under unperturbed or aberrant replication.

S4.4
Targeted genomic lesions - to ignore, to repair, to repair badly, or to mutate. Understanding DNA deaminase induced lesions and DNA repair choice

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DNA Editing in Immunity and Epigenetics, IFOM, Milano, Italy

DNA deaminase, deaminating dC to dU in single stranded DNA, are a major contributor to oncogenic mutations. Physiologically, DNA deaminases inactivate retro-elements and diversify the immunoglobulin (Ig) receptor. Aside from transcriptional pausing restarting, genetic evidence indicates that stalled DNA replication forks serve as platforms for DNA deaminase by exposing ssDNA. At sites of DNA replication - especially the lagging strand - the genome can be showered with mutations (kataegis), or initiate catastrophic oncogenic chromothripsis - the shattering and non-linear re-ligation of chromosomes. Although expression of the DNA deaminases is necessary, it is insufficient to explain the extraordinary mutation rate in kataegis. Our novel IVR (in vitro resolution assay) uncovered how dU lesion processing (DNA repair) is altered on a quantitative level. This includes: the cellular milieu with B cells preferring mutagenic repair; the presence of AID itself initiating altered DNA repair; the presence of nucleosome enhancing LP-BER; while DNA associated kinases DNA-PKcs inhibited MMR. These findings will be discussed in a unifying model and how this impacts on patient care.

S5 - Unfolding truth: making sense of intrinsically disordered proteins

S5.1
The tripartite degron model: the role of structural disorder in protein quality control

Peter Tompa, P. Bhowmick and M. Guharoy
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Intrinsically disordered proteins (IDPs) are prevalent in the proteome and often function by partner recognition and induced folding. Frequently, their recognition elements are comprised of a short sequence of residues, termed Eukaryotic Linear Motifs (ELMs). Protein turnover is regulated by sequence signals (degrons) that we suggest to have a "tripartite" nature. Tripartite degrons comprise: (1) a primary degron that specifies substrate recognition by cognate E3 ubiquitin ligases, (2) secondary site(s) comprising a single, or multiple neighboring, poly-ubiquitinated lysine(s), and (3) a segment that initiates substrate unfolding at the 26S proteasome. By analyzing relevant examples, we show that primary and secondary degrons are short motifs that tend to fall into locally disordered regions, whereas the tertiary degron is a disordered segment in the vicinity of the secondary one that is responsible for effective proteasomal engagement. The importance of degron motifs in disordered regions is shown by the high incidence of disease caused by mutations and alternative isoforms with abrogated degron components.

S5.2
Protein complementation and kinetics illuminate mechanisms of partner recognition by IDPs

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In spite of the increasing interest paid to intrinsically disordered proteins (IDPs), the precise mechanisms by which they recognize their partner(s) still remain poorly understood. IDPs often undergo folding upon binding to their targets. Folding coupled to binding can occur through two extreme mechanisms, i.e. conformational selection or folding after binding. Irrespective of the mechanism, the interaction can lead either to a compact form or to a fuzzy complex. Fuzzy regions flanking Molecular Recognition Elements may enable partner fishing through non-specific, transient contacts, thereby facilitating binding, but may also disfavor binding through various mechanisms. So far, few computational or experimental studies have addressed the effect of fuzzy appendages on partner recognition by IDPs. Likewise, relatively few experimental studies have attempted at discriminating between conformational selection and folding after binding. Using kinetic and mutational studies we have investigated the interaction between the intrinsically disordered C-terminal domain of the measles virus nucleoprotein and XD or Hsp70. Our results contribute to illuminate partner recognition by IDPs.

S5.3
Understanding the mechanisms of folding and binding of intrinsically disordered proteins

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Intrinsically disordered proteins become structured upon interacting with their partners. The mechanism of this 'folding upon binding' has not been fully characterised yet. In this talk, I will describe our work on the characterization of the folding mechanism of intrinsically disordered systems, in comparison to that of globular protein. This analysis will be based on our recent studies, which allowed us to investigate the role of the frustration arising from the competition between function, misfolding, and aggregation in proteins. Whilst the folding of globular proteins is strongly biased towards the native conformation via a robust nucleation-process, in the case of intrinsically disordered proteins, folding occurs by heterogeneous nucleation. . We suggest that this templated folding mechanism may enable intrinsically disordered proteins to achieve specific and reliable binding with multiple partners while avoiding aberrant interactions.

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S5.4
Cataloguing and annotating flavours of intrinsic disorder in proteins with MobiDB

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Intrinsic disorder (ID) in proteins can be defined in different ways, ranging from missing residues in X-ray crystallography to mobile regions in NMR structures and specific experiments which do not require structure determination (e.g. protease susceptibility). While most definitions broadly converge towards similar IDP definitions, the remaining differences can be confounding especially when training predictors used to annotate thousands of proteins. We have developed the MobiDB database (Potenza et al., NAR database issue 2015; URL:http://mobidb.bio.unipd.it/) to provide a broad and agnostic view on different IDP definitions. MobiDB is based on UniProt protein sequences using a three tier annotation pyramid. The top level is composed of ca. 700 ID proteins annotated manually by the DisProt database. Indirect sources, such as missing X-ray residues and mobile NMR regions, are extracted for all ca. 110,000 PDB structures to complement the manually curated data. At the lowest curation level, ID predictions are provided with 10 methods for ca. 80,000,000 UniProt sequences. Recent work has been directed at extracting additional statistics on the prevalence of different ID flavours in the database.

S6 - Plant adaptation and phenotypic plasticity to climate change

S6.1
Plant aquaporins as targets and players of cell signalling

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Aquaporins are water channel proteins which mediate the regulation by multiple hormonal and environmental signals of water permeability of plant roots, leaves and guard cells. Quantitative genetics approaches based on the natural variation of root water permeability in the model plant *Arabidopsis* were used to identify a novel RAF-like MAP3 kinase. This protein delineates a combinatorial signaling pathway integrating two soil signals, K^+ and O_2availability, to regulate root hydraulics. In leaves, three plasma membrane aquaporin (PIP) isoforms, including *At*PIP2;1, facilitate water efflux from veins. Light-dependent changes in phosphorylation of *At*PIP2;1 and expression in transgenic plants of aquaporin phosphorylation mutants showed that C-terminal diphosphorylation of this single isoform is necessary for light-dependent regulation of leaf hydraulics. A role for *At*PIP2;1 in stomatal closure induced by abscisic acid (ABA) or bacterial elicitor flg22 was also uncovered. We propose a mechanism whereby ABA and flg22 enhance phosphorylation of *At*PIP2;1 at a new site, to activate its water and hydrogen peroxide (H_2O_2) transport activities and induce guard cell closure.

S6.2
Climate change and forest decline: the physiology of tree mortality

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Large-scale tree die-back events and related forest decline have been reported with increased frequency after extreme drought and heat waves, raising new interest into the physiology of tree death, and the mechanisms responsible for tree resistance/resilience to severe water stress. Drought-induced decline of plant water potential causes symplastic damage and hydraulic failure due to xylem embolism. Non-lethal hydraulic damage can also cause prolonged stomatal closure. Hence, metabolic damage and reduction of photosynthesis might cause impairment of net carbon gain, and progressive depletion of non-structural carbohydrate reserves. Hydraulic failure and carbon starvation might co-occur to different extents in declining trees, depending on species-specific hydraulic strategy and drought intensity/duration. Hence, the view of hydraulic failure and carbon starvation as opposed and alternative causes of tree decline has been progressively abandoned. Currently developed theories, treating water and carbon metabolism as unavoidably interconnected processes, help to explain tree resistance/resilience to extreme droughts and open new possibilities for selecting and breeding tree and crop genotypes for a future warmer and drier climate.

S6.3
Exploring natural variation in *Arabidopsis*

Maarten Koornneef

The genetic variation of *Arabidopsis* in nature provides a useful resource for the functional analysis of genes. The genetic complexity of this type of variation requires Quantitative Trait Loci (QTL) analysis, using various types of mapping populations. In addition to the analysis of gene function, natural variation studies may reveal signatures of selection in nature that may explain local adaptation. To demonstrate the power of natural variation, examples on the analysis of plant architecture (plant length and branching and vein density patterns) will be presented. For plant length we found ample functional variation for the GA 20 oxidase (*GA5*) gene of which gene mutants have been exploited to generate modern short straw varieties in barley and rice. For branching an example on how to analyse such variation up to the gene level in *Arabidopsis* is the finding of the *AGL6* gene to be involved in this process. The complexity of the genetic interactions among natural variants and its consequences for evolution is shown by genetic incompatibilities that arise in certain combinations of genotypes.

S6.4
Dissecting GxE interactions in grapevine through a transcriptomic approach

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Grapevine, the most widely-cultivated perennial fruit crop, is also considered one of the most environmentally sensitive crop. It is characterized by remarkably phenotypic plasticity (i.e., the range of phenotypes a single genotype can express as a function of its environment) which in turn is believed to effectively buffer environmental extremes especially through transcriptomic and epigenomic reprogramming. Thus, the final phenotype (P) of a given grapevine plant is the result of the interaction between its genetic composition (G) and the environment (E). After assessing the plasticity of the grapevine transcriptome during berry ripening in the red berry variety Corvina, cultivated in 11 different vineyards over a 3-year experimental plan (Dal Santo et al, 2013), we analyzed Genotype x Environment (GxE) interactions in two grapevine varieties by characterizing their transcriptome plasticity when cultivated in different environments. Specifically, two genotypes (Sangiovese and Cabernet Sauvignon) were cultivated in three different locations in Italy (Bolgheri -littoral Tuscany-, Montalcino – Appennine Tuscany- and Romagna -foothill area-), trained in an almost identical manner, and sampled at four developmental stages over two grapevine growing seasons, 2011 and 2012, for a total of 144 samples that were analyzed by hybridization to a whole-genome microarray. In order to study the relationships among differential gene expression profiles and environmental cues, we have developed a new statistical data mining tool based on data reduction approaches which allowed a dissection of the transcriptomic data into stage-specific, cultivar-related and GxE important clusters of gene expression. This deep inspection of inner relationships between the different dataset variables allowed the identification of several candidate genes that could represent putative markers of berry quality traits in grapevine GxE interactions. Moreover, the methods used to establish our model provide a framework for the analysis of transcriptome plasticity in other crops as they respond to diverse environments.

Dal Santo S, Tornielli GB, Zenoni S, Fasoli M, Farina L, Anesi A, Guzzo F, Delledonne M, Pezzotti M (2013) The plasticity of the grapevine berry transcriptome. Genome Biol 14: r54

POSTER AND SELECTED SHORT TALKS

1 - Environmental Microbiology and Biotechnology

P1.1
Contrasting effect of diet shift on the gut microbiota of two amphoipods species *Orchestia montagui* and *Talitrus saltator*

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Talitrid amphipods are among the most important detritivorous living in the supralittoral environment of sandy beaches. We have previously reported that different talitrid species may host different gut bacterial communities. However, it is unclear if such differences arose because of some species-specific physiological features of host or/and in relation to possibly different foraging behavior. Consequently, we wanted to investigate the resilience of gut bacterial communities of talitrid amphipods, to shed some light on the effect that different alimentary sources feeding habits may have on species-specific gut microbiota patterns. We choose as model two species with contrasting food preferences and gut microbiota, *Talitrus saltator* (Montagu) and *Orchestia montagui* (Audouin) and challenged them with artificial food for two months to evaluate the resilience and the effect of dietary changes over the gut microbiota. Results, obtained by 16S rRNA gene metagenomics and analysis of cellulase-encoding genes, along five time points samples: natural habitats(T 0), after 24hours (T 1), after 7 days (T 2), after 23day (T 3) and after 1 day (T 4) showed a contrasting behavior of gut microbiota dynamics in the two talitrid amphipod species with *T. saltator* being more affected than *O. montagui* in terms of diversity of the microbiota. Concerning the taxonomic profiles, in *O. montagui* members of the class *Bacilli* resulted the most variable over time, while in *T. saltator* most of the variability was due to *Enterobacteriaceae*. Finally, cellulose-encoding genes (GHF48 family) were strongly increased in their abundance in *O. montagui* gut microbiota compared to *T. saltator*, mirroring the increase of Actinobacteria over time. In conclusion, we provide evidences that changes in food sources (in natural environments related to the availability of stranded organic material) may have a contrasting impact over the gut microbiota of different talitrid amphipod species, which could determine mid- or long-term changes in animal's physiology and on species' fitness in the environment.

P1.2
Lactobacillus gasseri SF1183 protects the intestinal epithelium and prevents colitis symptoms *in vivo*

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Lactobacillus gasseri strain SF1183 belongs to a subpopulation of human intestinal bacteria tightly associated to the ileal epithelium (1). It produces antimicrobials and forms biofilm in simulated intestinal fluid after exposure to gastric conditions (1). *In vitro* studies with human intestinal cells indicated that SF1183 secretes molecule(s) able to drastically interfere with the cell cycle (2). The human intestinal origin, the ability to survive gastric conditions and have metabolic activities in intestinal conditions and the *in vitro* results induced us to hypothesize an *in vivo* role of *L. gasseri* SF1183. To test this hypothesis we used a murine system of DSS-induced colitis and analyzed whether the oral administration of SF1183 cells had an anti-inflammatory, protective, role. Mice treated with SF1183 showed a strong reduction of the inflammation most likely due to the amelioration of the tight-junction assembly. Our results point to a role of SF1183 cells in protecting the epithelial barrier integrity and contributing to the reconstitution of the tissutal homeostasis.

1. Fakry et al. Res Microbiol. 160:817-823 (2009)
2. Di Luccia et al., PLoS ONE 8(7): e69102 (2013)

P1.3
Identification of FDA-approved compounds targeting the *pqs* quorum sensing system of *Pseudomonas aeruginosa*

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Hampering bacterial adaptability to the host environment is considered a promising strategy for the eradication of infections caused by drug-resistant pathogens. Quorum sensing (QS), a communication system that controls virulence factors production and biofilm formation in several pathogens, is an ideal target for the development of anti-virulence drugs. Here we describe the identification of new inhibitors of the *pqs* QS system of *Pseudomonas aeruginosa*, relying on 2-alkyl-4-quinolones (AQs) as signal molecules. Briefly, a reporter system based on the co-culture of *P. aeruginosa* PAO1 and of an AQs-biosensor, in which light emission depends on AQs produced by the PAO1 strain, has been used for the screening of a library of 1,600 FDA-approved drugs. Three hits specifically inhibit the *pqs* QS systems, and hence the expression of *pqs*-controlled virulence traits in *P. aeruginosa.* Preliminary analyses suggest that the newly identified inhibitors hamper the *pqs* system by targeting the transcriptional regulator PqsR. Further analyses proving the ability of the *pqs*-inhibitors to reduce *P. aeruginosa* pathogenicity in animal models of infection are in course.

P1.4
Fungal treatment for recalcitrant compounds removal In raw leachate and synthetic mixtures

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Recalcitrant compounds represent a serious concern in wastewater treatment since biological processes, based on bacterial degradation, are not suitable for their removal. Recently, the capability of white-rot fungi (WRF) in transforming recalcitrant pollutants generated a significant interest among bio-based industries. This study focused on the treatment of 3 effluents with the white-rot fungus *Bjerkandera adusta* MUT 2295 in batch tests. *B. adusta* MUT 2295 was selected during a previous experiment due to its ability to act towards a raw leachate sample (Italy). Treatment efficiency of *B. adusta* was evaluated on a) landfill leachate (Canada) and b) two synthetic recalcitrant solutions prepared with 1) tannic and 2) humic acid. Different parameters such as the pH of the treated effluent, its chemical oxygen demand (COD) , enzymatic activities and glucose consumption of *B. adusta* during the treatment were monitored for 10 days. COD removal was up to 49%, 61% and 49% in in raw leachate and the two synthetic solutions. Moreover, color removal between 25% and 49% was achieved in 1 week. Results obtained encourage further investigations on the use of the selected white-rot fungus.

P1.5
Environmental effects on the regulation of fatty acids biosynthesis in *Kluyveromyces lactis*

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Fatty acids (FAs) are essential components of functional cytoplasmic and organelle membranes. FAs vary in length and in number of double bonds depending on the species and also on the environmental conditions. A proper FAs membrane composition is critical for membrane function and consequently for cell viability. Unsaturated and polyunsaturated FAs determine functional membrane properties like fluidity. Although studies on lipid metabolism have been conducted in *S. cerevisiae*, this is not always the appropriate model yeast to study FAs properties conserved in evolution because FAs biosynthesis is restricted to saturated and monounsaturated FAs. In *Kluyveromyces lactis*, FAs composition is enriched with the polyunsaturated linoleic and α-linolenic acids generated by the Δ12 (Fad2) and ω3 (Fad3) desaturases. Our studies on this yeast indicate that environmental signals (glucose, oxygen and temperature) regulate FAs biosynthesis. Part of this regulation is mediated by the activity of the hypoxic regulator KlMga2. The effects on FAs composition and cellular fitness of the deletion of the *KlMGA2* gene and of the desaturase genes *FAD2* and *FAD3* have been studied. Work funded by MAECI (Direzione Generale per la Promozione del Sistema Paese).

P1.6
Energetic profile of *Saccharomyces cerevisiae* during batch cultivation on glucose

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In unicellular microorganisms, like *Saccharomyces cerevisiae*, there is a strict relation among cellular metabolism, growth and energy available. In this organism, the balance of energetic cofactors is quite complex. In fact, ATP, ADP and AMP concentration depends on the relative rate of catabolic and anabolic fluxes and, in turn, their variations deeply impact on the metabolic pathways. During the high growth rate phase of a batch cultivation on glucose, metabolism is mainly glycolytic with ethanol accumulation. Once this carbon source is depleted, the cells increase their respiration and activate gluconeogenesis, in order to consume the previously produced ethanol. In this work we collected data about the dynamic of energetic molecules (ATP, ADP, AMP) along the transitions of a diauxic batch growth in comparison with cultivation in totally respirative or anaerobic conditions and with chemostat cultivation at different dilution rate. Although relevant for an accurate modelling of yeast growth and metabolism, this energetic profile have not yet been systematically studied, probably because of the low cellular level and stability of these molecules.

P1.7
Mixed nodules in *Sinorhizobium meliloti - Medicago sativa* symbiosis suggest the presence of a cheating behavior

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In the symbiosis between rhizobia and leguminous plants host plants can form symbiotic root nodules with multiple rhizobial strains, potentially showing different symbiotic performances in nitrogen fixation. Here, we investigated the presence of mixed nodules, containing rhizobia with different levels of mutualisms, and evaluate their relative fitness in the *Sinorhizobium meliloti* - *Medicago sativa* model symbiosis. We used three *S. meliloti* strains, the mutualist strains Rm1021 and BL225C and the non-mutualist one AK83. We performed competition experiments involving both *in vitro* and *in vivo* symbiotic assays with *M. sativa* host plants. We show the occurrence of a high number (from 27% to 100%) of mixed nodules with no negative effect on both nitrogen fixation and plant growth. The estimation of the relative fitness as non-mutualist/mutualist ratios in single nodules shows that in some nodules the non-mutualist strain efficiently colonized root nodules along with the mutualist ones. In conclusion, we can support the hypothesis that in *S. meliloti – M. sativa* symbiosis mixed nodules are formed and allow non-mutualist or less-mutualist bacterial partners to be less or not sanctioned by the host plant, hence allowing a potential form of cheating behavior to be present in the nitrogen-fixing symbiosis.

P1.8
Structure and metabolic potentialities of microbial communities in natural arsenic-polluted freshwaters

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Arsenic (As) is one of the most toxic elements widely distributed in natural environments. It can exist in four oxidation states (+V, +III, 0, -III) and exhibit different mechanisms of toxicity to the biota, with AsIII more toxic than AsV. Microorganisms have developed mechanisms to tolerate and/or utilize As for respiratory metabolism. This work is aimed to analyze the structure and the metabolic potentialities of microbial communities exposed to natural high As levels in different freshwaters environments (i.e. groundwaters, surface and thermal waters). Single cell quantification was performed by flow cytometry and CARD-FISH. The abundance of arsenic-related genes involved in bacterial arsenic resistance and transformation (aio, ars, arr families) was estimated by qPCR. The main microbial functional groups (e.g. As-oxidizers and reducers, Fe-oxidizers and reducers, Mn-reducers, nitrate and sulphate reducers etc.) were evaluated by MPN (Most Probable Number) using selective growth media. Moreover, isolation attempts of AsIII oxidizing strains are in progress aiming to test their oxidative capability and to propose them as candidate species to improve bioremediation strategies.

P1.9
Generation of synthetic cells interfacing with bacterial pathogens for innovative drug-delivery approaches

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Liposomes are cell-like micro-compartments already used as drug-carriers in humans. We envisage the generation of liposome-based synthetic minimal cells (SMCs) able to monitor their environment and to release or synthesize antimicrobials only in response to bacterial pathogens. To reach this goal, a proof-of-concept is required showing the possibility of generating SMCs able to interface with natural cells. Here

we describe the generation of SMCs able to establish a "synthetic-to-natural" communication channel with the human pathogen *Pseudomonas aeruginosa*. We demonstrate that SMCs containing *i)* the gene coding for a synthase that catalyzes the production of a *P. aeruginosa* signal molecule, *ii)* the precursors of the signal molecule, and *iii)* a minimal cell-free transcription-translation apparatus, are able to synthesize the signal molecule, thus triggering the expected transcriptional response in *P. aeruginosa*. The generation of SMCs able to respond to signal molecules produced by *P. aeruginosa* is in progress. This further achievement will pave the way for the engineering of SMCs endowed with cognitive capacity, to be used as *softnanorobots* for intelligent drug-delivery approaches.

P1.10
Biofilm diversity in cooling towers industrial systems

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Biofilms are usually studied in medical field at biomolecular level and little information is available about multispecies biofilms in industrial settings. Biofilms colonize industrial cooling systems growing as aggregates on the inner surfaces, causing often biofouling with serious equipment damages, even the tower collapse. This study aims to analyze biofilm biodiversity in cooling systems and to understand the main driving adhesion mechanisms. The biodiversity was analyzed in four full scale cooling towers (pharmaceutical and refineries plants in Hungary, Croatia and Italy) by applying CARD-FISH combined with CLSM and Next Generation Sequencing. A complex architecture and diversity (heterotrophic and phototrophic organisms) were found in biofilms from refinery plants (cross-flow type tower). Considering the key-role of the signal molecule "c-di-GMP" in biofilm adhesion mechanism investigated in pure cultures of medical relevance bacteria, the quantification of this molecule in multispecies biofilm is in progress by a HPLC-UV on a lab-scale system designed and constructed to mimic real systems.

P1.11
Characterization of the microbiota from coelomic fluid of the sea urchin *Paracentrotus lividus*

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In this study we investigated the bacterial microbiota of *Paracentrotus lividus* coelomic fluid [1, 2] by coupling cultivation independent and dependent analyses and functional tests. Next generation sequence analysis of 16S rDNA from samples extracted from coelomic liquid lead to the identification of 56 bacterial taxa, classified to the genus level. Among these, the most abundant genera were *Propionigenium*, *Vibrio* and *Prolixibacter*. Culture-dependent analysis allowed the isolation of 8 Gram-negative bacterial strains, previously identified by culture independent method. In particular, 7 strains produce extracellular hydrolytic enzymes and 1 strain exhibits antibacterial activity.This research for the first time indicates that the coelomic fluid of a sea urchin does contain bacterial communities, suggesting a functional interaction between sea urchin and marine microorganisms. Moreover, it provides a novel source of biochemical diversity for the production of bioactive compounds and enzymes that can find biotechnological applications.

Remziye Deveci et al. (2015). *Journal of Morphology 276(5):583-8*
Stabili L et al. (1996). *Comp Biochem Physiol B Biochem Mol Biol 113(3):639-44*

P1.12
Application of plant-derived protein hydrolysate Trainer stimulates the growth of *Bacillus* with antagonistic activity against phytopathogenic bacteria and fungi

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The use of protein hydrolysates as biostimulant is proposed as an innovative solution to address the challenges to sustainable agriculture, to ensure optimal nutrient uptake, crop yield, quality and tolerance to abiotic stress. In this study we sequenced bacterial communities present on lettuce leaves of treated and untreated plants to evaluate the effect of a commercial plant-derived protein hydrolysate ("Trainer" by Italpollina, Rivoli Veronese- Italy) on the microbial biodiversity. Bacterial communities in the lettuce phyllosphere were dominated by a core microbiome of taxa including Actinobacteria, Bacteroidetes, Firmicutes and Gammaproteobacteria. Data obtained using culture-independent (Next-generation sequencing of 16S rDNA) and culture-dependent approaches indicated that foliar application of "Trainer" altered the composition of the microbial population and stimulated the growth of specific bacteria species of the genus *Bacillus* exhibiting biocontrol activity against *Erwinia amylovora* and several phytopathogenic *Fusarium* species.

P1.13
Yeast-based screens to identify natural compounds for Hailey-Hailey disease

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Hailey-Hailey disease (HDD) is a rare and chronic skin disorder, characterized by persistent blisters and skin erosions caused by suprabasal acantholysis. Such dominant autosomic disease has been linked to mutations in ATP2C1 gene encoding an ATP-calcium pump and the *PMR1* gene encodes its functional ortholog in yeast. In line with the notion that yeast represents a useful model for human diseases, *Kluyveromyces lactis pmr1*Δ mutant shows altered Ca^{2+} homeostasis and oxidative stress associated to impaired mitochondrial metabolism/structure and cell wall defects. In this study, *pmr1*Δ strain was exploited to screen a natural compounds library. FDA-approved collection includes inhibitors, activators and antagonists acting on molecular targets involved in different signaling pathways. Initially, the effect on cell wall defects of *pmr1*Δ cells was evaluated and molecules that resulted toxic were discarded. Six compounds showed a recovery of the phenotype and were accepted for further analysis. Their effect on other defects of *pmr1*Δ mutant strain was evaluated, ranging from mitochondrial functionality to sensitivity to the ROS-generator menadione, as well as alteration of Ca^{2+} homeostasis

P1.14
Development of a high throughput approach to investigated nitrogen metabolism in Lactic Acid Bacteria

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Lactic Acid Bacteria (LAB) are a heterogeneous group of Gram-positive bacteria, catalase-negative, non-spore-forming, low-GC, either rod-shaped or spherical. The traditional use of many LAB as starter cultures in food and dairy fermentations led to their widespread in human consumption. The knowledge of LAB nitrogen metabolism, including

their nutritional nitrogen requirements, constitutes a tool that could help the researchers and professionals of alimentary industries to produce foods with optimal qualities. Phenotype Microarray (PM) analysis, which is a high throughput method for microbial characterization, has been applied to the chemical sensitivity and carbon metabolism analysis of LAB. Nevertheless, such approach failed when nitrogen metabolism was investigated. Consequently, the aim of the present research is to define an efficient protocol for analysing LAB nitrogen metabolism using PM, evaluating appropriate tetrazolium dye, used as a reporter of metabolic activity, carbon sources, and buffer conditions. The results obtained will be showed and discussed.

P1.15
Anti-virulence activity of niclosamide in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

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The antibiotic-resistant chronic lung infection caused by *Pseudomonas aeruginosa* is the major cause of death in cystic fibrosis (CF) patients. The anti-helmintic drug niclosamide (NCL) inhibits the *P. aeruginosa* quorum sensing (QS) pathways based on *N*-3-oxododecanoyl-homoserine lactone (3OC12-HSL) and *N*-butanoyl-homoserine lactone (C4-HSL) as signals, hence reducing virulence *in vitro* and in an insect model of infection. However, since *P. aeruginosa* CF isolates show high genetic variability, the effect of NCL must be assessed in clinical isolates in order to support repurposing of this drug for CF therapy. Here 100 *P. aeruginosa* isolates from intermittent and chronic CF infections were collected and analyzed. Results showed that 63 strains produced both QS signals, while 22 and 6 strains produced only C4-HSL or 3OC12-HSL, respectively. QS proficient strains were found to be overall sensitive to NCL, with no effect on growth. However, great variability in the extent of NCL-mediated QS inhibition was observed among strains. Our results highlight that anti-QS molecules such as NCL have a good potential for CF therapy, though upon strain-specific susceptibility analysis.

P1.16
Immobilization of *Aspergillus niger* Cellulase on epoxy beads

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Cellulase (E.C. 3.2.1.4) is an important enzyme useful to obtain glucose from biomass and, thus, it is important for the circular economy, also known as bioeconomy. In fact, biomass can be used as substrate for the enzyme with the aim to obtain biofuel. Microorganisms provide powerful enzymes for different application. In this work *Aspergillus niger* cellulase has been chosen to be immobilized on epoxy beads. Enzyme immobilization is highly desired for industrial application to obtain high efficiency of the process at lower costs. Of course studies are need to find an efficient immobilization method that provides an enzymes exhibiting high performances. In this study we have used beads from ChiralVision (Immobeads-COV-2), made of a support that immobilizes covalently the enzyme. To quantify enzymatic activity we have used the 3,5-dinitrosalicylic acid based method (Ghose, 1987), while to determine the protein concentration, BioRad method was used (Bradford, 1976). Immobilization yield was equal to 42% while protein loading was in average 1 mg of enzyme per gr of beads. Experiments are in progress to establish the stability of the immobilized enzyme over the time.

P1.17
Environmental spread of antibiotic resistance genes (ARGs) in aquatic systems with different level of microbial contamination

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Aquatic ecosystem is considered as a significant reservoir of antibiotic resistance genes (ARGs), which could potentially be transferred from environmental microorganisms to human pathogens. In order to mitigate possible health problems, the environmental spread of the ARGs should be characterized and monitored. The purpose of the study was the assessment of ARGs occurrence in various water environments in Italy with different levels of microbial contamination (i.e. raw and treated wastewaters, surface waters and groundwaters). By using PCR and qPCR, the presence and/or abundance of 13 ARGs, selected on the basis of their environmental spread and clinical relevance, were investigated in comparison to total bacteria (16S rDNA gene) and a fecal contamination indicator (*E.coli* uidA gene). ARGs, comprising clinically relevant extended spectrum beta lactamases, were frequently detected in water environment. Overall, higher ARGs levels were associated to more contaminated waters (e.g. wastewater or contaminated surface water). Instead, limited or no correlation was found among the ARGs levels and *E.coli* levels, indicating different contamination sources and fate in the environments.

P1.18
Biodegradable films and containers from bitter vetch (*Vicia ervilia*) seed proteins

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Bitter vetch (*Vicia ervilia*; BV) seeds were analyzed as source to produce protein-based edible films and properly shaped biodegradable containers. Seed protein concentrates were prepared and analyzed for proteins, carbohydrates, phenols and other organic compounds, and protein film forming solutions were cast in the presence of different glycerol concentrations. Both lower plasticizer concentration and lamination by additional corn zein layer were found to reduce film moisture content and elongation at break, while both film tensile strength and water vapor barrier properties resulted enhanced. The obtained bioplastics were finally processed by a new laboratory plastic moulding equipment specifically designed and fabricated to convert films to shaped containers. The use of either lower glycerol concentration or zein lamination gave rise to satisfactory vacuum thermoformed containers with acceptable resistance and stability. These findings open new perspectives in using BV proteins as a sustainable alternative to fossil fuel based plastics to produce a variety of properly shaped biodegradable articles.
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P1.19
Profiling microbial communities iIn hyperalkaline waters of the Kizildag ophiolite complex (Turkey)

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It has recently been hypothesized that life on Earth could have been originated in hyperalkaline waters related to serpentinization of ophiolitic rocks, despite their extreme conditions (high pH and very low levels of nutrients). Five hyperalkaline springs of the Kizildag

ophiolite complex (Turkey) were characterized. The dominant gases are either H_2, CH_4 or N_2 . Bacterial diversity, analysed by RISA (Ribosomal Intergenic Spacer Analysis) revealed different profiles for each spring. Sequencing of excised DNA bands allowed to identifiy the presence of *Bacillus, Ralstonia, Pseudoalteromonas., Ureibacillus, Alicycliphilus, Anaerococcus*. 16S ribosomal DNA sequencing by Illumina is in progress. Three samples were also positive for the presence of *pmoA* (encoding the key enzyme of methane oxidation) confirming the presence of methanotrophs in accordance to gas analyses that showed clues of microbial methane oxidation in the isotopic ratio. The presence of methanotrophs in these hyperalkaline springs highlights the extraordinary capability of the methanotrophs to adapt to extreme conditions.

P1.20
Allosteric control in the synthesis and sensing of cyclic-di-GMP, a master regulator of bacterial growth and physiology

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Cyclic-di-GMP (c-di-GMP) is one of the most important regulators of bacterial adaptation strategies including biofilm formation and persistence. C-di-GMP is able to interact with a large variety of macromolecules *via* deeply different binding modes; the combination of c-di-GMP affinity and binding mode(s) along a complex signaling pathway leads to a wide variety of allosteric control mechanisms, yet to be identified and characterized in detail biochemically. Here we present mechanistic data on the protein domain involved in c-di-GMP synthesis (GGDEF domain) as i) a isolated catalytic unit or ii) a modulator of other domains. Our model systems belong to proteins (YfiN and PA0575) from *P. aeruginosa*, which are involved in biofilm formation during chronic infections. The mechanisms of single-domain regulation by domain-domain interactions have been investigating, integrating advanced biochemical and molecular biophysics methodologies with structural biology. We aim at defining the structural determinants required to "handle" c-di-GMP in biological systems to ultimately being able to predict the mode of action of a given GGDEF-containing protein in different bacterial species.

P1.21
Microbial *terroir* of red and white wines from Tuscia

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Grapevine (*Vitis vinifera*) represents one of the most economically and culturally important fruit crop worldwide. The *V. vinifera* phyllosphere is colonized by bacteria and fungi that substantially modulate vine growth, berry development and grape and wine quality (Barata *et al.,* 2012). Thus, metagenomic approaches that can reliably analyze the microbial community structure are essential for studying microbial "*terroir*" and developing tailored strategies to improve wine quality. Next-generation sequencing (NGS) of nucleic acids has facilitated major advances in our understanding of microbial ecology and it is now widespread in biotechnological applications from medicine to foods. In this work, NGS analysis and conventional techniques (culture-dependent methods) were used to analyze microbial systems inhabiting two Italian grape varieties (Grechetto and Sangiovese) from the same Tuscia vineyard. Results demonstrated that the importance of indigenous microorganisms on wine's "*terroir*" could be only evaluated using NGS analysis of both bacteria and yeast populations.

P1.22
Recovering dredged sediments contaminated by total petroleum hydrocarbon to productive soils: the mycoremediation approach in the Bioresnova project

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Chemo-physical treatments to remove salinity and metal contamination from dredged sediments were applied in combination to bio-based approaches. New fungal specimen were isolated from the contaminated sediments and re-inoculated to remove the Total Petroleum Hydrocarbon (TPH) contamination. Toxicological assays were exploited to estimate the effectiveness of the process. In fact, the only chemical characterization of polluted matrices does not allow to predict its real toxicity eventually related to the original pollutants, their degradation intermediates and the synergic actions of the both. Higher plants were exploited as indicators of toxicity of the process and for the evaluation of the eco-safety of the final product. Genotoxicity and clastogenicity were monitored by the detection of chromosomal aberrations in mitotic cells and of micronuclei formation, detectable in interphase cells of root tips. The combination of the Chemo-physical and the Bio-based approach was able to remove the organic contamination (TPH) and the excess of sodium salts. The sediments were detoxified and converted in a humified productive soil, suitable for a safe re-allocation in the environment.

P1.23
Annurca apple (M. pumila Miller cv Annurca) extracts act against stress and ageing in *S. cerevisiae* yeast cells

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Over the years, a number of studies on the relationship between diet and ageing are growing and it was demonstrated that calorie restriction and some antioxidants extend lifespan in yeast and also in others model organisms. In particular, fruit and vegetable consumption has been related with improving health thank to polyphenols that have been demonstrated to possess a wide range of biological activities which may contribute to health beneficial effect against diseases including cancer, cardiovascular disease, diabetes, pulmonary disorders, Alzheimer's disease and other degenerative diseases. In particular it has been demonstrated that apples and derivates have a role on ageing, cell stress and on different diseases such as cancer, degenerative and cardivascular diseases. We used yeast, a unicellular eukaryotic organism, as a model to study the effect of apple supplement on ageing and cells oxidative stress and we showed that apple extract increases lifespan, reduces the accumulation of reactive oxygen species, and protect cells from regulated cell death.

P1.24
Improvement of actinorhodin production yield in *Streptomyces coelicolor* by immobilized-cell cultivations by using PCL- and PLA-based films

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Actinomycetes are Gram-positive bacteria producing most of naturally occurring antibiotics (Donadio *et al.*, 2010). At industrial level, antibiotics are produced by submerged fermentations where the actinomycete filamentous morphology negatively affects bioproductivity (van Dissel *et al.*, 2014). Microporous membranes for bacterial cell-immobilization were already proven increasing bioproductivity in *Streptomyces coelicolor*, that is a model actinomycete producing the blue pigmented actinorhodin (ACT) antibiotic (Scaffaro *et al.,* 2016). To develop an immobilized-cell bioreactor system, different kinds of polycaprolactone (PCL) and polylactic acid (PLA) films were produced by an electrospinning-based approach. *S. coelicolor* cells immobilized on PCL and PLA membranes formed a dense biofilm as observed by scanning electron microscope. An increased biomass content and more than 4-fold ACT yield was obtained in comparison with free-cell cultivations for all the membranes, with O_2-plasma treated PLA membranes as the most effective. Therefore, the membranes are suitable for bioproduction improvement in actinomycete-based fermentation and encourage studies for process scaling-up.

P1.25
Ground Tire Rubber biodesulfurization by *Gordonia desulfuricans* 213E and *Rhodococcus* sp. AF21875

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The aim of the present study was to test a ground tire (GTR) biodesulfurization processes using two different strains: i) *Gordonia desulfuricans* 213E, a strain described in a biodesulfurization process patent and ii) *Rhodococcus* sp. AF21875, a strain isolated from tire factory wastewater. GTR is a no sterile material and it is impossible to autoclave the material at industrial scale. Therefore, we used ARISA and Illumina sequencing to monitor persistence of inoculated strains and change in bacterial community during the GTR treatment. We used a gene implicated in the DBT biodesulfurization (*dsz*A) to quantify the desulfurizing populations in the bioreactor by qPCR. In addition, the abundance of total bacteria (16S rRNA gene) were estimated. The ARISA e Illumina sequencing showed that *G. desulfuricans* 213E was able to persist, whereas analysis of the bioreactor containing AF21875 was confounded due to the presence of matching ARISA fragments and *Rhodococcus* genus in the untreated GTR. The increase of *dsz*A copy numbers in both bioreactors could indicate a selection of desulfurize bacterial inside the bioreactor.

P1.26
Effects of *Lactobacillus delbrueckii* subspecies on the nematode *Caenorhabdtis elegans*

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The nematode *Caenorhabditis elegans* is widely used as a model system for research on different fields. Recently, its use in evaluating the beneficial effects of probiotics makes it an outstanding biotechnological tool to screen for new strains for industrial purposes. In this study, we evaluated the effects of the most representative single species of a cheese-derived bacterial consortium, namely *Lactobacillus delbrueckii*, *L. fermentum* and *Leuconostoc lactis*, on worm's physiology. *L. delbrueckii* induced phenotypes more similar to those observed with the whole consortium. Identification of *L. delbrueckii* as subspecies *lactis* prompted us to compare the effects induced by a commercial *L. delb lactis* or by the probiotic *L. delb bulgaricus* strain. Our findings demonstrated that *L. delb bulgaricus* diet exerted probiotic features on nematodes in terms of lifespan and gut colonization capacity, whereas both *L. delb lactis* strains affected lifespan, larval development and fat accumulation. Metabolomic analyses revealed differences in metabolic pathways (i.e. folate, aminoacid and sugar metabolism) that could be implicated in the observed effects on host physiology.

P1.27
Response of rumen microbial ecosystem to diets integrated with chestnut or quebracho tannins in dairy ewes

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The aim of this study was to evaluate the response of ewe rumen microbial communities to diets integrated with chestnut (CHT) or quebracho (QUE) tannins, to increase the quality of dairy products and to reduce methane emissions in the atmosphere. Three fistulated ewes fed 3 diets based on chopped grass hay *ad libitum* administered and bentonite (CON) or CHT or QUE were used in a 3 X 3 Latin square experimental design. At the end of each experimental period, rumen liquor was analysed for fatty acid profiles by gas chromatography, and microbial diversity using a DGGE approach. CH_4 emission was also predicted on the basis of the molar stoichiometric relations between rumen volatile fatty acid and CH_4. The microbial profile was affected by the presence of tannins. The rumen liquor bacterial communities of QUE and CHT samples were correlated to C18:1 trans-11, C18:2 cis-9-trans 11 and C18:2 trans-11 cis-15. Moreover, the bacterial communities as affected by CHT resulted mainly correlated to C4:0, C3:0 and with potential CH_4 emission. In contrast, the bacterial communities as affected by CON resulted mainly correlated to C18:2 cis-9 cis-12 and C18:0 production.

O1.1
Light promotes the growth of heterotrophic bacteria and pollutant biodegradation on glacier surface

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Biological processes on glacier surfaces affect glacier reflectance, influence surface energy budget and glacier response to climate warming, and determine glacier carbon exchange with the atmosphere. Here we present a whole metagenomic analysis of tiny wind-blown supraglacial sediment (cryoconite) from Baltoro (Pakistani Karakoram) and Forni (Italian Alps) glaciers providing evidence for the occurrence in these environments of different and previously neglected metabolic pathways. Indeed, we observed high abundance of heterotrophic anoxygenic phototrophs, suggesting that light might directly supplement the energy demand of some bacterial strains allowing them to use as carbon source organic molecules which otherwise would be respired. In addition, data suggest that CO_2 could be produced also by microbiologically mediated oxidation of CO, which may be produced by photodegradation of organic matter. Furthermore, in situ microcosms studies showed that light both led to a significant photodegradation of the currently used pesticide chlorpyrifos and promoted its biodegradation in cryoconite. Annotation of reconstructed genomes from metagenomics data confirmed the

ability of highly abundant *Burkolderiales* photoheterotrops to degrade clorpyrifos on glacier surface.

O1.2
How bacteria can respire O_2 in sulfide-rich environments: a new role for *bd*-type terminal oxidases

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Many prokaryotes synthesize H_2S and inhabit sulfide-rich environments, like the human colon where high sulfide levels are reached due to the intestinal microbiota. As H_2S potently inhibits the heme-copper mitochondrial cytochrome *c* oxidase, we hypothesized that bacteria are endowed with sulfide-insensitive oxidases enabling O_2 respiration in sulfide-rich environments [1]. The hypothesis was tested on *Escherichia coli*, encoding one heme-copper (bo_3) and two *bd*-type respiratory oxidases. The *bd* oxidases are prokaryotic only and promote virulence, conferring resistance to oxidative/nitrosative stress. Working on the purified enzymes, we found that, unlike the bo_3 oxidase, both *E. coli bd* oxidases are sulfide-insensitive. In *E. coli* respiratory mutants, aerobic respiration and growth were impaired by sulfide when respiration was sustained by the bo_3 oxidase alone, but unaffected even at high sulfide levels when either *bd* enzyme acted as the only terminal oxidase. Consistently, O_2-limiting conditions favoring the expression of *bd* oxidases resulted in sulfide-insensitive respiration and growth in wild-type *E. coli*. We conclude that *bd*-type oxidases enable sulfide-resistant O_2-consumption and growth in bacteria. The physiological significance and broad impact of this discovery in life sciences will be discussed.

1. E. Forte et al. (2016) Sci Rep 6, 23788

O1.3
Photosynthesis on extrasolar planets: state of art and preliminary results of a pioneering experiment

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The recent discovery of a large number of exoplanets orbiting in the habitable zone of their stars and the finding of extremophiles thriving hostile environments, analogues to non-Earth planets, are expanding our concept of the limits of life. This offers new challenges to answer the question if life is unique of Earth or could exist in other worlds. In this frame we recently started a collaboration aiming to perform biological experiments under laboratory simulations replicating the environmental conditions of an Earth-like planet orbiting around the habitable zone of a M star. M stars represent about the 85 % of our Milky Way stars, have smaller mass and are dimmer and redder than the Sun, strongly radiating in the infrared. Preliminary results on the ability of selected photosynthetic microorganisms to grow and evolve oxygen, under a custom developed M star light simulator, will be presented. Data will be compared with growth and photosynthetic performances of the same organisms exposed to far-red or solar light. The importance of these experiments for remote detection of exotic life will be discussed.

O1.4
Localization of a red fluorescence protein adsorbed on wild type and mutant spores of *Bacillus subtilis*

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Bacterial spores have been proposed as vehicles to display heterologous proteins for the development of vaccines, biocatalysts, bioremediation and diagnostic tools. Two approaches have been developed to display heterolougus proteins on the spore: a recombinant approach, based on the construction of gene fusions, and a non-recombinant approach, based on spontaneous spore adsorption. We used the Red Fluorescent Protein (RFP) and *Bacillus subtilis* spores of a wild type and an isogenic mutant strain with altered spore surface to characterize the spore Adsorption process.A collection of isogenic strains carrying GFP fused to proteins restricted in different compartments of the *B. subtilis* spore was used to localize adsorbed RFP molecules. Our results indicate that RFP molecoles infiltrate through the outer coat localizing in the inner coat and point to the concept that different spores can be selected for different applications. Wild type spores are preferable when a very tight protein-spore interaction is needed, while *cotH* mutant spores are instead preferable when the heterologous protein has to be displayed on the spore surface or has to be released.

O1.5
In vitro evaluation of the activity of thiosemicarbazone derivatives against mycotoxigenic fungi affecting cereals

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With a steadily increasing world population, a more efficient system of food production is of paramount importance.One of the major causes of food spoilage is the presence of fungal pathogens and the production and accumulation of mycotoxins. Several strategies have been adopted to control mycotoxin contamination, starting from the breeding of resistant cereal varieties, to the adoption of more suitable agronomic practices including the application of fungicides.The aim of the present work was therefore to evaluate the potential of a panel of thiosemicarbazones, differing in their functionality, for crop protection and food spoilage control, with a particular focus on the biological activity of these compounds as antifungal and anti-mycotoxin agents . In particular we report a study on the activity of a series of functionalized thiosemicarbazones (namely cuminaldehyde, trans-cinnamaldehyde, quinoline-2-carboxyaldehyde, 5-fluoroisatin thiosemicarbazone and 5-fluoroisatin N4-methylthiosemicarbazone), as antifungal and anti-mycotoxin agents, against the two major genera of cereal mycotoxigenic fungi, i.e. Fusarium and Aspergillus. These thiosemicarbazones display different patterns of efficacy on fungal growth and on mycotoxin accumulation depending on the fungal species. Some of the molecules display a greater effect on mycotoxin synthesis than on fungal growth.

O1.6
Antimicrobial activity of unifloral honeys extracts from Campania against pathogenic bacteria and fungi

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The alarming phenomenon of antibiotic resistance has renewed the interest in the study of many natural extracts with antimicrobial properties, such as honey. Several honeys have been approved for clinical application, but the incomplete knowledge of the antibacterial compounds is however major obstacle for applicability of honey in medicine. The aim of this study has been to evaluate the antimicrobial profile of hydro-alcoholic extracts prepared from unifloral honeys from Campania, particularly acacia, chestnut and sulla. HPLC chromatograms of the extracts showed different phenolic compounds, that could represent markers of product traceability. The microbiological assays were conducted on clinical isolates of some bacteria, such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli*. To verify the antifungal effect of the honeys, still not well known in the literature, the extracts have been used against the strains UEF88662 of *Aphanomyces astaci* and SMM2 of *Fusarium avenaceum,* fungi responsible of different diseases in crayfish and plants. Preliminary results of *in vitro* microbiological tests demonstrate that the hydro-alcoholic extracts of unifloral honeys are able to effectively counteract the growth and survival of different pathogenic microrganisms although more or less appreciable effects were observed depending on the honey variety.

2 - Genomics, Proteomics and Systems Biology

P2.1
CrocusExpress: a coprehensive online resource for saffron transcriptomics

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Crocus sativus yields saffron, the world's most expensive spice, reaching 20,000 €/Kg. Worldwide production is about 250 tons per year. It takes about 150,000 flowers to produce a kg of saffron. Its origin dates back more than 3,000 years ago when it may have emerged via plant breeding, selected for its elongated stigmas. Since then, it has been used in foodstuff, medicine, textile and cosmetics. *C. sativus* is a sterile triploid plant with undetermined phylogenetic ancestry and *C. cartwrightianus* is believed to be a parental species. It is rich in apocarotenoids, high nutraceutical value molecules; its characteristic red color is primarily due to α-crocin; picrocrocin is responsible for saffron's flavour while safranal, a volatile compound, contributes much of its distinctive aroma. To help understanding the molecular basis of its phylogenetic origin and its metabolism, we took some RNA-seq data recently made available in the literature together with new ones to make a new, refined transcriptome assembly. The results, encompassing expression values for different tissues and different stigma stages, will be made shortly available online to researchers on the CrocusExpress database.

P2.2
Human gut microbiota across omic approaches

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The proteomic snapshot of gastrointestinal tract together with its genomic source offer the chance to study at the same time the microbiome production and potential, as two sides of the same coin. Here we analysed ten gut microbiotas in the healthy adult status with metagenomic and proteomic approaches. Our bioinformatics workflow, based on Pathway tools environment, allowed us to identify and quantify protein and read abundances. *Ad hoc* databases have been built up and used in each sample to create and compare individual specific metagenomic and proteomic pathways. Looking at reaction discrepancies we evaluated per-sample differences in anabolic and catabolic processes. On the other hand a punctual database comparison among samples allowed us to estimate their divergence. Commonly, metagenomic studies focused on taxonomic diversity, instead our two-pronged approach using both protein and genomic data added to taxonomy metabolomics resolution. Moreover, using our strategy we were able to characterize the items, which point to unknown and putative gene and protein entries usually filtered out in most of metagenomic studies.

P2.3
Protein contact networks approach applied to enzymes belonging to GH32 family

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Glycoside hydrolases are enzymes whose main activity is the hydrolysis of the glycosidic bond between two carbohydrates or a carbohydrate and a non-carbohydrate moiety. We have focused on GH32 family, including enzymes very similar in both sequence and structure, each having however clear specificities of substrate preferences and kinetic properties. Structural and topological differences among GH32 proteins have been here identified by an emerging approach (Protein Contact Network - PCN) based on the formalization of 3D structures as contact networks among amino-acid residues. The PCN approach succeed in reconstructing the functional domains and in identifying the structural counterpart of still elusive properties of GH32 enzyme. The main outcome of the study was the discovery of the activation of the border region between the two domains upon binding. This feature is characteristic of allosteric enzyme, suggesting that this mechanism (not yet highlighted in biochemical studies) might also be active in the GH32 family. PCN approach is also able to recognize a topological signature of the different affinity of the enzymes towards different enzyme substrates as well as to measure the energetically favorable or unfavorable complex formation after ligand binding.

P2.4
Treatment of pancreatic cells with palmitate alters acetylation on mitochondria

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Palmitate has often been used to examine the mechanisms of lipotoxicity, and it has been proposed that it may cause an alteration in mitochondrial function. In a previous work, with a proteomic approach, we highlighted mitochondrial changes in INS-1E beta cell exposed to palmitate. Our hypothesis was that high levels of fatty acids may be expected to promote lysine acetylation. Actually, a particular increase of acetylation was observed in mitochondria from INS-1E beta cell after palmitate treatment. Subsequently, we have started to analyze pancreatic islets from human donors. The aim was to verify if we could confirm the previous results obtained in the animal model. With this aim, proteins from human islets were separated by two-dimensional electrophoresis, transferred onto nitrocellulose membranes and incubated with the specific anti-acetylated lysine antibody. We found 136 acetylated spots, 16 had a significant difference in intensity and all these 16 spots were up-regulated in cells treated with palmitate. Among the hyper-acetylated proteins, consistent with our previous work, we found glutamate dehydrogenase, superoxide dismutase, sterol regulatory element-binding protein 1, and ATP synthase. In conclusion, this preliminary study seems to confirm the hypothesis that changes in mitochondrial lysine acetylation can contribute to the lipotoxicity.

P2.5
Human melanoma and endothelial cells proliferation is inhibited by PDGFR-alpha via CXCL10/IP-10: a multi-*omics* approach

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Most of the skin-cancer related mortality is associated to the advanced stages of melanoma. PDGFR-alpha is known to strongly inhibit

melanoma- and endothelium- proliferation. Human melanoma-biopsies show a significant reduction of PDGFR-alpha expression. In the current study overexpressing PDGFR-alpha in HUVEC and melanoma (SKMel-28, A375, Preyer) human-cells showed strong anti-proliferative effects, with profound transcriptomics and miRNomics deregulation. PDGFR-alpha overexpression affected expression of 82 genes in HUVEC (41 up-, 41 down-regulated), and of 52 genes in SKMel-28 (43 up-, 9 down-regulated). CXCL10/IP-10 at both transcript and protein level was increased by 10-to-20 fold. miRNome profiling in cells overexpressing PDGFR-alpha showed 14 miRNAs up-regulated and 40 down-regulated. Integrating transcriptomics and miRNomics data highlighted different pathways affected, according to KEGG and Gene-Ontology analysis, and identified a validated miRNA target, as the one underlying the anti-melanoma activity of PDGFR-alpha. This study demonstrates for the first time that PDGFR-alpha strongly inhibits endothelial and melanoma cells proliferation in a CXCL10/IP-10 dependent way.

P2.6
RNA editing erofiling in Alzheimer's disease

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RNA editing is a co/post-transcriptional process that modifies RNAs by nucleotide insertion/deletion or base conversion. In human, A-to-I changes represent over 99% of editing events and are prominent in brain. Alterations of editing pattern are associated to different neurodegenerative diseases, including Alzheimer's Disease (AD), that is the major form of senile dementia worldwide. To investigate RNA editing levels in AD, we analyzed RNA-Seq data obtained from hippocampus of 6 AD patients and 6 controls, querying two different databases: RADAR and the human inosinome Atlas database (1,2). We found the vast majority of A-to-I changes in repeated sequences and introns and an overall decrease of editing levels in protein coding regions of AD patients. We used a targeted resequencing strategy of a selected set of edited sites in coding regions to better quantify differences of A-to-I editing levels in different AD brain regions. We observed an overall decrease of editing levels in these sites in AD patients, mainly in the hippocampus and to a lesser degree in the temporal and frontal gyrus.

1. Ramaswami G, Li JB 2014NAR 42: D109-113
2. Picardi E et al. 2015Sci Rep 5: 14941

P2.7
A multidisciplinary approach to study Sporadic Amyotrophic Lateral Sclerosis in patients with common geographical origin

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Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disorder, is object of intensive research as the causes are still unknown and a treatment not available yet. This project is aimed to study, with a multidisciplinary approach, a small cohort of ALS subjects with a common environmental exposure. For metal quantitation, samples of serum and whole blood were analyzed by ICP-MS. For proteomic analyses, immobilized pH gradient covered the 4-10 and 3-7 pH range. Arsenic concentration resulted significantly lower in patients than in controls. Also, Mn and Hg showed lower levels in patients. Levels of plasma APOA2 protein resulted decreased in patients with respect to controls, whereas SAMP showed a significant decrease only in the late onset group. APOA1 and TTHY also were decreased, the latter in late-onset patients. RET4 was decreased only in the early-onset group. When evaluating APOE genotype we found a 3-fold increase in the frequency of E3/E4 genotype in the patient's group. DNA oxidative stress has been evaluated through a Comet Assay. The multidisciplinary approach applied in this study allowed to dissect different aspects of ALS, often are evaluated separately and in heterogeneous cohorts of patients.

P2.8
Unravelling the effects of food-related engineered nanoparticles on the GUT interactive ecosystem

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The ever-growing use of nanotechnology in the agro-food sector will increase the oral exposure to engineered nanoparticles (ENPs). The average person in a developed country consumes over a trillion man-made food-related ENPs every day. The published literature on the safety of oral exposure to food-related ENPs currently does not provide sufficiently reliable data to allow a clear safety assessment. Our aim is to provide scientific evidences in order to elucidate the effects of sub-lethal concentrations of AgNPs on the human gut interactive ecosystem. The effects of 1mg/ml of AgNPs on the human gut ecosystem have been investigated in term of modulation of the proteome of two interdependent components: 1) a mono intestinal *E.coli* biofilm grown for 96 h on TSB medium (control), for 96 h on TSB medium + AgNPs (chronic effect) and for 72 h on TSB medium without AgNPs followed by 24h with AgNPs (acute effect); 2) a monolayer of differentiated human intestinal Caco-2 cells treated for 24h with TSB (control) and TSB + AgNPs. We also analyzed Caco-2 cells monolayer treated with permeates collected from biofilms grown on TSB with or without AgNPs.

P2.9
A longitudinal population genomic study of *Stenotrophomonas maltophilia* in cystic fibrosis patients: expanding our knowledge about an emerging opportunistic pathogen

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Stenotrophomonas maltophilia has been recognized as an emerging multi-drug resistant opportunistic pathogen associated with nosocomial infections. It occurs in about the 10% of CF patients, often co-isolated with P. aeruginosa. The genomic determinants associated with antibiotic resistance and virulence have been poorly investigated to date. We report a comparative genomic analysis based on Whole Genome Sequencing of 93 S. maltophilia isolates from 10 CF patients over an 11–years period. Genome-wide SNPs and the analysis of the pangenome demonstrated that this population is composed of 3 major phylogenetic lineages. MLST analysis revealed that 48 isolates belonged to a new sequence type, SNP-based phylogenetic tree clustered together samples from the same patient and/or having the same ST. Genomes were mined for antibiotic resistance and virulence genes, and their association with the corresponding phenotype was investigated. Our study highlights a much greater genetic diversity within this species than previously thought. The improved understanding of the biology of this bacterium will support the development of new therapeutic strategies in the future.

P2.10
Are metabolic processes affected during astrogliosis? DiVaMo, a non-standard analysis method, identifies new modifications in metabolic pathways in LPS and MCAO models of gliosis

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Astrogliosis has been recently investigated to identify genes that are over- or under-expressed and to derive the biological processes involved. In this line of research, we have considered a data set of gene expression in the neuroinflammation model induced by LPS and the ischemic stroke model (MCAO) (Zamanian et al., 2012), where standard biclustering methods fail to attribute a relevant role to the main metabolic pathways in separating models from controls. We analyze this data using DiVaMo, a method inspired by a covering-based feature selection approach (see Bertolazzi et al., 2016). The method identifies group of genes based on their integrated capability to differentiate between model and control samples, based on the expression ratios between models and controls. A single threshold allows a very straightforward control of the sensitivity and the robustness of the results. The separation power of a set is thus derived as a non-additive measure of the power of its genes; application to metabolism-related pathways (Glu/GABA, Glycolysis, TCA Cycle, PPP cycle, Lipid metabolism, NGF-TrkA/p75) identifies those that are significant for one of the two models, or for both.

P2.11
Why glutamate cannot be used as both a carbon and nitrogen source in budding yeast?

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Saccharomyces cerevisiae is unable to grow on media containing glutamate as the only carbon, nitrogen and energy source. However, stoichiometric considerations and computational simulations using an FBA model of yeast metabolism suggest that growth in this condition is theoretically possible. To investigate the reasons for growth inability of wild type yeast on media containing only glutamate, vitamins and supplements, we applied a directed evolution approach and isolated four independent mutants able to grow in this condition. Growth kinetics of these mutants in shaking flasks and in bioreactor confirmed their ability to use glutamate as the only carbon, nitrogen and energy source, even if the growth rate is strongly reduced compared to glucose-containing media. In order to clarify the molecular reasons allowing the newly isolated mutants to use glutamate as both a nitrogen and carbon source, a combination of physiological, molecular and omic analyses - including transcriptional and metabolomic analyses - are under way. Preliminary results suggest that an altered 2-oxo-glutarate usage and activation of nutrient-sensing pathway(s) may underlie the growth properties of the mutants.

P2.12
Secretome profiling of malignant mesothelioma cell lines

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Malignant pleural mesothelioma (MPM) is a locally aggressive malignancy arising from the surface serosal cells of the pleura with dismal prognosis. Lack of accurate pre-clinical biomarkers makes diagnosis, prognosis, and treatment decisions challenging. MPM has a highly secretory cell type. The cancer cell secretome has emerged as an attractive subproteome for discovery of candidate blood-based biomarkers. The aim of the present study was to compare the secretome of human MPM cell lines H28 and H2052 with normal mesothelial cell line Met5A in order to identify novel tumor-derived potential biomarkers. Secretomes from MPM and control cell lines were compared by two dimensional gel electrophoresis. Normalized spot volumes were analyzed using the ANOVA test to detect the proteins significantly ($p < 0.05$) more abundant in H28 and H2052 secretomes than in control. Selected spots were excised from gels and are currently under identification by MS/MS. Since matrix metalloproteinases (MMPs) play a significant role in tumor invasion and angiogenesis, MMP secretion was also investigated by zymography. Thus, we found H2052 cells secreted greater amounts of MMP-2 and MMP-9 than H28 cells.

P2.13
Unraveling the olfactory repertoire of the tiger mosquito *Aedes albopictus*

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The Asian tiger mosquito *Aedes albopictus* is a highly invasive species and competent vector of several arboviruses (e.g. dengue, chikungunya, Zika). Complex mosquito behaviours like host seeking, feeding, mating or oviposition rely on sensory functions carried out by olfactory neurons (ONs) localized mainly on antennae, mouthparts and maxillary palps. Typically, volatile odorants cross the cuticle and through Odorant Binding Proteins (OBPs) reach specific Odorant Receptors (ORs) on dendritic membranes of ONs. In order to characterize the main *Ae. albopictus* olfactory gene families we analyzed by RNA-seq female antennae and maxillary palps to assemble a transcriptome of 33846 contigs. Overall 79 OBPs, 88 ORs, 62 Ionotropic Receptors (IR) and 30 Gustatory Receptors (GR) were identified by comparative genomics and transcriptomics. Contigs upregulated in the antennae (620) and maxillary palps (268) were identified by differential expression (DE) and PFAM enrichments verified. We believe that a deeper knowledge of the olfactory repertoire of the tiger mosquito may help to better understand its biology and possibly it may pave the way to design new attractants/repellents.

P2.14
Mitochondrial proteome: characterization of the impairment due to shRNA of eIF6 using SWATH-MS analysis

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Eukaryotic Initiation Factor 6 (eIF6) is an initiation factor that binds 60S ribosomal subunits and has an anti-association property, by impeding 60S premature joining to 40S. In general, eIF6 is rate limiting for tumor onset and progression. eIF6 haploinsufficient cells are normal, but not efficiently transformed *in vitro*.

Mitochondria are the main compartments of energy production, and some lines of evidence have shown that mitochondrial alterations contribute to the development of metabolic syndrome. To this aim, we analysed, by using the SWATH-MS (Sequential Window Acquisition of all Theoretical fragment ion spectra) analysis, the expression of mitochondrial proteome of AML-12 (non-tumourigenic murine liver hepatocytes) cell line where eIF6 was down-regulated by shRNA. The SWATH-MS is a high throughput label-free method for protein quantitation that combines the traditional shotgun proteomics with the quantitative accuracy and reproducibility of selected reaction monitoring (SRM). We found that depletion of eIF6 by shRNA induce profound and varied impact on mitochondrial proteome, triggering the impairment of mitochondria.

P2.15
LTR-retrotransposons as major drivers of genome diversification across the genus *Helianthus L*

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Transposons play a key role in the evolution of species leading to rapid genome remodeling. Herein, we study the variability of the repetitive fraction of the genome in the genus *Helianthus* which recently has emerged as model for studying the genetics of speciation and adaptation. After determining the relative genome size of ten species and one subspecies of *Helianthus*, different assembling and clustering approaches were carried on by using next generation sequencing techniques to explore the repetitive component of the genomes. On average, repetitive DNA in *Helianthus* species represented more than 75% of the genome, with long terminal repeat retrotransposons (LTR-REs) being the vast majority of repetitive sequences. Prevalence of *Gypsy* over *Copia* superfamily was observed; and, among *Gypsy* lineages, *Chromovirus* was by far the most represented in each analyzed species. Moreover, considerable variability in the abundance of diverse LTR-RE lineages was found across the genus, showing differences especially between annual and perennial species. In some cases, such variation produced relevant effects on species genome size which was only partly related to the ploidy level.

P2.16
Effects of dexamethasone on gene and protein expression in ataxia telangiectasia

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Ataxia Telangiectasia (AT) is a rare genetic disease caused by biallelic mutations in the ataxia telangiectasia mutated (*ATM*) gene that codes for a protein kinase belonging to the PI3-kinase family. Unfortunately, no therapy is currently available to treat this condition. Glucocorticoid analogues have been shown to improve the neurological symptoms of patients. In the present study two WT and five AT lymphoblastoid cell lines were used as a cellular model and treated with dexamethasone (Dexa). Integrated analyses by transcriptomic (Affymetrix platform) and proteomic (2D-PAGE, MS/MS) approaches were carried out. We found out that Dexa was able to restore some of the AT impaired gene expression and protein behaviour. The identified differentially expressed genes and proteins were also functionally networked by Reactome FI, DAVID and STRING in order to evaluate the molecular functions and the biological processes influenced by Dexa in AT.

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P2.17
Proteomic profiling of zirconia nanostructure-induced neuronal differentiation and neuritogenesis in different cellular models

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Biophysical signals, including microenvironmental nanotopography, exert strong impact on cellular mechanics and behavior. The aim of the present research is to understand how nanoroughness can guide cellular activities in the context of neuronal differentiation in PC12 and rat hippocampal neuronal cells. The proteome of cells grown on neuritogenesis-inducing zirconia nanostructure (nrZr), flat zirconia (flZr) and Poly-L-Lysine (PLL) in the presence of Nerve Growth Factor (NGF) was investigated by label free quantitative high resolution tandem mass spectrometry. An Anova test was carried out to identify proteins differentially expressed among the various conditions. Congruent with the nature of the biophysical signal input, most of the differentially expressed proteins are involved in adhesome and/or cytoskeletal organisation and their up- or down regulation is in line with their functions in neuronal differentiation processes and/or neuritogenesis.
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P2.18
Glutamate rewires yeast metabolism and positively affects biomass yield

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The yeast *Saccharomyces cerevisiae* is sensible to the nature and amount of the available nitrogen source. Different sources are usually divided into preferred and "poor" ones. The preference for a nitrogen source is manifested either quantitatively by an enhanced growth rate in media containing the preferred source or qualitatively by the ability of the preferred source to induce repression of genes required for catabolism of other nitrogen sources. To understand the underlying mechanisms of yeast adaptation to different nitrogen sources (ammonium and glutamate) we took advantage of genome-wide and core FBA models of yeast central metabolism, constrained with fluxes obtained from glucose-limited chemostat cultures at different dilution rates. The model precisely describes the biomass yields obtained with different nitrogen sources as a consequence of the varying extracellular carbon source (glucose or ethanol). Moreover, the model describes the metabolic strategies, in terms of intracellular fluxes, that are adopted by the cells to fully exploit nutrients to achieve maximal biomass. Model predictions are being validated by physiological, biochemical and omic analyses.

P2.19
The ß-importome of mitotic HeLa cells

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Importin ß (ImpB) is the major vector for nuclear protein import. In interphase, it associates with protein cargos in the cytosol and transports them into the nucleus. In mitosis, when the nuclear envelope breakdowns and nuclear transport ceases, release of key mitotic factors from ImpB concurs in spindle organisation. We recently addressed the ß-importome in HeLa cells through Proteomics, providing a robust experimental frame on the recruitment of

specific proteins by ImpB at sub-stages of the cell cycle. At interphase, the ImpB complexes are characterised by a highly heterogeneous composition, overwhelmed by factors established to concur in nuclear protein import. Conversely, at mitosis this approach enabled identification of almost 500 partners of ImpB *per* replicate. This latter more informative set of data is then expected to evoke an accurate picture for the largely unknown role of ImpBeta in splindle organization and for the downstream effects induced by mutations at its multiple domains. Moreover, comparative profiles of ß-importomes alternatively fished with a plethora of commercially available ImpB antibodies, may support future structural studies on this multipurpose protein.

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P2.20
Nanorough Zirconia surfaces modulate mechanotransduction processes in human islets of Langerhans

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Cells are competent to perceive biophysical signals of their microenvironment (including nanotopography) and to convert them into biochemical cellular responses. Here we investigate the influence of zirconia surfaces nanoroughness, produced by supersonic cluster beam deposition (SCBD), on mechanotransduction processes in islets of Langerhans. NanoLC-ESI tandem mass spectrometry allowed comparing the proteome of pancreatic islets grown on zirconia nanostructure surfaces (nrZr15nm), flat zirconia (flZr) and matrigel (Gel); 65, 66 and 50 proteins are exclusively expressed in nrZr, flZr, Gel, respectively, while 101 out of 1406 common proteins differ with statistical significance (Anova test, FDR 0.05). The current study provides a first quantitative proteome comparison of human islets grown on a nanosubstrate and on matrigel. Though the quantitative architecture of their core proteome is highly conserved, cells show remarkable differences unraveling some interesting candidates for more detailed analysis of mechanotransduction processes induced by nanostructured surfaces. Acknowledgments: funded by Program FP7-NMP-2013-LARGE-7

P2.21
Multi-level approach for the identification of structure-function relationships in neurofibromin type I protein

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Neurofibromatosis type 1 is a dominant autosomal inherited disorder affecting approximately 1 in 3000 individuals, correlated to mutations in the *NF1* gene, encoding neurofibromin, a still poorly characterized large multidomain protein. In order to improve the understanding of NF1 functions and regulation, we exploited a system-level approach spanning from sequence and structure analysis to intracellular networks of the protein. First we collected and finely structured information available in literature and database, concerning gene organization, tissue-specific splicing variants, functional regions/domains organization, binding partners, missense and non sense mutations associated to neurofibromatosis type I. We demonstrated that NF1 is highly conserved in phylogenetically distant organisms in term of secondary structure and thus likely of global folding, much beyond the expected RasGAP domain. These results strongly support the notion that NF1 large multidomain protein organization has been conserved during evolution, suggesting that all parts of the protein are required to structurally and/or functionally regulate NF1 activities.

P2.22
Genome-wide analysis of DIV-like and RAD-like transcription factors in orchids

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The establishment of floral symmetry in dicots is related to transcription factors belonging to the TCP (CYC) and MYB (DIV and RAD) families. In orchids, studies on these genes are completely missing. Although extremely diversified, the orchid flowers share a common structure and a bilateral symmetry. To explore the *DIV*-like and *RAD*-like genes in orchids, we analyzed the available genomes of *Phalaenopsis equestris* and *Dendrobium catenatum* (Epidendroideae) and the floral transcriptome of *Orchis italica* (Orchidoideae). The copy number of the *DIV*-like genes varies between 7 and 8, whereas that of the *RAD*-like genes between 4 and 5. Their genomic organization is well conserved, with the presence of a single intron, except in one *RAD*-like gene that does not show any intron. Alternative splicing was detected for two *DIV*-like genes, with different expression pattern for each transcript isoform in the orchid floral tissues. Evolutionary analysis shows that purifying selection is acting on these genes; however, relaxation of selective constraints is detected between different paralogs and orthologs. The expression profile of these genes seems to confirm their conserved function in orchids.

P2.23
Changes in sunflower transcriptome during arbuscular mycorrhizal colonization

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Arbuscular mycorrhizal (AM) fungi establish beneficial symbioses with the roots of most land plants, including major food crops. The goal of this work is to study, by using a transcriptomic approach, gene expression variations in sunflower (*Helianthus annuus*) roots colonized by the arbuscular mycorrhizal fungus *Rhizoglomus irregulare*. First, a transcriptome (named HanMyc) was established by de novo assembling the reads obtained by Illumina sequencing of the RNAs isolated from colonized and control roots. Contigs were annotated by comparing them with sequences available in public databases. Qualitative analyses showed that, even if most genes expressed in the roots did not show changes following AM colonization, several genes were specifically expressed in mycorrhizal plants. Quantitative analyses allowed the identification of overexpressed genes in the early stage of mycorrhizal establishment, compared with controls. Such an overexpression largely increased as colonization proceeded. Gene Ontology analysis of differentially expressed genes showed significant differences, specifically in genes involved in *cellular process*, *metabolic process*, *membrane*, *binding* and *catalytic activity*.

O2.1
Comparative genome-scale modelling of *Staphylococcus aureus* strains identifies strain-specific metabolic capabilities linked to pathogenicity

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S. aureus is a bacterial pathogen colonizing diverse ecological niches within its host. We describe the pangenome of *S. aureus* based on genome sequences from 64 strains of *S. aureus* spanning a range of ecological niches, host types, and antibiotic resistance profiles. Based on this, *S. aureus* have an open pangenome composed of 7411 genes and a core genome composed of 1441 genes. Metabolism was highly conserved; however, differences were identified in amino acid and nucleotide pathways. Genome-scale models (GEMs) of metabolism were constructed for the 64 strains of *S. aureus*. These GEMs enabled a systems approach to characterize the core and pan metabolic capabilities of the species. All models were predicted to be auxotrophic for niacin and thiamin, whereas strain-specific auxotrophies were predicted for riboflavin, guanosine, leucine, methionine, and cysteine, among others. GEMs were used to analyze growth capabilities in more than 300 growth-supporting environments. The results identified metabolic capabilities linked to pathogenic traits and virulence acquisitions. Such traits can be used to differentiate strains responsible for mild vs. severe infections and host preference. GEMs analysis of multiple strains of a species can thus be used to identify metabolic determinants of virulence and increase our understanding of why certain strains of this deadly pathogen can spread rapidly.

O2.2
Dynamic networks dealing with oxidative stress: From design principles to personalised therapies for Parkinson's disease

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We have built a dynamic model of energy metabolism coupled to an intracellular signalling network controlling the response to oxidative stress and identified seven design principles of Reactive Oxygen Species (ROS) management in the context of ROS-related death of dopaminergic neurons, such as in certain cases of Parkinson's disease (PD). These are: (i) Mitoptosis and limited mitochondrial synthesis stabilize ROS management and protect against PD; (ii) Keap1-Nrf2 axis enables homeostatic dynamic adaptation; (iii) NFkB signalling prevents necrosis at high ROS levels, yet making the cell liable to a sudden increase of ROS; (iv) DJ-1 protects from a sudden increase of ROS; (v) Strong adaptation runs out with ageing, e.g. depletion of p62 occurring only after many years would explain the sudden onset of PD upon ageing; (vi) Inter-individual variation in ROS-managing network may well cause disease variability between individuals; and (vii) Preconditioning (e.g. coffee-induced Nrf2 activation) may play a protective role against PD. Here we show how these principles may help to design personalised PD therapies.

O2.3
Using CRISPR/Cas9 to determine the order of specific events in a cellular system

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It is difficult to assess the order of events leading to a specific phenotype. Currently, the only feasible manner is to interrogate each event individually, but this approach presents limits. We designed an assay to obtain *a posteriori* the order in which a series of events have occurred in a mammalian system. The assay exploits the Cas9/sgRNA system, a versatile genome-editing tool, to induce recombination of an artificial DNA cassette. The cassette bears barcodes interspersed among Cas9/sgRNA target sequences. The Cas9 is targeted to the cassette by different sgRNAs, individually linked to specific events. Thus, upon induction of the sgRNAs, the onset of double-strand breaks on the cassette will induce its sequential recombination, and the presence of the barcodes in the terminally recombined cassette is determined by the order in which the sgRNAs have been induced. Our system is currently designed to analyse either two or three events. Here we present proof of principle of the system, and we discuss how the system can be used to assess the transcriptional activation of different factors involved in complex phenotypes, such as self-renewal and cancer-transformation.

O2.4
Multi-level modeling of Metabolism, Growth and Cycle in *Saccharomyces cerevisiae*

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The construction of comprehensive, molecular whole cell models would help to extract understanding from the wealth of omic data by structuring them in mechanistic relations. A bottom-up approach to modelling, similar to that used for *Mycoplasma genitalium* ((Karr, J.R. et al. *Cell* 150, 389-401 (2012)), appears impracticable for more complex organisms, such as the model organism *Saccharomyces cerevisiae*. Here we present a step-wise approach, entailing the construction of a top-down, coarse-grained model, linking metabolism and cell mass growth to cell cycle dynamics and asymmetric cell division. The model accurately predicts nutritional modulation of protein distributions, a sensitive marker of growth and cell cycle dynamics in proliferating yeast populations (Porro, D. et al,, *Cytometry* A 75, 114-120 (2009)). Substituting a molecular model of the G_1/S transition (Palumbo, P. et al. *Nat Commun* 7, 11372 (2016)) in place of the corresponding coarse-grained module provides the proof-of-principle that the integrated coarse-grained model can act as a scaffold to structure and constraint molecular findings to facilitate the construction of a Yeast Whole Cell Model.

O2.5
Computational design of short linear D-tripeptides as binding moieties for protein pockets

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Because of their straightforward synthesis and purification procedures, short synthetic peptides can be used to rapidly explore their potential affinity for protein pockets. To best exploit this aspect, we have developed a strategy based on: 1) a new computational approach for the generation of small sets of D-tripeptides designed *ad hoc* to complement protein cavities, 2) the rapid parallel synthesis and purification and 3)

their testing by label-free multiwell Epic Corning technology. The computational part uses optimized FLAPdock software, generating sets of D-tripeptides ranked for their ability to complement the physico-chemical properties of the target cavities. As model targets for the design and testing of our pipeline, we have chosen Gadd45b/MKK7[1] and AIF/Cyclophilin A[2] protein complexes, characterized by specific complementary cavities on their surface, each with their own chemical features. We have generated 4 sets of tripeptides for Gadd45b, 3 sets of tripeptides for MKK7, one set for AIF and 2 sets for CypA. The first 8 tripeptides from each set have been synthetized and tested for their ability to bind the corresponding target proteins. Some peptides have been further experimentally tested, identifying some interesting new protein binders and validating the overall strategy.

References

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3 - Chromosome Biology, Cell Division and Cell Cycle

P3.1
Gene expression profiling of aneuploid IMR90 cells induced by CENPE depletion

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Segregation errors of chromosomes into daughter cells lead to aneuploidy that is considered a major feature of solid tumors. The Spindle Assembly Checkpoint (SAC) is a cellular surveillance mechanism that ensures faithfully segregation of chromosomes. Weakening the SAC by reduced expression of some of its components like CENPE induces aneuploidy. How diploid cells face chromosome mal-segregation and the mechanisms driving aneuploidy tolerance in tumor cells are not yet completely defined. Thus, an important goal in cancer genetics is to identify gene networks leading to aneuploidy as well involved in its tolerance. We induced aneuploidy by CENPE partial depletion in IMR90 primary cells and analysed gene expression profiles at 72h and two weeks when cells are still aneuploid. By using DNA microarrays we identified differentially expressed genes, up or down regulated, in CENPE depleted IMR90 cells. Normalized data were also analysed with the Gene Set Enrichment Analysis (GSEA) software to detect pathways/gene-sets deregulated and associated with the aneuploidy phenotype. GSEA analysis suggested the existence of a common gene signature underlying aneuploidy induction and tolerance

P3.2
Identification of Hec1-microtubule targeted anticancer compounds

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Highly Expressed in Cancer protein 1 (Hec1) is a subunit of the kinetochore (KT)-associated Ndc80 complex, which ensures proper segregation of sister chromatids during mitosis and is highly expressed in cancer. Structural reconstitution of Ndc80 complexes bound to microtubules (MTs) has identified a region of Hec1 calponin homology domain that binds a task on the wall of the MT, interacting with negatively charged residues on the MT. We have performed a virtual screening on this interaction domain and identified one positive compound from which a series of analogues has been developed. We have investigated the biological activity of the lead compound and the analogue molecules on human cancer cells and identified two highly cytotoxic small molecules (SMs). The two SMs produce chromosome segregation defects and mitotic catastrophe. However, a clear inhibition of mitotic entry associated with the occurrence of cell death from interphase was also recorded in time lapse experiments. Cold-induced MT depolymerization experiments demonstrated an hyper-stabilization of both mitotic and interphase MTs, suggesting that the two SMs may work by stabilizing the MT-KT interaction or the MT itself.

P3.3
The GTPase RAN, nuclear transport receptors and kinetochore function during mitosis

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The GTPase RAN and its effectors regulate the organization and activity of the mitotic spindle in mammalian cells. The establishment of interactions between the spindle MTs and chromosomal kinetochores is crucial during mitosis and is accompanied by the recruitment of numerous proteins to kinetochores. Among those, a relevant complex comprises RANBP2, a RAN-binding nucleoporin with SUMO E3 ligase activity, and SUMO-conjugated RANGAP1, a regulator of RAN. SUMO-RANGAP1/RANBP2 are thought to modulate the functional status of kinetochores. We have developed proximity ligation assays (PLA) to investigate how this recruitment is itself regulated. We find that Importin beta and exportin 1/CRM1 play antagonistic roles in RANBP2/RANGAP1 localization at kinetochores. Introducing an imbalance between importin beta and CRM1 impairs RANBP2/RANGAP1 localization to kinetochores, microtubule/kinetochore interactions, mitotic progression and chromosome segregation. We also find that RANBP2 plays additional roles in SUMO conjugation of chromosomal passenger complex proteins. Our results indicate that a finely tuned cross-talk between RAN effectors regulates KT functions in mitosis.

P3.4
SAMHD1 regulates DNA precursors in human cells according to cell cycle progression

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Sterile alpha motif and HD-domain containing protein 1 (SAMHD1) is a triphosphohydrolase that degrades DNA precursors (dNTPs) in all human tissues. Mutations in the SAMHD1 gene are associated with the autoimmune disorder Aicardi-Goutières Syndrome (AGS) and to various types of cancer. Our group investigates the role of SAMHD1 in cultured human cells and found that it is the major catabolic regulator of dNTP concentrations. The absence of SAMHD1 leads to oversized and unbalanced dNTP pools in different cell lines (AGS fibroblasts, THP1 KO monocytes, SAMHD1-silenced fibroblasts) especially when DNA is not replicating. Despite the abnormal pools the rate of replication forks and cellular growth are unaffected. The total amount of SAMHD1 protein remains relatively unchanged during cell cycle progression and increases in not proliferating cultures. The phosphorylation of SAMHD1 on Thr592 is related to the cell cycle. It is absent in G1, appears at G1/S transition and remains untill G2/M phase. Overexpressed SAMHD1 is largely phosphorylated and only slightly decreases the dGTP, suggesting a downregulation of its dNTPase activity related to the proliferation state of the cells.

P3.5
Study of molecular mechanisms behind X-Y chromosome segregation defects in mammals

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Homologous recombination (HR) is an essential step in meiosis to ensure proper chromosomes segregation in the gametes. A fundamental component of this process is Spo11 who induces DNA double strand breaks (DSBs) onto chromosomes, initiating HR. In mammals, Spo11 gene produces different alternative transcripts. Spo11β and Spo11α are the major isoforms. Spo11-/- mice are infertile as germ cells undergo massive apoptosis. The goal of the project was to understand the distinct function of Spo11 splice isoforms in meiosis. We generated two knock-in models: Spo11βki and Spo11αki. The analysis of Spo11βki mice revealed a reduced testis size and increased apoptosis, due to the impairments of recombination/synapses of the autosomes (40%) and defective segregation of X-Y chromosomes (60%). Nevertheless mice were fertile. As opposite, Spo11αki mice were sterile, and phenotypically resembled Spo11-/-. We conclude that Spo11β is required but not sufficient to promote proper synapse of the chromosomes. Spo11α, is

unable to generate DSBs and likely require Spo11β for its function. Further ongoing studies will elucidate their functional interplay. Since alterations of the expression of Spo11 splicing variants cause X-Y segregation defects, we hypothesize that Spo11 gene mutations might be at the bases of some human diseases such as Klinefelter or Turner syndromes, whose genetic origin is unknown.

P3.6
Importin beta regulates mitotic spindle assembly and activity via distinct molecular mechanisms

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Importin beta is the main protein import vector in interphase nuclei and a RAN GTPase effector. When nuclear transport ceases, importin bet acts at several steps of mitosis, but the molecular mechanisms are unclear. Importin beta is overexpressed in many cancer types with high genetic instability. We have generated inducible cell systems expressing importin beta, either wild-type or defective for nucleoporin binding. Using interactomics, time-lapse imaging and functional approaches, we identify factors acting in two major processes: SUMO pathway factors, required for both microtubule assembly and stabilization, interact with both wild-type and mutant importin beta; microtubule stabilization also requires DLGAP5/HURP, a selective interactor of wild-type but not mutant importin beta. Both pathways, when impaired, ultimately hinder chromosome segregation, thus increasing genetic instability through cell generations. These approaches identify concomitant pathways in mitotic progression that would otherwise be difficult to disentangle, and contribute to clarify mechanisms through which importin beta influences genetic instability in cancer cells in which it is overexpressed.

O3.1
A dysregulated dNTP pool hinders DNA replication in cell cycle-reactivated terminally differentiated cells

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Terminally differentiated (TD) cells are defined by their inability to proliferate. When forced to reenter the cell cycle, they generally cannot undergo long-term replication. Our previous work with myotubes has shown that TD cells fail to proliferate because of their intrinsic inability to complete DNA replication. Moreover, we have reported pronounced modifications of deoxynucleotide metabolism during myogenesis, with marked down-regulation of thymidylate synthesis. Here, we investigate the causes of incomplete DNA duplication in cell-cycle reactivated myotubes (rMt). We find that rMt possess extremely low levels of thymidine triphosphate (dTTP), correlating with very slow replication fork rates. Increasing deoxynucleotide availability allows extended and faster DNA replication. Inadequate dTTP levels are caused by failure to re-express specific synthetic enzymes upon cell cycle reentry, due to a selective epigenetic silencing that resists cell cycle-triggered reactivation. We conclude that lack of dTTP is at least partially responsible for the inability of myotubes to proliferate, and speculate that it constitutes an emergency barrier against unwarranted DNA replication in TD cells.

O3.2
Alternative lengthening of telomere (ALT) implicated in telomere length modulation induced by x-rays in human primary fibroblasts

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Most human tumors (80-85%) maintain their telomeres by expressing telomerase, whereas a significant minority of cancers (15-20%) utilizes the Alternative Lengthening of Telomeres (ALT) pathway. Although the telomerase is well known, the molecular details of ALT remain poorly described. Previous studies demonstrated that X-rays in human primary fibroblasts modulate telomere length at 15 days after exposure. In order to explore the modulation induced by X-rays and to understand the mechanisms responsible for such modulation, we treated HFFF2 with 4Gy of X-rays and analyzed telomere length, telomerase activity and ALT markers (APBs, T-SCEs and C-circles) in a time period from 3 to 15 days after irradiation. Results demonstrated that X-ray irradiation modulates telomere length. These data confirm our previous hypothesis that ALT is a mechanism activated by normal primary cells as a response to supraphysiological telomere damage.

O3.3
CK2 Phosphorylation of MUS81 regulates its activation for proper resolution of DNA intermediates in mitosis

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The faithful transmission of genetic information to daughter cells is central to maintaining genomic stability during each cell cycle. MUS81 endonuclease is important to process perturbed forks and promotes replication restart under persisting arrest or checkpoint deficiency. However, unscheduled MUS81 activation generates chromosomal damage and thus it needs to be tightly controlled. Analysis by MS identified several phosphorylated residues, including S87, which lie within a putative CK2 consensus sequence. Functional analysis on cells expressing unphosphorylable (S87A) or phosphomimetic mutant (S87D) of MUS81 revealed that phosphorylation at S87 is cell cycle dependent and is required for resolution of mitotic recombination intermediates. Indeed, S87A mutant affects mitotic progression, as revealed by accumulation of bulky anaphases bridges, ultra fine bridges and 53BP1 NBs. Also, MUS81^{S87D} is constitutively active, and this causes its inappropriate activation during DNA replication, as emphasized by accumulation of DSBs in S-phase. Altogether, our results demonstrate that, in mitosis, MUS81 phosphorylation is crucial to facilitate proper DNA segregation under replication stress.

O3.4
Microcephaly with neurodevelopmental impairment shows microtubule assembly defects with h-prune mutations during mitosis.

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Autosomal recessive primary microcephaly MCPH is a rare hereditary neurodevelopmental disorder with a marked reduction in brain size and intellectual disability.Perturbations that upsets the balance between symmetric and asymmetric division,defects in cell proliferation,enhanced apoptosis,abnormal neuronal migration and differentiation contribute to MCPH.MCPH-causing mutations were identified in twelve genes encoding proteins involved in cell cycle regulation.Variants in h-Prune were identified in families with MCPH. We identified mutations in Prune(D30N,R297W,D106N)in three families with MCPH from Oman,India,USA.Prune-1 belongs to DHH-phosphoesterase superfamily,is expressed in fetal brain and is involed in proliferation and motility interacting with NME1 and GSK3.We show that Prune1 is a novel interactor of α/β-tubulin altering microtubules polymeration during mitosis.These mutations result in mitotic defects impairing microtubules nucleation and polymeritation,the proper spindle length,with its biochemistry related to polymerization.As result,the mutations reduce cell proliferation and migration.Our data establish Prune1 biochemical activity necessary for a correct cell division.

O3.5
CSA and CSB proteins localize to the midbody during cytokinesis and regulate abscission through PRC1 degradation

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Here, we report that CSA and CSB proteins, so far considered primarily nuclear proteins, localize to the midbody, a transient structure that connects two daughter cells at the end of cytokinesis. Our findings further demonstrate that CSA and CSB participate in the ubiquitination and degradation of protein regulator of cytokinesis 1 (PRC1) which plays a fundamental role in the regulation of abscission. Accordingly, loss of function of CS proteins results in abscission impairment and citokinesis failure resulting in mitotic abnormalities that lead to formation of multinucleated cells and multipolar mitotic spindles. Altogether these findings reveals an unexpected roles of CS proteins in the context of mitosis and open a new scenario in the understanding of Cockayne syndrome.

4 - Epigenetics and Epigenetic Therapies

P4.1
Up-modulation of KDM5A is a key determinant for temozolomide-resistance in glioblastoma

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Glioblastoma (GB), the most common malignant brain tumor, remains a clinical challenge because of its aggressiveness and resistance to the clinical treatments. In spite of current multimodal treatment (surgery, radiotherapy and chemotherapy with temozolomide) the median survival of glioblastoma patients is about 14 months due also to the emergence of cell clones resistant to treatment. Therefore, understanding the mechanisms underlying chemoresistance can contribute to improve treatments' outcome. In an *in vitro* model we demonstrated that TMZ resistance in GB is partially reverted by "drug wash-out" suggesting the contribution of epigenetic mechanisms in drug resistance and supporting the possibility of TMZ rechallenge in GB patients after prior drug exposure. In this dynamic process some lysine histone demethylases, KDM5A in particular, are involved and up-modulated in TMZ-resistant cells. The modulation of KDM5A expression restores the sensitivity to TMZ resistant cells as well as HDACi treatment. Attempts to inhibit this epigenetic modifier are under investigation to develop novel combined adjuvant therapies for this rapidly progressing and invariably lethal cancer.

P4.2
Secondary epimutation of the *Igf2/H19* Imprinting Control Region in a mouse model of the Beckwith-Wiedemann and Silver-Russell Syndromes

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Genomic imprinting is an epigenetically regulated process determining allele-specific expression in a parent-of-origin dependent manner. Epigenetic alterations affecting the *IGF2/H19* imprinting locus at chromosome 11p15.5 cause two imprinting disorders with opposite growth phenotypes, the overgrowth-associated Beckwith–Wiedemann syndrome (BWS) and the undergrowth-associated Silver–Russell syndrome (SRS). Since the architecture of 11p15.5 imprinting cluster is similar between human and mouse, we have generated a knock-in mouse model, in which the *Igf2/H19* ICR (mIC1) is replaced by the orthologous human sequence either wildtype (hIC1wt) or carrying a mutation (hIC1mut) found in a familial BWS case. While the mice carrying hIC1wt showed normal phenotype, the mice with hIC1mut had increased IC1 methylation, *Igf2* activation, *H19* repression and overgrowth resembling BWS, upon maternal transmission. In contrast, paternally transmitted hIC1wt and hIC1mut led to decreased IC1 methylation, *Igf2* repression, *H19* activation and undergrowth resembling SRS. However, growth restriction was less severe in the mice carrying hIC1mut that were fully viable although never caught-up in growth.

P4.3
E2 induces chromatin remodelling at the Neuroglobin-regulated genomic regions in neuronal cells

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Neuroglobin (NGB) has a neuroprotective factor that is regulated by 17b-estradiol (E2), but little is known about its transcriptional regulation. E2 genomic pathway in gene expression regulation is mediated by estrogen receptors (ERα and ERβ) that bind to specific regulatory genomic regions. We focused our attention on E2-induced NGB expression in human differentiated neuronal cell lines (SK-N-BE and NT-2) and in mouse GN11. Previously, using bioinformatics analysis we identified a putative enhancer in the first intron of NGB locus. Therefore, we observed that E2 increased the enrichment in active epigenetic marks on promoter, H3K4me3, and on the intron enhancer, H3K4me1 and H3K27Ac. In these NGB regulatory regions, we found estrogen receptor alpha (ERa) binding suggesting that ERa may mediate chromatin remodeling to induce NGB expression upon E2 treatment. Altogether our data show that NGB expression is regulated by ERa binding on genomic regulatory regions supporting hormone therapy applications for the neuroprotection against neurodegenerative disease.

P4.4
The *in vivo* reactivity of *S. cerevisiae* DNA topoisomerase 1 is affected by chromatin structure

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DNA topoisomerases ensure the proper level of supercoiling required during basic DNA transactions such as replication, transcription and recombination. Despite the deep knowledge of their mechanism of action, elements regulating their *in vivo* accessibility toward DNA have to be better understood. Determining which conditions are suitable to maximize their activity could improve anti cancer therapies, based on DNA topoisomerases inhibitors. These drugs stabilize the DNA-enzyme complex (that would be transient in physiological conditions) formed after the breaking reaction and before the rejoining one. In *S. cerevisiae,* Nhp6A and B proteins (homologs to the mammal Hmgb1p) are abundant chromatin components. *S. cerevisiae Nhp6ab* double mutants show altered gene expression, genome instability, shortened replicative life span and a reduced histone amount, as well as mammals HMGB1-/- cells. Given that Topo1 and Topo2 activity depends on nucleosome presence, we wanted to investigate whether the DNA Topoisomerase 1 activity could be altered in a *Nhp6ab* background and found that Nhp6 proteins affect Topo1 reaction.

P4.5
Nhp6 proteins negatively contribute to transcriptional regulation of histone genes in *Saccharomyces cerevisiae*

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Maintaining a stable and balanced histone pool is of paramount importance for genome stability and fine regulation of DNA replication and transcription. The regulation of histone genes involves a complex regulatory machinery, exploiting transcription factors as well as histone

chaperones, chromatin remodellers and modifiers. Although a series of regulative elements have been found to be involved in histone gene expression, the functional details of this machinery are as yet unclear. In a our previous study we reported that histone amount decreases in mammalian and yeast HMGB family mutants, HMGB1$^{-/-}$ and *nhp6ab* respectively. In this study we decided to explore the reasons of the histone decrease in *nhp6ab* mutant of *S. cerevisiae*. We found that Nhp6 proteins control histone gene expression by affecting nucleosome stability at regulative regions of the histone clusters. In addition, we observed that histone overexpression is associated with H4K16 hypoacetylation, a feature of new regulated gene family: the hypoacetylation-activated genes (HAAG). Our observations allow us to incorporate Nhp6 proteins in the large group of chromatin factors that tightly regulate histone gene expression.

P4.6
The epigenetic landscape of equid centromeres: a molecular approach

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Mammalian centromeres are typically associated with highly repetitive DNA (satellite DNA), which has so far hindered a detailed molecular analysis of this chromatin domain. Previously (Piras et al PLoS Genetics 2010) we showed that, during the evolution of the genus *Equus*, several centromeres moved to new sites lacking satellite DNA. In this system the epigenetic marks related to the centromere can be studied by comparing the centromeric domain of a species with the non-centromeric orthologous locus in another species. We also demonstrated (Purgato et al Chromosoma 2015) that the location of the CENP-A binding domain can vary in different individuals giving rise to *epialleles*, proving that centromeres are autonomous relative to the DNA sequence. Here we present ChIP-seq experiments with anti-CENP-A antibodies in donkey and zebra cell lines. We identified several satellite-less centromeric sequences and compared them with the orthologous non-centromeric loci of the horse, evaluating the role of sequence composition, DNA breakage, DNA and histone methylation, transcription in the formation of new centromeres. The inheritance of centromeric domains in a hybrid family was also studied.

P4.7
Epigenetic mechanisms in metabolic regulation: the involvement of HAT Gcn5 in yeast respiration

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Metabolic pathways provide energy to cell functions and alterations in their regulation are found in a variety of human diseases. Epigenetic mechanisms play a primary role in metabolic regulation by histone and non-histone proteins acetylation. Budding yeast is a suitable model system to study the interplay between epigenetic and metabolism. The findings obtained in yeast can be successfully translated to human cells because of the highly conservation of fundamental functions. SAGA is one of the major acetylation complex in yeast *S. cerevisiae* and we found that it is needed for growth in respiratory condition and for oxygen consumption. In particular, we focalized on acetyltransferase Gcn5 (SAGA HAT domain) showing that its regulation is dependent on the carbon source in growth medium. Indeed, Gcn5 is specifically required for respiration and alteration of its HAT activity leads to defective phenotype.

P4.8
Unveiling the folding mechanism of the bromodomains

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Bromodomains (BRDs) are the only known small protein interaction modules that selectively targets ε-N-acetylation of lysines. BRDs have a pivotal role in the regulation of the transcription of growth-promoting genes and cell cycle regulators because they "read" acetylation of lysine, one of the most frequently occurring post-translational modifications that controls gene transcription and chromatin structure. Several studies are focusing on BRDs, but little is known about the dynamic properties of these proteins. In our study we present a thermodynamic characterization of domain 2 of BRD2 and domain 1 of BRD4 that belong to one of the eight major subfamilies identified in the human genome, the BET family. They are structurally very similar, so they are ideal experimental systems to investigate conservation (if any) of the folding mechanism among members of a fold family. In this study the quantitative analyses of stopped-flow mixing experiments and ultra-rapid temperature-jump data allowed us to show that the folding mechanism of both BRDs are consistent with the presence of a folding intermediate, transiently populated in the sub-milliseconds time-regime.

P4.9
Unexpected effect of dexamethasone in ataxia telangiectasia cell lines

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Ataxia-telangiectasia (AT) is a rare autosomal recessive disorder caused by mutations in the ataxia-telangiectasia mutated gene (ATM), which codifies for a protein kinase mainly involved in DNA damage response. Patients with the classical form of AT survive until their second-third decade of life, and no established therapy is currently available for this disease. Recently, short term treatment with Dexamethasone (Dexa) was shown to improve the symptoms of this syndrome. Nevertheless, the molecular mechanism involved in Dexa action in AT patients is not yet know. Here we examined the effects of dexamethasone treatment in human primary fibroblast ATM$^{-/-}$ GM02052, GM0648 and in the control cell line ATM$^{+/+}$ AG09429. We observed, for the first time, a nucleoplasmic accumulation of Lamin A only in ATM muted cell lines treated with Dexa. In order to provide insight into this outcome, we verified the Lamin A/C phospho-dependent nucleoplasmic localization in Ser22 and Ser404. Dexa significantly triggered the phosphorylation of Lamin A Ser22 in ATM$^{-/-}$ cell lines and a marked phosphorylation in Ser404 in GM0648 cells. Lamin A Ser404 is a nuclear target of AKT, a kinase involved in cell survival, proliferation and metabolic responses downstream the phosphoinositide-3-kinase (PI3 kinase) signaling pathway. Dexa resulted capable to exclusively activate AKT phosphorylation in ATM$^{-/-}$ GM0648 cell line, endorsing the Lamin A accumulation. Some of the established Lamin A/C regulated genes were evaluated by qPCR. Finally new emerging epigenetics roles of Lamin A prompted us to investigate if the noticed Lamin A accumulation was involved in chromatin modulation in Dexa treated AT cells. ChIP-seq analysis were performed for this purpose.

P4.10
Analysis of epigenetic modifications associated with Amyotrophic Lateral Sclerosis (ALS) onset and progression

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ALS is an adult-onset neurodegenerative disease, characterized by the progressive degeneration of upper and lower motor neurons. ALS is predominantly sporadic and environmental triggers may be involved in disease initiation, althought no clear environmental risk factors have been identified for ALS, perhaps because the triggers may act only in genetically susceptible individuals. In this respect since aberrant epigenetic patterns are acquired throughout life, the understanding of their role in motor neuron can be fundamental to improve our knowledge about ALS pathogenesis. The major objective of our work is: (i) to investigate whether in cellular and animal models for ALS is possible to highlight chromatin modifications associated with disease onset and progression and to study the "epigenetic" status of key genes known to be involved in ALS development, (ii) to describe the interaction between the ALS-causative genes and the epigenetic machinery. In order to to achive these objectives, we use the following cellular and animal models able to mimic the genetic alterations that cause ALS: (i) *Transgenic mice SOD1-G93A*; *(ii) adenoviral delivery of ALS causative-gene* in neuronal cells.

P4.11
Unraveling the function of m6A modification in acute myeloid leukemia

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N6-methyladenosine (m6A) is a well-known RNA modification that can affect mRNA stability and translation (Yue et al., Genes Dev. 2015). In mammals, the m6A writer is a multicomponent complex composed of the two methyltransferases METTL3 and METTL14 and the regulatory protein WTAP. WTAP has been recently described as an oncogenic factor in AML suggesting that m6A modification might play crucial role in leukemogenesis (Bansal et al., Leukemia 2014). Notably, we also found that METTL3 and METTL14 are upregulated in primary AML samples. Here, we analyzed the functional role of m6A during myeloid differentiation of AML cell lines. Impairing the expression of the methylation complex components by RNAi affected consistently myeloid differentiation and induced massive apoptosis. Moreover, in AML cell lines METTL3 mislocalized in the cytoplasm and associated with polysomes. These data indicate that the misregulation of m6A methylation may contribute to leukemogenesis, but also highlight a putative m6A-independent role for METTL3. Our data also pave the way to the development of new therapies for AML through the inhibition of the methylome complex.

P4.12
Chemosensitization of sarcoma cells by ITF2357 histone deacetylase inhibitor

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Soft tissue and bone sarcomas are rare tumors with generally poor prognosis, for which current therapies have shown limited efficacy. Histone deacetylase inhibitors (HDACi) are emerging as a prominent class of therapeutic agents for several cancers; however, little is known about HDACi activity in sarcomas. We show that, ITF2357 potently inhibited survival of sarcoma cells *in vitro* and induced expression and nuclear translocation of the FOXO3 tumor suppressor gene. Notably, ITF2357 cytotoxicity was independent on p53 status, despite its ability to inhibit the expression of mutated p53 protein. ITF2357-mediated cell death implied the activation of mitochondrial apoptosis, as attested by upregulation of pro-apoptotic BH3-only proteins Bim and PUMA, and caspases-dependent cell death. ITF2357 also induced a canonical-autophagic process, which protected sarcoma cells from apoptotic cell death and enhanced cell survival. Finally, ITF2357 also synergized with doxorubicin to induce cell death of sarcoma stem cells obtained from sarcoma patients, and to inhibit tumor growth *in vivo*. These studies identify an effective combination therapy for the most aggressive form of sarcoma.

O4.1
Genomic regulatory regions role in Th17 and Treg cells balance during pregnancy of multiple sclerosis patients

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The unbalance between Th17 and Treg cells is associated with outcome and progression of multiple sclerosis (MS) and estrogens play a role in the reduction of relapse rates among women with multiple sclerosis (MS) during the last trimester of pregnancy. Our hypothesis is that estrogen receptor alpha (ERα) may bind at cell-type-specific regulatory regions crucial for Treg and Th17 cells identity, defining as super-enhancers (SE). In order to identify SE, we used an integrative approach based on ENCODE and ROADMAP datasets and it helps us to build a transcription factors network. We selected and validated cell-type-specific genomic regulatory regions at FOXP3 and RORC loci, respectively lineage-determining transcription factors for Treg and Th17 cells. E2 induces an active chromatin state in FOXP3 regulatory regions, while enhance a repressive state on RORC regulatory regions in Th17 purified cells and in peripheral blood mononuclear cells (PBMC). In addition, we found that ERα binds at these genomic regulatory regions upon E2 treatment and in MS patients. These data suggested that Th17 cells may shift to regulatory T cells by E2-induced chromatin remodeling.

O4.2
The epigenetic landscape of equid centromeres: a cytogenetic approach

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The centromere, the chromosomal locus required for chromosome segregation, is defined epigenetically, centromeric chromatin being distinguished by the presence of the modified histone CENP-A. Transcriptional competence is a requisite for centromere function (Quénet & Dalal, Elife 2014) and centromeric chromatin is characterized by a peculiar histone code (Sullivan & Karpen, Nat Struct Mol Biol 2004). The DNA of most eukaryotic centromeres is composed of extended arrays of tandem repeats. We previously studied the organization of the centromere of different species of the genus Equus which, due to the coexistence in a single karyotype of satellite DNA based and satellite-less centromeres, revealed to be a unique model system for the analysis of centromere function (Piras et al, PLoS Genet 2010; Cerutti et al, Mol Cytogenet 2016). Using a molecular-cytogenetic approach, based on multiple colour immunofluorescence, here we investigated the architectural organization of different histone modifications at the centromeres of horse and donkey chromosomes. Our results, while confirming literature data, shed new light on the debated question of centro-chromatin organization.

O4.3
Epigenetic control of hyaluronan synthases

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Epigenetics has emerged as a key aspect in the synthesis of hyaluronan (HA), which is controlled by gene expression of hyaluronan synthases 1,2 and 3 on cell membranes. The amount of HA in tissue has a critical role in several pathologies. UDP-sugar availability as well as the cellular

energy are crucial for the synthesis of HA and for HAS2 activity. The AMP activated protein kinase, a sensor of the energy status of the cell, leads to HAS2 T110 phosphorylation, which specifically inhibits HA secretion. However, UDPGlcNAc, the most general sensor of cellular nutritional status, can lead to intracellular protein glycosylation (O-GlcNAcylation). O-GlcNAcylation of serine 221 residue of HAS2 induces a dramatic stabilization of the enzyme on the membranes and an increase of HA production. Eventually we found a long non-coding RNA (NAT) positively controls in cis the HAS2 expression involving p65 and NFkB pathway. Beside the effect of antisense, the histone acetylation by P300 and histone de-acetylation by HADC and sirtuins have a critical effect. The transfection of P300 increased the HAS2 expression and HA synthesis whereas transfection of HDAC1 has opposite effects.

O4.4
Use of JARID histone demethylases inhibitors to enlighten the biological role of these enzymes in yeast and mammalian cells with focus on transcriptional regulation.

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Histone N-terminal tails are subjected to several covalent modifications which form a sophisticated combinatory code interpreted by a plethora of regulatory protein complexes. Among the various modifications, Lysine (K) methylation is particularly interesting, due to its widespread roles in transcriptional regulation, DNA repair and epigenetic inheritance. For many years, histone lysine methylation has been considered irreversible and persisting through cell division. Recently, two families of histone demethylating enzymes (HDMs) have been identified in eukaryotes: the LSD1 family and the Jmjc-domain-containing family. JHDMs are potential therapeutic cancer targets and among them, those capable to demethylate specifically H3K4 (*JARID 1A-1D,*) look particularly interesting. In order to discover inhibitors specific for H3K4 histone demethylation we set up a *in vivo* screening system which tests the effects of candidate small molecules inhibitors on a *S. cerevisiae* mutant strain (Mannironi et al., 2014). With this system we selected compounds which appears specific and we are testing their biological and transcriptomic effects on a breast cancer cell line which over-expresses Jarid 1B.

O4.5
Unraveling the role of NAP1L1 into the chromatin of mammals

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Nucleosome assembly protein 1-like1 (NAP1L1) belongs to a mammalian family of histone chaperone whose members are homologous to the highly conserved yeast NAP1 protein. Different reports show its involvement in transcriptional regulation, cell cycle progression and differentiation. Intriguingly, NAP1L1 displays not only assembly but also disassembly activity in vitro. However, little is known regarding the mechanism by which NAP1L1 regulates chromatin in mammals. We have generated a mouse line knockout for the Nap1l1 gene. Mutant animals born from crosses of heterozygous exhibit a pre- and post-natal partial lethality and are 15-20% smaller at birth compared to wild-type. To assess the role of NAP1L1 into chromatin and nucleosomal organization, we have isolate MEF cells and quantified histones levels relative to DNA content. Preliminary data in Nap1l1 mutant cells demonstrate an increase in the total amount of histones that may result in higher nucleosomal occupancy, as suggested by the observed 40% increase in protection from micrococcal nuclease digestion. These findings raise the prospect that in mammals NAP1L1 could act mainly as a disassembly chaperone.

5 - Oncogenes and Tumor suppressors

P5.1
Sphingosine-1-phosphate in the tumor niche promotes glioblastoma malignancy

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Glioblastoma (GBM) is characterized by the presence of cancer cells and a considerable amount of parenchymal cells. Microglia and endothelial cells contribute for GBM tumor growth and spread. The signals regulating the interplay between cells in the GBM niche are little known. Sphingosine-1-phosphate (S1P) has emerged as a crucial mediator of the hallmark capabilities of GBM. We investigated the capacity of parenchymal cells to act as source and/or target of S1P. We found that GBM-derived tumor cells, stem cells, endothelial cells and microglia are able to synthesize and secrete S1P. GBM stem cells and endothelial cells were found to be effective in releasing S1P extracellularly. After co-culture, GBM and parenchymal cells exhibit enhanced expression of S1P receptors, and of S1P secretion, respectively. Extracellular S1P is able to promote growth, stemness and survival of tumor cells, migration and vasculogenesis of endothelial cells, and inflammatory properties of microglia. Our data demonstrate that different cell types of the GBM niche and their cross-talk contribute to the S1P enrichment of the GBM niche, where S1P prompts multiple processes which favor GBM malignancy.

P5.2
Regulatory mechanisms of the Aurora-A/TPX2 complex: influence on chromosome stability and implications for cancer

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The Aurora-A kinase controls several mitotic events and hence chromosome stability. It is overexpressed in cancer and Aurora-A chemical inhibitors are in clinical trials as anti-cancer agents. Aurora-A activity, stability and localization are regulated by TPX2. We collected evidence of Aurora-A and TPX2 co-overexpression in cancer and proposed the Aurora-A/TPX2 complex as an oncogenic unit. To address this issue we generated non-transformed hTERT-RPE1 cell lines stably expressing Aurora-A, TPX2 or the whole complex. Our analyses show defects in mitosis and potentially pro-tumorigenic aneuploidy induction in the following interphases, with the most severe phenotypes induced by overexpression of the complex. We also observe that co-overexpression of Aurora-A and TPX2 influences Aurora-A stability and localization in interphase in a manner that can be relevant for transformation. In parallel studies, we highlighted potential caveats in the use of classical Aurora-A inhibitors; we are therefore currently investigating the possibility to inhibit Aurora-A activity by an alternative approach, based on impairing the interaction with TPX2.

P5.3
SOD1 and *GALNT7* gene: RNAi screening on mesothelioma cell lines

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The aggressiveness of Malignant Pleural Mesothelioma (MPM) and the inefficacy of the current therapies highlight the necessity to identify new biomarkers for MPM. Through a previous research, novel genes upregulated in MPM have been identified. Among them we focused our attention on *SOD1* and *GALNT7*. In order to evaluate their role in MPM tumorigenesis, we performed the caspase assay, the proliferation assay, the wound-healing and the colony formation assay. These phenotypic studies were carried out on a panel of four MPM cell lines (Mero-14, Mero-25, IstMes-2, NCI-H28) and one normal mesothelial cell line (Met-5A) following gene knockdown by siRNA. *GALNT7* depletion did not show any significant phenotypic change. Interestingly *SOD1*-silencing caused a statistically significant decrease of proliferation in Mero-25 and an increment of caspase activity in Mero-14 and NCI-H28 cells. Moreover the colony formation ability of Mero-25 cells was affected by *SOD1*-silencing showing a decrease of clonogenicity. Further studies are needed to ascertain the potential role of *SOD1* in MPM tumorigenesis suggested by our preliminary results.

P5.4
Expression of proline dehydrogenase in non-small cell lung cancer: immunohistochemical characterization and possible regulators

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Non-Small Cell Lung Cancer (NSCLC) is one of the most frequent cancers in developed countries and the main cause of cancer related deaths worldwide. The two main NSCLCs histotypes are adenocarcinoma (ADC) and squamocellular carcinoma (SCC). Proline dehydrogenase (PRODH) is a mitochondrial flavoenzyme that catalyses the key step in proline degradation and is involved in the regulation of cell survival, autophagy and apoptosis. We recently found extensive immunostaining for PRODH protein and high levels of transcript in the majority (70%) of lung ADCs. TTF-1 is a homeodomain-containing transcription factor essential for lung morphogenesis, differentiation and physiology. Based on their similar expression pattern in normal lung tissues (type II pneumocytes and Clara cells) and in NSCLC, their involvement in the same tumor or genetic pathologies and the presence of putative TTF-1 response elements in the PRODH gene we hypothesized that PRODH may be a novel TTF-1 transcriptional target. Preliminary experiments show that TTF-1 transfection indeed leads to PRODH upregulation in two ADC cell lines, warranting further investigations on the mechanisms underlying this regulation.

P5.5
cAMP-dependent kinase: an helper for cancer survival in stress environment

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Cancer cells often rely on glycolysis to obtain energy and support anabolic growth. Several studies showed that glycolytic cells are susceptible to cell death when subjected to low glucose availability. However, some cancer cells, including glycolytic ones, can efficiently acquire higher tolerance to glucose depletion, leading to their survival and aggressiveness. Although increased resistance to glucose starvation has been shown to be a consequence of signaling pathways and

compensatory metabolic routes activation, the full repertoire of the underlying molecular alterations remain elusive. Using omics analysis, we found that cAMP-PKA axis activation is fundamental for cancer cells resistance to glucose starvation and *anoikis*. Here we show that such a PKA-dependent survival is mediated by concurrent activation of autophagy and glutamine utilization that in concert concurs to attenuate the ER stress and to sustain cell anabolism. Importantly, both processes, together with a PKA-dependent activation of Src, actively participate also to protect cancer cells from *anoikis*. Our results reveal for the first time an important role of PKA in cancer cells survival under stress environment.

P5.6
S. cerevisiae as a tool to select inhibitors of the deneddylating activity of the COP9 signalosome

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The CSN complex plays a key role in various cellular pathways: through a metalloprotease activity of its Csn5 deneddylating enzyme, it regulates the activity of Cullin-RING ligases (CRLs). Indeed, Csn5 has been found amplified in many tumors, but, due to its pleiotropic effects, it is difficult to dissect its function and the involvement in cancer progression. Moreover, while growing evidences point to the neddylation function as a good target for drug development; specific inhibitors have not yet been developed for the CSN. Here, we propose the yeast *Saccharomyces cerevisiae* as a model system to screen libraries of small molecules as inhibitors of cullins deneddylation, taking advantage of the unique feature of this organism to survive without a functional CSN5 gene and to accumulate a fully neddylated cullin substrate. By combining molecular modeling and simple genetic tools, we were able to identify two small molecular fragments as selective inhibitors of Csn5 deneddylation function.

P5.7
Hyaluronic acid metabolism is important for the p63-dependent tumorigenesis

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p63 is a transcription factor belonging to the p53 family and its oncogenic isoform ΔNp63 plays a crucial role in squamous cell carcinomas survival and progression. By combining RNA-seq approach with co-expression studies in human primary tumors, we identified the hyaluronan synthase 3 (Has3) and the hyaluronidase1 (Hyal1) as novel ΔNp63 regulated genes. Has3 catalyzes the synthesis of hyaluronic acid (HA), while Hyal1 catalyzes the degradation of HA. In tumor cells, ΔNp63 directly induces Has3 expression and concomitantly represses the expression of Hyal1, resulting in the increase of the extracellular level of HA. In primary squamous tumor datasets, the expression of Has3 is positively correlated with that of p63. The interaction through HA and its receptor CD44 is important for chemoresistance by sustaining the activation of tyrosine kinase receptors and regulating the expression of ABC transporters. ΔNp63, through its action on HA metabolism, regulates tyrosine kinase receptors activation and ABC transporter expression, promoting thus tumor chemoresistance. In human tumors data sets, the positive correlation between p63 and Has3 expression or the negative correlation between p63 and Hyal1 expression, is a negative prognostic factor on patient survival, suggesting that the p63/HA signaling axis is an important determinant of the p63-driven tumorigenesis.

P5.8
Analysis of N6L interaction with nucleophosmin and its effect on AML cell linesAnalysis of N6L interaction with nucleophosmin and its effect on AML cell lines

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Nucleophosmin (NPM1) is a nucleolar protein implicated in ribogenesis and export, DNA damage response and response to stress stimuli. The *NPM1* gene is mutated in one third of acute myeloid leukemia (AML) patients. Mutations map to the C-terminal domain of the protein and lead to its aberrant cytoplasmic localization. N6L is a synthetic pseudopeptide that interacts with cell–surface nucleolin and inhibits cell growth. N6L also binds NPM1 and this could explain, at least in part, its toxicity. Here, we investigate the interaction between N6L and NPM1and show that N6L binds the N-terminal domain of the protein at two sites with high affinity. One of these sites overlaps with the NPM1 protein-protein interaction surface, as assessed by competition studies. We also analyzed the effect of N6L treatment in AML cells with mutated NPM1 (OCI-AML3) in comparison with cells bearing the WT protein (OCI-AML2). N6L is shown to be more effective in OCI-AML2, when administered alone. However, we also show that N6L strongly sinergizes with doxorubicin in inducing cell death in OCI-AML3 cells, suggesting that this compound might sensitize cells harbouring *NPM1* mutation to chemotherapeutic treatment.

P5.9
Itch/βarrestin2-dependent non-proteolytic ubiquitylation of SuFu controls Hedgehog signalling and medulloblastoma tumourigenesis

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Suppressor of Fused (SuFu), a tumour suppressor mutated in medulloblastoma, is a central player of Hh signalling, a pathway crucial for development and deregulated in cancer. Although the control of Gli transcription factors by SuFu is critical in Hh signalling, our understanding of the mechanism regulating this key event remains limited. Here, we show that the Itch/β-arrestin2 complex binds SuFu and induces its Lys63-linked polyubiquitylation without affecting its stability. This process increases the association of SuFu with Gli3, promoting the conversion of Gli3 into a repressor, which keeps Hh signalling off. Activation of Hh signalling antagonizes the Itch-dependent polyubiquitylation of SuFu. Notably, different SuFu mutations occurring in medulloblastoma patients are insensitive to Itch activity thus leading to deregulated Hh signalling and enhancing medulloblastoma cell growth. Our findings uncover new mechanisms controlling the tumour suppressive functions of SuFu and reveal that their alterations are implicated in medulloblastoma tumourigenesis.

P5.10
The p14ARF tumor suppressor promotes cell spreading and protects cells from anoikis

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p14ARF is among the most important tumor suppressors in humans. Nevertheless, recent studies show that ARF may possess a pro-survival function. While to achieve its tumor suppressor functions it has been shown to be required its nuclear localization, we show that during adhesion/spreading p14ARF localizes at focal adhesions where it interacts with the Focal Adhesion Kinase in several cell lines. We observe that knocking down ARF expression induces cells to acquire a round morphology accompanied by reduced pFak levels, defects in cellular spreading and actin cytoskeleton organization leading to apoptosis. While spreading defects are common to several cell lines, in H1299 cells, where the activated FAK expression level is very low, we do not observe cell death. These data suggest that the ARF involvement in adhesion/spreading does not depend on the level of FAK activation but rather relies on different ARF functions. Thus we propose two novel ARF functions: in cell morphogenesis and shape maintenance and in cell survival.

P5.11
The nucleolar localization signal (NoLs) of fbw7γ interacts with nucleophosmin N-terminal domain

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Nucleophosmin (NPM1) plays a number of roles in ribogenesis, centrosome duplication, DNA damage repair and response to stress stimuli. The NPM1 gene is the most frequently mutated one in acute myeloid leukemia (AML). Mutated NPM1 aberrantly translocates from nucleoli to the cytosol of AML blasts, due to the loss of its nucleolar localization signal and the acquisition of a novel nuclear export signal. NPM1 interacts with several protein partners and when its mutated counterpart translocates in the cytosol, a number of these partners are also delocalized and then degraded. Among them, Fbw7γ is a nucleolar E3-ubiquitin ligase controlling c-MYC levels. As a consequence of Fbw7γ degradation, an important tumor suppressor pathway is hampered in leukemic blasts and the c-MYC oncogene product is stabilized. Here, we characterized the NPM1-Fbw7γ interaction and identified key residues implicated in both proteins. We also propose a structural model for the interaction, which is coherent with experimental data. The NPM1-Fbw7γ interaction surface may be targeted for the treatment of AML with NPM1 mutations.

P5.12
STAT3/ERP57/TPX2 axis and process of "androgen escape" in prostate cancer

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The mechanisms of Prostate Cancer (PCa) progression through hormone-dependent to hormone refractory form is still unclear. Many data indicate that JAK/STAT signaling contributes to tumor resistance and STAT3 hyperactivation is observed in a variety of human cancers. Moreover, several authors suggested that ERp57 (GRP58/PDIA3), a disulfide isomerases protein, is associated with modulation of STAT3 activity. We investigate the role of STAT3-ERp57-TPX2 axis in the hormone-responsive and androgen-refractory tumor using human PCa cell lines, LNCaP (androgen-sensitive) and PC3 (androgen-refractory), untreated and stimulated with IL-6 and EGF. Immunoblotting and CoIP analysis were performed to confirm STAT3 activation and ERp57-STAT3 interaction. To investigate the physiological relevance of STAT3-ERp57-TPX2 axis, we inhibited STAT3 or ERp57 activity and the expression levels of TPX2 was monitored by qRT-PCR. The results showed that increased STAT3-ERp57 complex association determines an TPX2 overexpression. In conclusion, this study showed that STAT3-ERp57-TPX2 axis is correlated with tumor progression and it suggests a functional role of STAT3/ERp57 complex in the Androgen Escape.

P5.13
ID4-driven cross-talk between breast cancer cells and tumor-associated macrophages

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ID4 is a member of ID family of proteins (Inhibitors of Differentiation, ID1 to 4) that act as dominant negative regulators of bHLH transcription factors. We previously reported that ID4 is transcriptionally regulated by mutant-p53 in breast cancer (BC) contributing to an enhancement of angiogenesis by the regulation of proangiogenic cytokines and by the induction of new microvessels formation. Numerous studies have shown that the tumor microenvironment and Tumor Associated Macrophages (TAMs) are important for regulating the process of angiogenesis in BC. We here investigated the role of ID4 in the crosstalk between BC cells and TAMs and its contribution to the angiogenic pathway activation in macrophages (Mφ). We first demonstrated by migration assay that ID4 expression in BC cells plays a central role in promoting migration of Mφ *in vitro* and enhancing Mφ recruitment *in vivo*. We also analyzed 62 triple negative BC (TNBC) cases from Italian National Cancer Institute Regina Elena by Immunohistochemistry, observing a significant correlation between the expression of ID4 protein and the human macrophage marker CD68. Angiogenesis-related genes and microRNA expression changes were observed in Mφ co-coltured with BC cells depleted or not of ID4 expression. In conclusion, we demonstrated that ID4 expression in BC cells promotes recruitment of macrophages and enhances Mφ angiogenic potential.

P5.14
Role of the estradiol in the development of papillary thyroid carcinoma in women

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Sex hormones should have an important role in papillary thyroid carcinoma (PTC) given the higher incidence in women than in men. On the basis of a GWAS carried out by our laboratory in collaboration with DKFZ, we evaluated the effect of estradiol (E2) on the expression of selected genes associated with the risk of PTC in women. 12 genes bearing the highest number of SNPs associated with the risk of PTC in women (OR>1), were selected. The NTHY-ORI3 cell line was cultured in a medium lacking hormones and treated with E2. Candidate genes silencing, was performed with siRNA before and after E2 treatment. Cell viability, apoptosis and cell cycle, were evaluated. Each experiment was carried out in triplicate, and its significance was assessed by analysis of variance one-way ANOVA and t-test. *ATG5*, *TCF4* and *CD83* showed an increase in expression after 24 and 48 h of E2 (1uM) treatment ($p<0.03$). Cell viability increased after *CD83* silencing (140% $p=0.05$) and decreased after *ATG5* and *TCF4* silencing (50%, $p<0.05$) in combination with E2. *In silico* studies (Dragon ERE finder) showed no estrogen-

responsive element in the promoter. *ATG5*, *TCF4* and *CD83* could have a role in the response to E2 in the aetiology of PTC.

P5.15
Skeletal muscle atrophy: a role for the receptor subtypes specific for sphingosine 1-phosphate

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Skeletal muscle (SkM) atrophy, caused by several and heterogeneous conditions, such as cancer (cachexia), neuromuscular disorders and aging, is characterized by protein synthesis suppression, protein degradation and expression of atrogenes, such as the ubiquitin ligase Atrogin-1/MAFbx. Sphingolipids represent a class of bioactive molecules capable of modulating the destiny of many cell types, including SkM cells. In particular, sphingosine 1-phosphate (S1P), formed by sphingosine kinase (SphK), is able to act as trophic and morphogenic factor in myoblasts. Here, we report that the inhibition of SphK1 drastically reduced myotube size and increased Atrogin-1/MAFbx. Reduction of active SphK1 was also observed in muscle fibers obtained from cachectic mice inoculated with C26 adenocarcinoma. In addition,we found that SkM atrophy was accomplished by changes in the pattern of expression of S1P receptor subtypes (S1P1,2,3) indicating a crucial role played by the downstream signalling triggered by S1P1 and S1P2 in SkM atrophy. These findings provide the first evidence that S1P/SphK1/S1PR axis acts as a molecular regulator of SkM atrophy, thereby representing a new possible target for therapy.

P5.16
Expression of the FGFR2c mesenchymal splicing variant in human keratinocytes inhibits differentiation and promotes invasion

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The altered isoform switching of the fibroblast growth factor receptor 2 (FGFR2) and aberrant expression of the mesenchymal FGFR2c isoform in epithelial cells induces epithelial-mesenchymal transition (EMT). Here we analyzed the effects of the forced expression of FGFR2c on human keratinocyte differentiation and stratification. Phase contrast immunofluorescence and western blot approaches demonstrated that, differently from cells overexpressing the epithelial variant FGFR2b, keratinocytes ectopically expressing FGFR2c are not able to form a monolayer, as a consequence of the changement in epithelial morphology and growth mode, and display decreased expression of the early differentiation markers keratin 1 (K1) and desmoglein-1 (DSG1). This impaired ability to enter the early differentiation program is related to an increased expression of the transcription factor ΔNp63. In addition, FGFR2c-expressing keratinocytes undergo defective stratification and invasion of the collagen matrix in 3D organotypic cultures, further supporting the hypothesis that the appearance of the mesenchymal FGFR2c variant in the epithelial context would drive the early steps of cancerogenesis.

P5.17
Functional cooperation between p53 and MYC in cancer-associated cell competition

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Cell competition is a mechanism conserved from *Drosophila* to mammals, based on the comparison of relative fitness between neighbouring cells, leading to the apoptotic elimination of the weaks. Several molecules are involved in these competitive interactions: in particular, cells expressing high levels of MYC grow at the expense of surrounding cells. In *Drosophila* epithelia, MYC-Mediated Cell Competition (MMCC) selects cells undergoing clonal expansion, and p53 function is necessary in MYC-overexpressing cells to sustain their competitive advantage. Malignant cells often upregulate MYC and results obtained in our lab suggest that MMCC can shape tumour expansion and evolution. p53 is one of the most frequently mutated genes in human cancers, but the function of its several mutant products and dominant negative forms is not clear. Through IHC analysis on several kinds of human carcinomas, *in vitro* co-culture assays and *Drosophila* experiments, we observed that loss of p53 in the winner cells is sufficient to make them unable to grow and out-compete the neighbours, thus suggesting a functional cooperation between MYC and p53 in cancer-associated MMCC. Our results show an oncogenic side of the p53 *wild-type* protein that appears to help shape cancer progression through selection of the most competitive cells.

P5.18
Distinct roles of v-Jun:ATF and v-Jun:Fos dimers in skeletal muscle differentiation

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The Jun oncoprotein is the major component of the transcriptional complex AP-1, which is involved in several cell functions. Constitutive activation of AP-1 is required for cell transformation, and also occurs in distinct human tumor cells. Jun is able to dimerize with different partners including Fos family members and ATF proteins providing AP-1 with high flexibility in gene regulation and functions. Particularly, by using mutants selective for Fos proteins or ATF2-like proteins, it has been demonstrated that v-Jun-induced transformation phenotype consists of, at least, two distinct, complementing genetic programs. It has been long known that transformation of myogenic cells by v-Jun, as well as by activated c-Jun, prevents terminal differentiation. In order to better dissect the transformation activity exerted by Jun in myogenic cells, C2C12 cells were infected with retroviral vectors expressing v-Jun mutants selective for Fos proteins or ATF2-like proteins. The analysis of the different cell population revealed opposite phenotypes induced by the different mutants. Data supporting the major role of v-Jun:ATF dimer in the inhibition of terminal differentiation will be presented.

P5.19
MYC ectopic expression establishes a precancerous field leading to multifocal lesions in a *Drosophila* epithelial model

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The term "field cancerisation" describes a precancerous area in which genetically altered but histologically normal tissues proceed towards the development of multiple malignant foci. In the early stages of tumour progression cells may indeed acquire genetic damages that allow them to proliferate, gradually replacing normal tissue. This mechanism invokes MYC-mediated cell competition (MMCC), a phenomenon characterised in *Drosophila* consisting in fitness confrontation between cells sharing the same tissue, with cells expressing high MYC levels ultimately killing and replacing cells showing lower MYC activity. These features make it a candidate mechanism pioneering field cancerisation. Here we mimic field formation by upregulating MYC in a territory of the larval wing epithelium of *Drosophila*. Analysis of specific markers usually found in mammalian precancerous areas confirmed that MYC upregulation is sufficient to trigger specific cellular responses. Moreover, MYC-expressing fields were susceptible to the development of multifocal tumours upon induction of different second mutations, a typical trait correlated to mammalian field cancerisation. In summary, our study identified an undescribed early genetic change implicated in field cancerisation and established a genetically amenable model which may help study the molecular basis of the initial tumourigenic events.

P5.20
MYC-containing double minute chromosomes: origin, structure and impact upon transcriptome in acute myeloid leukemia patients

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Despite the emergence of double minutes (dmin) harboring amplifications was explained through chromothripsis, our recent results excluded it in tumor cell lines with *MYC*-dmin. In addition, *MYC* is not overexpressed when amplified in leukemia, suggesting a nonlinear correlation between amplification and overexpression. By integrating DNAseq, SNP array, RNAseq, FISH and PCR, we reconstructed the internal structure of head-to-tail amplicons in 23 leukemia cases with *MYC*-dmin, evaluated chromothripsis, identified fusion transcripts involving 8q24 amplified genes, and performed differential expression analysis of cases with and without dmin. Our bioinformatics analyses led us to exclude chromothripsis as the driving force underlying amplicon genesis in our samples. In the 55% of the cases, amplicons were deleted in one of the two chromosome 8 homologs, suggesting an excision from the original chromosomal location. Moreover, dmin were accompanied by novel 8q24 fusion transcripts, sometimes recurrent in a few cases and with multiple transcript isoforms, although not corresponding to genomic rearrangements originating fusion genes. This result clearly suggests the involvement of post-transcriptional events that could lead to a dramatic remodeling of transcriptome. In this view, a possible impact of these RNA entities upon *MYC* expression pattern needs to be further explored.

P5.21
Chromothripsis and genomic amplifications behind a case of aggressive AML-MCR

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Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) is a heterogeneous disorder defined by morphologic, genetic, and clinical features. Patients with AML-MRC have a higher frequency of unfavourable cytogenetics, generally associated with poor prognosis. Here we describe a case of AML-MRC showing a complex karyotype and a poor outcome. By combining FISH, SNP array and exon array, we disclosed chromosomes 11q, 19q and 22q amplifications mapping on a marker chromosome resulting in overexpression of several oncogenes, including *MN1*. Moreover, we unveiled a chromothripsis event accompanying a complex t(5;7) translocation, leading to the deletion and downregulation of several tumor suppressor genes. The differential gene expression profile analysed by IPA disclosed the significant inactivation of Wnt pathway, already described in MDS-MSCs. To the best of our knowledge, the one reported here is the first case of AML-MRC harboring a chromothripsis rearrangement. Our results suggest that several mechanisms might cooperate to originate the complex karyotype observed in AML-MRC, leading to the deregulation of specific cancer related genes.

P5.22
TRIM8 restores p53 tumour suppressor function through quenching of N-MYC activity and blunts tumorigenic potential and chemo-resistance in Renal Carcinoma

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Clear cell Renal Cell Carcinoma (ccRCC) represents the most common and aggressive subtype of renal cancers and is characterized by high chemo- and radio-resistance. Inactivation of the p53 tumour suppressor network seems to be a hallmark of this cancer although the gene is rarely mutated. We have previously demonstrated that ccRCC expresses reduced levels of TRIM8, a key player in controlling the p53 molecular switch that sustains the transcriptional activation of cell cycle arrest genes and the response to chemotherapeutic drugs. Here we report that miR-106b-5p and miR-17-5p up regulation, whose expression is promoted by the oncoprotein N-MYC, directly inhibit the expression of TRIM8 in ccRCC cancer cells causing chemoresistance. We mechanistically demonstrate that upon inhibition of miR-17-5p and/or miR-106b-5p, ccRCC cells recover the p53 tumour suppressor activity in a TRIM8-dependent fashion and turn off the oncogenic action of N-MYC resulting in the reduction of cells proliferation. The block of cell proliferation in human tumour xenografts overexpressing TRIM8 provides conclusive evidence of its pivotal role in controlling tumour growth. We conclude that the abrogation of TRIM8 function, which in turn shuts off p53 activity and switches on N-MYC, leads to an uncontrolled cell proliferation responsible for renal cancer progression and chemo-resistance.

O5.1
CSB ablation induced apoptosis is mediated by increased endoplasmic reticulum stress response

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The DNA repair protein Cockayne syndrome group B (CSB) has been recently identified as a promising anticancer target. Suppression, by antisense technology, of this protein causes devastating effects on tumor cells viability, through a massive induction of apoptosis, while being non-toxic to non-transformed cells. To gain insights into the mechanisms underlying the pro-apoptotic effects observed after CSB ablation, global gene expression patterns were determined, to identify genes that were significantly differentially regulated as a function of CSB expression. Our findings revealed that response to endoplasmic reticulum stress and response to unfolded proteins were ranked top amongst the cellular processes affected by CSB suppression. The major components of the endoplasmic reticulum stress-mediated apoptosis pathway, including pro-apoptotic factors downstream of the ATF3-CHOP cascade, were dramatically up-regulated. Altogether our findings add new pieces to the understanding of CSB mechanisms of action and to the molecular basis of CS syndrome.

O5.2
ERAP1 is a novel drug target in the oncogenic Hedgehog signaling pathway

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Endoplasmic reticulum aminopeptidase ERAP1 is essential for the maturation of a wide spectrum of peptides and is involved in several biological functions such as antigen processing, cytokine receptor shedding and neo-angiogenesis. ERAP1 enzymatic activity contributes to the pathogenesis of several major human diseases ranging from infections to autoimmunity and cancer. Here, we show a new biological role for ERAP1 in a not-immune mediated control of cancer and identify ERAP1 as a novel activator of the Hedgehog signaling, an essential pathway in both development and tumorigenesis. Mechanistically, we demonstrate that inhibition of ERAP1 impairs the Hedgehog signaling leading to an increased E3-ubiquitin ligase β-TrCP1 protein levels, which results in higher levels of the repressor form of the transcription factor Gli3. Notably, both pharmacological and genetic inhibition of ERAP1 reduce Hedgehog-dependent tumor cells growth *in vitro* and in allograft and xenograft medulloblastoma model *in vivo*. These data identify a novel molecular mechanism in the regulation of Hedgehog signaling and strongly support ERAP1 as a novel drug target in this oncogenic pathway.

O5.3
Role of STAT3 in the cross-talk between cancer associated fibroblasts and cancer cells

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Tumor interactions with stromal cells, including Cancer Associated Fibroblasts (CAFs), promote tumor progression enhancing EMT, tissue invasion, dissemination, metastasis, drug resistance and relapse. The pro-oncogenic transcription factor STAT3 is frequently constitutively active in both tumor and stromal cells, including CAFs, often as part of a feed forward loop with the pro-inflammatory cytokine IL-6. We therefore decided to assess the role of STAT3 in regulating CAF-tumor cross-talk and in promoting and maintaining CAFs' activated state. We established an *in vitro* model based on the exposure of cancer cells to the conditioned medium derived from CAFs silenced or not for STAT3, followed by the assessment of cell migration, invasion, proliferation, anchorage independent growth and extravasation. CAF-conditioned medium enhances all these pro-oncogenic features in a STAT3-dependent way. Differential gene expression analysis revealed several target genes encoding for secreted proteins, potentially involved in mediating the STAT3-dependent effects of CAF-CM, which are being functionally analyzed in primary and immmortalized CAFs.

O5.4
MYC-mediated cell competition as an evolutionary trait of cancer

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MYC-Mediated Cell Competition (MMCC) is a phenomenon of fitness comparison occurring between adjacent cells showing different levels of MYC activity. It describes a mechanism, conserved from *Drosophila* to mammalian development, through which cells characterised by high expression of MYC induce apoptotic death of neighbouring low MYC-expressing cells and acquire an advantage in space occupancy. Though it is widely speculated that this phenomenon is relevant to cancer, its characterisation during tumour progression is still missing. Here we show the presence of markers of MMCC in human carcinomas and demonstrate through experiments in human cancer cell lines that MYC modulation is *per se* sufficient to induce competitive behaviours in both genetically distant and identical cells. Noteworthy, MYC inhibition in the fittest cell line is sufficient to reverse its competitive *status*. Moreover, data obtained in a *Drosophila* cancer model indicate that MMCC is normally at work during tumour growth and that induction of high or low-MYC expressing cells in the growing masses deeply alters the final tumour size, supporting a role for MMCC in cancer evolution.

O5.5
Human lung adenocarcinoma cell cultures derived from malignant pleural effusions as model system to predict patients chemosensitivity

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Lung cancer is the leading cause of cancer related deaths and Malignant Pleural Effusion (MPE) is a frequent complication. Current therapies are inefficacious in a great percentage of cases, especially at late stages. Moreover, patients' responses vary and the outcome is unpredictable. Therefore, it is essential to identify patients who will benefit most of chemotherapy treatment. In this study, using malignant pleural effusions (MPE) from non-small cell lung cancer (NSCLC) patients, we established and characterized 16 primary cultures of patient-derived adenocarcinoma cultures for their sensitivity to chemotherapeutic drugs and their mutational pattern for most common driver mutations in lung cancer; in addition, we established some patient derived xenografts (PDX) in rag2/IL2 knock-out mice. For six patients we found a correlation between drug response in vitro and response to therapy in the clinic. Drug response and mutation profile highlight the heterogeneity of NSCLC in advanced stages, and this strategy may provide a potentially useful approach for evaluating individual pharmacogenomic profile and tailor the therapy accordingly. Furthermore, extensive characterization of MPE-derived primary cultures may support the identification of novel targets for late stage NSCLC.

6 - Plant Metabolism and Environmental Stress

P6.1
Reduction of the geomagnetic fields (GMF) delays flowering time in *Arabidopsis thaliana*

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The GMF influences plant biological processes and has been suggested to play a role in plant evolution (Occhipinti et al., 2014, *Trends Pl. Sci*., 19:1-4). In *Arabidopsis thaliana*, near-null magnetic field (NNMF, <100 nT) causes a delay in the transition to flowering, but the expression of genes involved in this response has rarely been studied. To investigate this aspect, we built a triaxial-Helmholtz coils system and grew *A. thaliana* Col 0 under NNMF and GMF conditions. We evaluated NNMF time-course effects on *Arabidopsis* morphology and qPCR expression of 25 rosette and meristem flowering-involved genes. NNMF significantly reduced leaf area index and stem length. Under NNMF, flowering delay was retained in generations experiments. Down-regulation of *FT* and *Ga20ox* (involved in flowering promotion) and *FLC* (encoding a flowering repressor) in the flowering meristem and *TSF* (encoding a floral inducer), *FLC* and *Ga20ox* in the rosette correlated to flowering delay. Our results confirm the influence of GMF variations on plant flowering time and support the stimulating hypothesis of GMF involvement in plant evolution during paleosecular GMF variations (Maffei, 2014, *Front. Pl. Sci*. 5:445).

P6.2
Alternative electron transport pathways around Photosystem I in *Physcomitrella patens*, driving forces in terrestrialization process

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Alternative electron transport pathways around photosystem I (PSI) control the ATP/NADPH ratio, avoid electron transport chain over-reduction in the chloroplast and the formation of reactive oxygen species. Flavodiiron proteins (FLVs) can redirect electrons toward oxygen reduction and water production. FLVs are distributed among different groups of photosynthetic organisms but they are absent in flowering plants. In order to understand the role of alternative electron transports in the early stages of land colonization process, we generated *Physcomitrella patens flv* knock-out mutants. FLVs were extremely active as electron sinks downstream of *P. patens* PSI. Electrochromic changes measured in intact plants of *P. patens* showed that FLVs are responsible for a large fraction of the transported electrons in the first seconds after a sudden increase of light intensity. If exposed to fluctuating light, *flv* mutants showed dramatic light sensitivity, PSI photoinhibition and impaired growth compared to the wild-type. It appears that before natural selection, different alternative electron transport strategies were present and have steered the evolution of photosynthesis in early land plants.

P6.3
Profiling volatile terpenes and the expression of terpene sythase genes in conifers from Calabria forest stands

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Forest ecosystems have recognized roles at national and international scale for environmental protection and for the development of sustainable economies. The "ALForLab" Project, co-funded by the National Operational Programme for Research and Competitiveness 2007-2013, aims at developing and integrating innovative technologies for improving mobilization and utilization of forest resources in the Calabria region, as well at monitoring and preserving the environmental services they provide. Terpenes and terpenoids maybe important descriptors of the ecophysiological status of forest stands, because they not only are essential for plant growth and development, but also play fundamental roles in plant-environment interactions, among which attraction of pollinators, protection against photooxidative stress, mediating thermotolerance, and defense against microbes and insects. Here, we preset emission profiles of volatile terpenes from conifers [*Pinus nigra* Arn. ssp laricio (Poir.) Maire, Austrian pine, and *Abies alba* Mill., European silver fir] populating Calabria forests, matched with isolation, characterisation and expression analysis of genes coding for terpene synthases

P6.4
Changes in gene expression and metabolic responses in tomato plants (*Solanum lycopersicum* cv. Micro-Tom) treated with a new biostimulant

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The effects of EXPANDO®, a new biostimulant containing different bioactive compounds and developed mainly for fruit growth were investigated both in greenhouse and open field. Preliminary agronomic trials on different crop species showed an enhancement in plant growth and an increase in final yield. Since there was no information available at transcriptomic level, a microarray analysis was perfomed to investigate gene expression changes in control and EXPANDO®-treated tomato plants (*Solanum lycopersicum* cv. Micro-Tom) grown in a growth chamber. A preliminary analysis of the microarray data showed that EXPANDO® was able to modulate the expression of about 4,000 genes (>1,700 up-regulated and >2,000 down-regulated) involved in several biological processes like transcription, stress responses, signal transduction, carbohydrate metabolism, transport, protein metabolism and secondary metabolism. Moreover, to investigate the metabolic responses of tomato to treatment, several biochemical parameters were evaluated in control and treated plants. In general, a good correlation was found between the expression level of ROS scavenging enzyme genes and the corresponding enzymatic activity.

P6.5
AtCuAOδ participates in abscisic acid-induced stomatal closure in Arabidopsis

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Copper amine oxidases (CuAOs) catalyse the oxidation of polyamines to aminoaldehydes, producing ammonia and hydrogen peroxide (H_2O_2). CuAOs are induced by stress-related hormones such as methyl-jasmonate and abscisic acid (ABA). In this study, we have analysed the role played by an Arabidopsis CuAO, AtCuAOδ, in the ABA-mediated stomatal closure. ABA induced *AtCuAOδ*-gene expression at two different concentrations (10μM, 100μM). Moreover, ABA-induced stomatal closure was analysed in *atcuaoδ* T-DNA insertional mutant lines. Under physiological conditions no differences between WT and mutants were observed, while mutants were less responsive to ABA-induced stomatal closure. Treatment with the H_2O_2 scavenger *N,N'*-dimethylthiourea reversed in part the ABA-induced stomatal closure in WT plants. Moreover, H_2O_2, normally detected in guard cells of WT plants upon ABA treatment, was absent in guard cells of ABA-

treated mutants. *AtCuAOδ* over-expressing plants showed an increase in stomatal closure level along with a detectable H_2O_2 production in guard cells under normal growth conditions. These data suggest that AtCuAOδ may play a role as H_2O_2 source in ABA-induced stomatal closure.

P6.6
Population traits shape the elevation effect on non-structural carbohydrates (NSC) and flavonoids of *Vaccinium myrtillus* stands in Alpine tundra

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Environmental stressors induce plants to acclimate by both morphological and physiological modifications. Along an elevation gradient (as a proxy for temperature stress), we demonstrated that changes of two ecophysiological stress indicators (NSC in underground stems and leaf flavonoids) of *Vaccinium myrtillus* were affected not only by altitude, which, in some cases, acts as secondary player. In particular, plant traits (age, width of xylem rings, length of stem shoot) of the populations and interspecific competition (shrub density) can shape the effect of elevation. Glucose content was positively correlated with altitude, but negatively with competition. Instead, sucrose decreased at high altitude and in older populations. Starch content increased along with ring width and decreased with high shrub density. Flavonoid content was mainly related to elevation and plant trait of the population. NSC and flavonoids exhibit different patterns with respect to elevation and plant traits. In conclusion, we suggest that bulk NSC can not be considered as indicators of stress and that plant traits would represent modulators of species response.

P6.7
Expression Analysis of Stress-Related genes involved in the response of Durum wheat to salinity and high light

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The study of stress-related genes is critical to understand the molecular mechanisms of stress tolerance in plants. Several studies have demonstrated the important role of asparagine synthetase, glutamate decarboxylase and Δ^1-pyrroline-5-carboxylate synthase genes signalling in response to environmental stresses. In order to investigate the expression changes of these genes in durum wheat under salinity and high light, a semi-quantitative RT-PCR analysis was performed. High light increased the gene expression level of *TdAsn1* alone (2.6 fold) and in combination with salinity (2 fold) in comparison with the control at low light. The isoforms *TdAsn2* was expressed at low levels compared to *TdAsn1* and the transcript was present only in leaves in control conditions or simultaneous stresses . A trend similar to that of *TdAsn1* was observed for *P5CS* expression. On the contrary, *GAD* expression was decreased by salinity and high light (1.1 and 1.5-fold, respectively) and even more under the two combined stresses (3.7 fold) compared to control. The expression levels were compared to the respective enzymatic activities. Our expression data confirmed the pivotal role of the studied genes in the response to abiotic stresses in *durum wheat.*

P6.8
Metabolic engineering of the phenylpropanoid pathway in *S. lycopersicum* using CRISPR/Cas9 mediated genome editing

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CRISPR/Cas9 system is a powerful tool enabling efficient and precise genome editing in many organisms and with many applications in several fields, including metabolic engineering. In plants one of the most important secondary metabolic pathways is the phenylpropanoid pathway that produces many nutraceutical compounds. To modify this pathway, we edited the *SlHQT* gene involved in the biosynthesis of caffeoylquinic acids (CQAs), the most abundant polyphenols in many plant species. We induced mutagenesis of the *SlHQT* gene at two different positions directing the cas9 cut in the *SlHQT* genomic sequence. Undertaking full genotypic analysis of a large number of T0 transformed tomato plants, we observed a very high mutation frequency but also considerable variability in terms of the number of alleles and types of mutations in any one plant; this variability correlated very well with the CQA accumulation. Here we show that the CRISPR/cas9 system can be used successfully for metabolic engineering of the phenylpropanoid pathway affecting the caffeoylquinic acid biosynthesis and possibly altering the production of other phenolic compounds with pontential impacts on nutritional value.

P6.9
Subcellular localization analyses of a *Hordeum vulgare* P2-G6PDH isoform by transient expression of reporter fusion proteins

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In plants different glucose 6-phosphate dehydrogenases (G6PDH) exist. Among them, plastidic P2 isoforms are considered an essential source of NADPH in heterotrophic plastids. Barley P2-G6PDH displays an unusually long plastidic transit peptide (>95aa), considering these sequences generally comprise less than 60aa. We investigated the subcellular localization of *Hv*P2-G6PDH reporter fusions in Arabidopsis protoplasts and tobacco leaves; specifically, whether this protein may be directed to heterotrophic plastids only, or to other compartments as well. The results obtained in protoplasts suggest that *Hv*P2-G6PDH with GFP fused to the C-terminus may localize to chloroplasts. However, upon Agrobacterium infiltration, the fusion protein was detected in heterotrophic plastids of the epidermis and in the cytosol of mesophyll cells. Interestingly, with GFP fused to the N-terminus, the fusion protein partially co-localised with peroxisomes, indicating presence of a peroxisomal targeting signal. Moreover, initial co-expression experiments suggest interaction with inactive *Hv*P0-G6PDH. Further studies are needed to confirm the P2-P0 interaction, and to specify localization of the heterodimers.

P6.10
Transgenerational responses to nitrogen deprivation in *Arabidopsis thaliana*

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Nitrogen deprivation represents one of the major stresses to which plants are exposed. As plants respond to this stress, it would be profitable to retain a *memory* of the response to prime following generations. Therefore, an experimental setup considering successive generations of control or stressed *Arabidopsis* plants was used to evaluate the establishment of a transgenerational memory of N-deficiency stress response. Results demonstrated a faster induction of a high nitrate uptake capability as a result of multigenerational stress exposures. This behaviour was paralleled by changes in the expression of nitrate responsive genes. Transcriptional analyses revealed an enduring modulation of genes in downstream generations, despite the lack of stress stimulus in these plants. Using genome-wide detection of DNA methylation we could

show a correlation between stress treatments and DNA methylation changes. This suggests the involvement of epigenetic responses, while the mechanism underlying the maintenance of expression changes in the progeny of stressed plants remains elusive and compels further investigation.

P6.11
Cyclic AMP deficiency negatively affects thermotolerance by altering redox homeostasis

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Heat stress (HS), affecting different physiological, biochemical and transcriptional pathways can have deleterious effects on plants. To cope with high temperatures, plants have a number of defense mechanisms, including the synthesis of heat shock proteins (HSPs) and the maintenance of an opportune redox balance. Cyclic AMP is involved in the acquisition of thermotolerance; in Arabidopsis, cAMP increases during HS and activates CNGC6, inducing Ca^{2+} influx and HSPs expression. To get more insight into the role of cAMP in HS response, tobacco BY-2 cells have been transformed with the "cAMP-sponge", a genetically encoded tool, able to selectively bind cAMP. The cAMP content in the transgenic (cAS) lines is lower than in WT lines. At optimal temperature, inhibition of cell division and enhancement of antioxidants occur in cAS cells, suggesting that cAMP deficiency is sensed as a stress condition. HS inhibits cell division both in WT and cAS cells. However, cAMP deficiency makes BY-2 cells more susceptible to HS, determining an increase in cell death. The failure in the control of redox homeostasis in response to HS seems to be the cause of the low thermotolerance in condition of cAMP deficiency.

P6.12
Glutamate dehydrogenase isoenzyme 3 (GDH3) of Arabidopsis thaliana is less thermostable than GDH1 and GDH2 isoenzymes

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NAD(H)-glutamate dehydrogenase (GDH; EC 1.4.1.2) is an abundant and ubiquitous enzyme that may exist in different isoenzymic forms. Variation in the composition of the GDH isoenzyme pattern is observed during plant development and specific cell, tissue and organ localization of the different isoforms have been reported. However, the mechanisms involved in the regulation of the isoenzymatic pattern are still obscure. In *Arabidopsis thaliana*, three genes (*GDH1*, *GDH2*,*GDH3*) encode three different GDH subunits (β, α and γ) that randomly associate to form a complex array of homo- and hetero-hexamers. In order to asses if the different *Arabidopsis* GDH isoforms may display different structural properties we have investigated their thermal stability. Differences among the various GDH isoforms were observed. In particular, the γ subunit containing isoforms were less stable than the α or β containing isoforms. The stability of GDH1 (6β) and GDH3 (6γ) isoenzymes was then studied using site-directed mutagenesis in a heterologous yeast expression system. It was established that the carboxyl terminus of the GDH subunit is involved in the stabilization of the oligomeric structure of the enzyme

P6.13
Ultrastructural and physiological changes in barley seedlings exposed to cadmium

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Cadmium pollution is one of the main menaces for the natural ecosystems, because its relatively high solubility, and its strong toxicity for the living organisms. Barley (*Hordeum vulgare*) is one of the first 5 crops cultivated worldwide, and it is particularly resistant to abiotic stress. Therefore, barley plants could represent an interesting model in the evaluation of cadmium accumulation in a widely cultivated crop. The effects of exposition for 7 days at different Cd levels were analysed on barley seedlings. Morphological, and TEM - SEM analyses revealed a massive damage to leaf chloroplasts. Cadmium exposed roots show irregular vacuoles and damaged endomembrane system structure. Physiological and biochemical analyses were related to leaf water content, ultrastructural damage, measurements of photochemical indices, content in pigments and starch accumulation. Nitrate reductase activity, proline and Heat Shock Protein 70 levels suggested a dose-dependent effect of Cd. Finally, the cadmium change root structure by increasing the number of lateral roots. In conclusion, our data suggest that barley can be used as a useful tool to assess and monitor pollution by cadmium.

P6.14
Comparison of functional properties of δ^1-pyrroline-5-carboxylate reductases from plants and bacteria

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Besides being needed for protein synthesis, a wide array of plants and microorganisms accumulate high intracellular levels of free proline to cope with osmotic stress conditions. Proline production is tightly regulated at both the transcriptional and the translational levels, yet the mechanisms for post-translational regulation of the enzymatic activities involved have not been fully elucidated. Two main routes lead to proline synthesis using glutamate or ornithine as the precursor, respectively. These two pathways share the terminal step, the conversion of δ^1-pyrroline-5-carboxylate (P5C) to L-proline, catalyzed by P5C reductase (EC 1.5.1.2). The genes coding for P5C reductase in *Bacillus subtilis*, *Streptococcus pyogenes*, *Arabidopsis thaliana*, *Oryza sativa* and *Medicago truncatula* were isolated and expressed in *E. coli*. The functional properties of the affinity-purified proteins were characterized and compared, with special emphasis on the use of NADH or NADPH as the electron donor and the occurrence of inhibitory or stimulatory effects by increasing concentrations of products (proline, NAD^+ and $NADP^+$), cations and anions. This work was supported in part by AGER, grant # 2010-2369.

P6.15
Salt tolerance in an Italian rice variety is provided by rapid and specific stress responses

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Soil salinity is a major constraint for crop production overall the world and rice is the most sensitive to salt among cereals. To identify mechanisms involved in salt tolerance, we compared the response of two contrasting Italian rice genotypes at physiological and molecular level. Upon salt stress the susceptible cultivar, Vialone Nano, showed growth arrest and leaf yellowing, due to Na^+ accumulation in leaves leading eventually to a drop in photosynthetic efficiency. The tolerant variety, Baldo, sacrificed the oldest leaves, excluded Na^+ from the new leaves and resumed growth after two days. This variety seems to react promptly to salt stress by stopping growth and closing stomata rapidly. Therefore, these plants put in place an adaptation programme by changing root architecture and activating a NPQ response in new leaves. A quick Na^+ compartmentalization in root vacuoles along with changes

in hormonal levels seemed to play an essential role in the regulation of this adaptive behaviour. In fact, genes involved in osmosensing and Na^+ translocation in vacuoles showed expression profiles peculiar of the tolerant plants and might be considered as markers of salt tolerance.

P6.16
Glutathione-Ascorbate cycle and lipid peroxidation in fruit of sweet cherry landraces of Campania region (Italy)

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Fresh fruits are living organs that continue their metabolism even after harvest. The advanced stages of ripening culminate into the senescence process, that eventually leads to the death of fruit. Several works highlight the importance of antioxidant metabolism in relation to the fruit quality and shelf-life: fruit ripening is accompanied by a progressive increase in oxidative stress that is controlled by a related induction of the antioxidant scavenging systems. Dysfunctioning of such systems in the later stages of ripening causes an increase of oxidation, that is among the most important factors of fruit decay, favouring also parasite attack and development. High antioxidant metabolite levels, in fact, could delay senescence and preserve nutritional and nutraceutical characteristics, significantly reducing fruit loss and cost. Sweet cherries fruits are excellent sources of phytochemicals: nutraceuticals and antioxidants. It has been demonstrated that the eating of cherries reduces the risk of cancer and the joint pains, and protects from cardiovascular and neurodegenerative diseases. The aim of this work was to characterize glutathione-ascorbate cycle as well as lipid peroxidation in mature fruits of the sweet cherry germoplasm of Campania region and their involvment in post harvest storage. Fruits from cherry landraces of Campania region were collected at commercial maturity and used for the analyses. Glutathione as ascorbate contents differed among the landraces as welle as glutathione reductase. Differences were also found in the lipid peroxidation activities using the MDA test. . the data of glutathione level and redox state and gluthione peroxidase, ascorbate level and redox state as ascorbate peroxidase activities, tocophrols and polyphenols, two groups of landraces have been evidenced. The first showed high polyphenol oxidases activities, that could indicate a higher risk of developing oxidative stress and, consequently, a higher susceptibility to the oxidative degradation during shelf-life. The second showed high ascorbic acid and tocopherols contents, and low polyphenol oxidase activities. The high metabolites concentration could reduce the risk of oxidative damages during storage, therefore they could show a longer shelf-life than the other tested fruits. These characteristics were probably due to endogenous characteristics, making these landraces particularly interesting for breeding programs aimed to improve sweet cherry shelf-life, highlighting also the value of genetic heritage of sweet cherry of Campania region.

P6.17
Adaptation to metalliferous soils and nutrient use efficiency in populations of *Silene paradoxa* L.

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Plants adapted to live in metal-enriched soils have to face both the stress due to the high metal concentrations and to nutrient deficiency. In this work, we investigated the difference in response to 3 macronutrients (Ca^{2+}, Mg^{2+}, K^+) deficiency stress by two metallicolous and a non metallicolous population of *Silene paradoxa* in order to see if the adaptation to heavy metal-enriched soils could have modified the physiology of nutrient utilization. Plants were grown in hydroponic solution, totally and partially (1/10) deprived of each nutrient and the nutrient content in roots and shoots was measured. Growth results show that the two metallicolous populations were less sensitive to the nutrient deficiency compared to the non metallicolous one, thus demonstrating that, despite the scarcity of nutrients in the soil, these populations are able to optimize the nutrient accumulation and allocation in their organs to maintain an adequate development and growth rate. The NUE (nutrient use efficiency) values demonstrated that metallicolous populations are indeed more efficient than the non metallicolous one in facing the nutrient deficiency stress.

P6.18
Pointing out the distinct features of plant and algae phosphoribulokinase

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Phosphoribulokinase (PRK) is an essential and highly regulated homodimeric enzyme of the Calvin-Benson cycle, conserved in all green photosynthetic organisms and required to re-generate the RuBisCO's substrate (Michelet et al., 2013). PRK is the only enzyme of the Calvin-Benson cycle whose 3D-structure is unknown and that cannot be predicted by homology modelling because of lack of similar proteins in other organisms. In the present study, two recombinant forms of PRK, one from the green alga *C. reinhardtii* (CrPRK) and one from the higher plant *A. thaliana* (AtPRK), were analysed and compared in order to highlight possible biochemical and structural differences. The two mature forms of PRK share 75% of amino acid identity. Low resolution structures obtained by Small Angle X-ray scattering (SAXs) showed appreciable differences in shape between CrPRK and AtPRK, the former being more anisotropic and less compact and the second showing a larger contact area between the two subunits. The activity of the two enzymes appeared differently affected by physicochemical parameters such as redox conditions, pH and temperature. Analyses of circular dichroism (CD) indicated a different binding of the substrates. A systematic experimental approach aimed in solving the first crystal structure of a plant PRK is underway.

P6.19
Effects of cGMP constitutive accumulation in *Arabidopsis thaliana* plants challenged with avirulent pathogens

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Cyclic guanosine 3',5'-monophosphate (cGMP) has been indicated as a second messenger induced by nitric oxide (NO) in incompatible plant-pathogen response. However, the role of cGMP in the signaling of plant defense response has not yet characterized. In this study a genetic tool has been used in order to constitutively increase cGMP level in plant. In particular, *Arabidopsis thaliana* plants expressing the rat soluble guanylate cyclase (GC) have been produced. In order to evaluate cGMP contribution in local and systemic acquired resistance, the transgenic plants have been subjected to the infection of an avirulent strain of *Pseudomonas siringae*. Even if phenotypic local response of GC plants did not show any difference compared to wild type line, glutathione metabolism resulted modified suggesting a crosstalk between cGMP and redox signaling. Large-scale transcriptomic and proteomic analysis highlighted significant modulation between wild type and GC overexpressing plant of both gene expression and protein abundance at the infection site. Moreover, high constitutive cGMP level in GC plants appeared to affect systemic acquired response of the plants to the pathogen.

P6.20
Chemical characterization and standardization of bioactive boswellic acids from *Boswellia* (Frankincense) species by HPLC-ESI-MS/MS

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Plant extracts are a rich source of secondary metabolites able to exert biological activities on humans. They also have a high economic impact on the dietary supplements market. Environmental factors and extraction methods can strongly affect the chemical composition of plant extracts; therefore, the use of accurate and sensitive analytical techniques for the characterization and quantification of bioactive molecules is a compulsory quality standard to assure to costumers both safety and bioactivity. Traditional medicine uses *Boswellia* spp. resin-gum extracts for anti-inflammatory, anti-proliferative, antiseptic and neuro-protective effects because of the presence of pentacyclic triterpenoids known as boswellic acids (BAs). *B. sacra* and *B. serrata* are characterized by significant amounts of 3-*O*-Acetyl-11-keto-β-boswellic acid (AKBA), α- and β-BAs and their acetylated derivatives. In market products, BAs percentages are often misinterpreted and it is not unusual to find claims of 70% BAs content. This study aims to quantify BAs content by using HPLC coupled to Tandem Mass Spectrometry in two commercial *Boswellia* species to achieve the accurate standardization of bioactive BAs.

P6.21
Sirtuin-mediated DNA damage response by modulation of glutamate dehydrogenase activity in *Arabidopsis thaliana*

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Sirtuins, ClassIII NAD-dependent deacetylases, play a central role in many metabolic pathways related to cell survival and are evolutionary conserved from bacteria to mammals. Among the seven human sirtuins, SIRT4 and SIRT6 share homology domains with the two sirtuins present in *Arabidopsis thaliana* plants, AtSRT2 and AtSRT1 respectively. With the aim to evaluate sirtuin functions in phylogenetically distant organisms, we report data on a corresponding role between *Arabidopsis* SRT2 and human SIRT4 genes. We find that AtSRT2 is involved in a defence process already known to be regulated by SIRT4. In fact the DNA Damage Response (DDR) in human cells induces SIRT4 that in turn limits proliferation via repression of glutamine metabolism (Jeong et al, Cancer Cell 2013, 23:450). In *Arabidopsis* seedlings, the induction of DNA damage promotes transcriptional activation of SRT2 gene and decreased activity of glutamate dehydrogenase (GDH), one of the enzymes that catalyze a-ketoglurate (aKG) production from glutamine. As aKG is a major anaplerotic component of TCA cycle in proliferating cells, the decreased GDH activity is coherent with the slowed cell proliferation that we observed. Moreover, in plants knock out for SRT2, GDH activity and cell proliferation are less affected by DNA damage, confirming the role of AtSRT2 in this metabolic pathway.

P6.22
Molecular insights on the role of *Arabidopsis thaliana* NAOD in fruit set

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Ornithine (Orn), an intermediate of arginine and polyamines (PAs) biosynthetic pathways, is produced in plants by N^2-acetylornithine:N-acetylglutamate acetyltransferase (NAOGAcT). In enteric bacteria, Orn is synthesised also by N-acetylornithine deacetylase (NAOD) via a linear pathway. The plants seem to be unable to use this pathway despite the presence of many NAOD-like genes identified in various plant species. We have studied the role of the putative NAOD of Arabidopsis (*At4g17830*) by analysing the effects of its downregulation *in vivo*. *AtNAOD*-suppressed plants displayed an impaired fruit setting. *AtNAOD* downregulation determined a reduced Orn content and altered PAs levels. To elucidate the role of *AtNAOD* in fruit setting, we compared the mRNA profile of fertilised flowers of *AtNAOD*-downregulated plants with that of wild-type. We found 63 genes significantly changed (fold change ≥ |2|). Our analysis revealed that the altered Orn and PAs metabolism in the reproductive organs of the *AtNAOD*-downregulated plants is associated with an impaired transcription of cysteine-rich signalling peptides involved in male-female cross-talk and perturbation of genes involved in regulating N:C status.

P6.23
Salt-tolerant related protein: a new protein involved in plant adaptation to low temperature

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Plants are constantly exposed to different environmental stresses such as extreme temperatures, high salinity, excess or lack of water, light and nutrients that strongly limit plant growth and significantly reduce crop yield. Plants respond with changes in their pattern of gene expression and protein products when exposed to these stresses. Thus, the ability to adapt has an impact on the distribution of the plant and crop yield. In a proteomic analysis of temperature stress-responsive proteins of *Arabidopsis thaliana* rosette leaves, an uncharacterized protein, known as salt-tolerant related protein (STRP), resulted to be strongly increased by cold treatment. The aim of this work was to clarify the role of this protein in Arabidopsis response to cold stress. By using polyclonal antibodies obtained against recombinant STRP protein, it was demonstrated that STRP is expressed in the cytosol but is also associated to the plasma membrane. Low temperature treatment greatly increased STRP levels in the cytosol and this effect was due to an enhanced protein stability rather than to activation of gene transcription. Moreover, STRP knock out mutant showed increased susceptibility to oxidative damage induced by cold stress, as demonstrated by the higher levels of lipid peroxidation and ion leakage. These results suggest that STRP may play a protective role in the plant response to cold stress.

P6.24
Identification and quantification of flavonoids in fresh leaves and fruits of *Cyclanthera pedata* Scrabs (Caigua) and in their commercial food supplement preparations

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Cyclanthera pedata Scrabs (Caigua) is a plant belonging to the Cucurbitaceae family largely cultivated in South America. The plant is used as food and in popular medicine to treat diabetes and to control high blood pressure and cholesterol. Hypoglycemic properties of plant extracts have been frequently related to their content in flavonoid glycosides which are known to posses antihyperglycemic activity. This poster reports the results of a study carried to identify and quantitate flavonoid glycosides extracted from fresh fruit and leaves of *Cyclanthera pedata* Scrab and from commercially available food supplements based on Caigua extracts. Plants of Caigua were grown either in a greenhouse or in an open field located in the vicinity of our University, whereas the food supplements were of commercial origin. Flavonoids were extracted using methanol or methanol-water mixtures and their separation and quantification were carried out by reversed phase high performance

liquid chromatography (RP-HPLC), using both UV spectrophotometry and mass spectrometry as the detection systems. The different occurrence and quantity of flavonoids in the investigated samples are reported and discussed.

P6.25
Can chemical xylem sap changes trigger grapevine isohydric and anisohydric behaviors under environmental water deficit?

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Grapevine cultivars have been classified as isohydric or anisohydric on the base of their different stomatal kinetics experienced in response to water deficit conditions. In this work, dynamic changes of stomatal responses to leaf water potential were monitored in six *Vitis vinifera* varieties in order to investigate a possible correlation between physiological parameters and biochemical features of xylem sap [apoplastic pH, abscisic acid (ABA) and soluble sugar concentrations]. Sap samples were collected in an experimental vineyard during the summer season at different levels of environmental water deficit: low (early summer), moderate (middle of the season) and recovery (one day after rainfall in late summer). Preliminary results showed that all varieties were characterized over the season by: a progressive xylem sap alkalization, a decrease of soluble sugar content and an increase in ABA level. However, when near-isohydric (Grenache) and anisohydric (Barbera) cultivars were compared, distinct profiles of all the analyzed parameters of xylem sap were observed, thus providing peculiar physiological responses of grape varieties upon progressive soil water scarcity conditions.

P6.26
Effect of zinc deficiency in *Hordeum vulgare*: are changes in morphological and physiological aspects related to alterations in nitrate uptake?

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Zinc (Zn) is an essential micronutrient for living organisms, involved in a wide variety of metabolic processes. Despite of the evidence that Zn is the second most abundant transition metal in nature, more than 3 million people worldwide suffer from Zn deficiencies. The main aim of this work was to analyse the effects of Zn deficiency in a plant of high agronomic relevance as barley (*Hordeum vulgare*). Thus plants were grown for 7 days in hydroponic culture in control, toxicity and deficiency conditions. We measured the levels of heat shock protein 70, growth inhibition factor, water content, photosynthetic pigments content, starch accumulation, nutrient uptake and expression and activity of several basal metabolism enzymes, such as glucose6 phosphate dehydrogenase. The results show under Zn deficiency several changes in both morphological aspects and in biochemical processes. Moreover, NO_3^- uptake changed suggesting that under Zn deficiency, nitrogen metabolism was altered inducing structural and physiological changes in barley plants.

P6.27
The existence of sirtuin activity in purified durum wheat (*Triticum durum* Desf.) mitochondria

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Sirtuins are a family of NAD^+-dependent deacetylases able to regulate proteins through the N^{ε}-deacetylation of lysine residues. Recently, it has been demonstrated that the *Arabidopsis* SRT2 (At5g09230) resides in the inner mitochondrial membrane and is involved in regulation of energy metabolism and metabolite transport. *In silico* analysis suggested that a functional SRT2 may exist in durum wheat having an N-terminal signal peptide that putatively directs it to mitochondria. So, we tried to assay for the first time a sirtuin activity in a highly purified mitochondrial fraction. To do this, we used the luminescent SIRT-Glo™ assay (Promega), based on the luciferin/luciferase reaction. We were able to measure a sirtuin activity that resulted *i)* linearly dependent on mitochondrial protein, *ii)* inactivated by protein denaturation by boiling mitochondria and *iii)* completely abolished by 100 mM nicotinamide, a known sirtuin inhibitor. On the basis of calibration by using human SIRT1 (Sigma-Aldrich) an unexpectedly high activity of durum wheat mitochondria (DWM) sirtuin equal to 0.165 ± 0.013 µg SRT1 eq./mg DWM was calculated, thus suggesting relevant role/s of this enzyme in DWM.

P6.28
From an expression-based reverse genetic study to the functional characterization of two determinants of osmotic stress tolerance

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Adaptation to osmotic stress requires an extensive alteration of gene expression. Previously, we identified several genes regulated in cells adapted to polyethylene glycol (PEG). Here, the functional role of fifty of these genes was verified. Using a large-scale phenotype screening, we have identified two genes: the splicing factor *IAG1* (*INSENSITIVE TO ABA IN GERMINATION1*) and the putative TOR-pathway component *XSA1*(*EXTRA SENSITIVE TO ABA1*). *IAG1* is induced upon long-term exposure to abscisic acid (ABA) and PEG and is mainly expressed in trichomes and stomata, organs controlling transpiration. Germination analysis of plants with altered expression of *IAG1* and protein interaction with the splicing factor SUA, suggest that IAG1 may be involved in pre-mRNA splicing of effectors of ABA response leading to germination inhibition. *XSA1* possibly affects pathways in ABA-mediated response to stress. *XSA1* is expressed in vascular tissues and is up-regulated by long-term exposure to NaCl and ABA. *xsa1-1* is ABA hypersensitive, indicating alteration in ABA biosynthesis and/or perception. Taken together, our results reveal promising mechanisms of plant adaptation to osmotic stress.

P6.29
Key role of pectin methylesterases in controlling ascorbic acid content in tomato fruit

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The potential increase of L-ascorbic acid (AsA) content in tomato is a common goal in breeding programs due to its beneficial effect on human health. In a previous study, one *Solanum pennellii* introgression line (IL12-4-SL) harbouring one quantitative trait locus that increases the content of AsA in the fruit was identified. In the present study, we confirmed the higher content of total AsA and of the reduced form of AsA in IL12-4-SL compared to the cultivated line M82 at three developmental stages (mature green, breaker and mature red). Genomic and transcriptomic analyses indicated in IL12-4-SL the involvement of genes controlling pectin de-methyl esterification/degradation in AsA accumulation. The expression of the identified candidate genes during fruit ripening was studied in relation with pectin methylesterase (PME) activity, degree of pectin methyl esterification and AsA content. A PME and a polygalacturonase were identified that may affect pectin-derived D-galacturonic acid (GalUA) content leading to AsA biosynthesis as metabolic precursor. This knowledge could provide novel tools for marker-assisted selection of tomato genotypes with a higher content of antioxidant in the fruit.

P6.30
Molecular insights into the interaction between KAT1 channels and their regulatory protein 14-3-3

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The voltage-gated inward rectifier potassium channel KAT1 of *Arabidopsis thaliana* plays a key role in stomatal movement, thus controlling carbon dioxide uptake for photosynthesis and water balance of the entire organism. In previous work we have shown that the cytosolic regulatory proteins 14-3-3 interact with the C–terminal portion of the KAT1 channel and modulate both its voltage dependent gating and the trafficking to the plasma membrane (1,2). By a combination of high resolution structural and functional studies we are now able to detail the molecular and structural basis for the channel/14-3-3 interaction. Biochemical binding assays with isothermal titration calorimetry (ITC), structural studies (crystallography) and electrophysiological (patch-clamp) monitoring of KAT1 function support a molecular model in which the 14-3-3 protein is binding to the C-terminus of KAT1 via a mode III binding motif. Strikingly, this interaction is strongly stabilized by the fungal toxin fusicoccin (FC) thereby potentiating the regulatory effect of 14-3-3 on the channel. Based on this detailed insight on protein/protein interaction we are now investigating the molecular basis of gating modulation that presumably involves the recruitment of additional partner proteins. Since 14-3-3 binds exclusively to KAT1 but not to the very similar channel KAT2, we are using KAT2 as a functional tool to investigate the molecular mechanism of 14-3-3 modulation of KAT1 channel opening.

[1] Sottocornola B. et al. *J Biol Chem.* 281(47):35735-41
[2] Sottocornola B. et al. *Plant Biol (Stuttg).* 10(2):231-6

P6.31
First evidence of a Ca^{2+}- induced loss of membrane potential in durum wheat mitochondria

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Durum Wheat (*Triticum durum* Desf.) Mitochondria (DWM) were recently shown to possess a Ca^{2+}-activated PLA_2 that releases FFAs and, under stress conditions, may activate both uncoupling protein (UCP) and mitochondrial potassium channel ($PmitoK_{ATP}$), thus damping protonmotive force and excess ROS production. Nevertheless, so far, no information is available about how the DWM-PLA_2 activity increases under stress. One possibility is that changes of Ca^{2+} concentration might regulate the enzyme. To study this possibility, DWM oxidizing succinate were added with $CaCl_2$ (0.1 and 0.5 mM) and a biphasic depolarization was observed under conditions contrasting possible occurrence of permeability transition (5 mM Mg^{2+}, no phosphate). A rapid membrane potential decrease ranging from 10 to 25 mV (depending on Ca^{2+} concentration) is observed, followed by a Slow Continuous Depolarization (SCD). Interestingly, 1 mM ATP (in the presence of atractyloside, oligomycin and Ap5A), able to inhibit UCP and $PmitoK_{ATP}$, together with 0.1% BSA, able to bind FFAs, were able to rapidly reverse the SCD phase. This is compatible with the hypothesis that PLA_2/UCP/$PmitoK_{ATP}$ pathway might be involved in the SCD phase.

P6.32
Calcium signaling in response to abiotic stress during plant evolution

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The colonization of land environment has been a key step in the evolution of photosynthetic organisms. Plants acquired novel abilities that were not essential to their aquatic progenitors, such as resistance to dehydration. Such a massive change clearly involved an adaptation of the interaction of organisms with the environment. In order to investigate the evolution of mechanisms of external stimuli perception we exploited *Physcomitrella patens*, a moss belonging to the phylum *bryophytes*, thougth to maintain the characteristics of the first plants colonizing land. In particular we focused on the study of the response to osmotic stresses in term of Ca^{2+} dynamics, as univocal signals activating metabolic response. To this aim we generated mosses stably expressing the YC3.6 Cameleon variant, a ratiometric FRET-based Ca^{2+}-probe, targeted to different subcellular compartments. By confocal microscopy, we verify the specific localization of Cameleon in the cytosol, mitochondria or nuclei. By analyzing the plants at the protonema stage, we could observe, in all cell compartments analyzed, a transient increase in Ca^{2+} concentration triggered by both dehydration and rehydration treatments.

P6.33
Does carbon starvation during prolonged drought prevent embolism repair pushing trees toward irreversible hydraulic failure?

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Recent episodes of anomalous drought and heat waves have caused, on a global scale, widespread mortality of plants. Drought-induced tree death is a complex event and recent hypotheses suggest that hydraulic failure and carbon starvation are co-responsible of tree decline. Progressive decline in soil water reserves increases xylem tension inducing xylem embolism and stomatal closure. Reduced CO_2 uptake can lead to impoverishment of non-structural carbohydrates (NSC) reserves. Plants can cope with hydraulic failure by reversing xylem embolism via refilling upon stress relief. Embolism reversal is likely based on an osmotic mechanism, and thus requires availability of soluble sugars. We tested the hypothesis that embolism reversal represents the mechanistic link between carbon starvation and stem hydraulics. Measurements were performed on laurel plants exposed to carbon starvation caused by a prolonged water stress. Plant water status, gas exchange, leaf water potential isotherms, loss of hydraulic conductivity and NSC content of stems were measured in control versus drought-treated plants, before re-irrigation and eventual recovery. Our findings suggest a strong correlation between NSC availability and embolism reversal ability, opening interesting new scenarios on mechanisms responsible for tree resistance and resilience to severe water shortage.

P6.34
Strigolactones are needed for miR156 inducibility by drought in tomato

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Strigolactones (SL) are terpenoid hormones influencing many aspects of plant development, biotic interactions and responses to environmental stress, namely drought. MicroRNAs (miRNAs) are a set of small, non-protein-coding RNAs that regulate gene expression at the post-transcriptional level. Given the inducibility of certain miRNAs by environmental stress, and the pervasive effects of SL on plant acclimatization to drought, we asked whether the latter may have a role in miRNA metabolism in such conditions in tomato. The results show that SL synthesis in the shoot, but not in the root, is needed for the accumulation of miR156 (but not miR166) in both organs under drought. A preliminary analysis of pre-miR156 levels indicates that stress triggers precursor accumulation only in shoots, which implies that mature miR156 moves rootwardly under drought. Additionally, pre-miRNA inducibility by drought seems to be only marginally affected by SL availability, suggesting that the latter may rather play their role later in miRNA maturation - a process whose efficiency towards specific miRNAs is sensitive to cellular context.

O6.1
Crocin biosynthesis in saffron stigmas: a tale of three compartments

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Crocus sativus stigmas are the source of the spice saffron, and owe their characteristic color, taste and aroma to the apocarotenoids crocetin, crocins, picrocrocin and safranal. The identification of genes involved in apocarotenoid biosynthesis and sequestration is a necessary prerequisite for the pathway engineering in heterologous systems. By transcriptomics we identified candidate genes for the whole biosynthetic pathway. The first step is catalyzed by a plastidial carotenoid cleavage dioxygenase (CCD2), able to cleave zeaxanthin yielding crocetin dialdehyde, when expressed in *E. coli* or in *Z. mays*. The second step, dehydrogenation of crocetin dialdehyde into crocetin, was investigated *in bacterio* by coexpression of different stigma aldehyde dehydrogenases (ALDH) with CCD2. By this approach we identified a cytosolic ALDH that efficiently catalyzes the reaction. At the other end of the pathway we identified putative vacuolar transporters expressed when glycosylated crocins are stored in the vacuole. Heterologous expression in yeast, coupled with *in vitro* transport assays, was used to dissect their substrate specificities, showing for one of them the ability to transport crocins.

O6.2
The mitochondrial nucleoid associated WHIRLY2 affects morphology and dynamics of mitochondria

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Variations in amount and structural integrity of organellar DNA are tightly regulated by nuclear-organelle cross-talk. WHIRLY proteins are DNA binding proteins that were shown to play a role in organellar DNA maintenance and organization. Arabidopsis thaliana has three WHIRLY proteins with different subcellular localization: WHIRLY1 and WHIRLY3 are targeted to chloroplasts, while WHY2 is targeted to mitochondria. WHIRLY2 gene expression is related to early plant development, being expressed in imbibed seeds, shoot apex and roots of young seedlings. A T-DNA insertion mutant for the WHIRLY2 gene does not show an obvious phenotype except for shorter root length compared to wild type. In fusion with GFP, WHIRLY2 shows a localization to mitochondrial nucleoids. In confocal microscopy analyses, mitochondria of mutant plants appear larger and less dynamic. In order to investigate the impact of WHIRLY2 on mitochondria in more detail, electron microscopy was performed. TEM images revealed that in the absence of WHIRLY2 mitochondria appear less dense and with less cristae, compared to wild type mitochondria. Moreover, alterations of mtDNA content depending on plant organ and developmental stage have been observed. These results suggest that WHIRLY2 in mitochondria plays a similar role as WHIRLY1 in plastids where it was shown to be a major organizer of nucleoids.

O6.3
β-amylase-1-dependent starch degradation in mesophyll cells releases carbon skeletons required for the production of proline

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In Arabidopsis leaves, transitory starch degradation is predominantly nocturnal and involves ß-amylase-3 (BAM3) (Fulton et al., 2008). However a second ß-amylase isoform, named ß-amylase-1 (BAM1), is synergistically involved with α-amylase 3 (AMY3) in diurnal starch degradation that occurs in chloroplasts of guard cells, a critical process required for the rapidity of stomatal opening (Horrer et al., 2016). Behind its role in the regulation of stomatal pores, BAM1 has also been proposed to take part in diurnal starch degradation pathway in response to osmotic stress (Valerio et al., 2015). In this study, the response to mild and prolonged osmotic stress in wild-type, *bam3* and *bam1* plants were analysed (Zanella et al., 2016). In comparison to both wild-type and *bam3* plants, *bam1* mutants showed lower proline accumulation, higher lipid peroxidation, diurnal starch accumulation and lower levels of soluble sugars at the end of the day in response to drought stress. Taken together, these data strongly suggest that carbon skeletons deriving from diurnal starch degradation catalysed by BAM1 support the biosynthesis of proline required to face the osmotic stress.

O6.4
Acidification of xylem sap pH observed in woody plants provides apoplastic environment for facilitating recovery from water stress

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Under water stress in many herbaceous plants xylem sap becomes more alkaline, thus affecting the abscisic acid (ABA) level and its activity in leaves This in turn triggers stomatal closure and limits water loss in drying soils. Sap alkalization is not a universal phenomenon, and in some woody plants sap becomes more acidic upon drought. We assumed that sap acidification is one of the symptom/signal of severe water stress in plants experiencing embolism, and that the resulting physiological changes promote the recovery process. To test our hypothesis, we followed the dynamics of the apoplastic pH during water stress treatment in woody plants characterized by alkalization (*grapevine*), acidification (*poplar*) and unknown response of sap (*olive*). Upon drought, poplars decreased sap pH and increased the sugar concentration in the xylem apoplast. This represents a different strategy to cope with drought than what we observed in grapevines, where xylem sap pH and ABA level increased together with a decrease in sugar concentration. Further analyses on olive plants are ongoing to complete our investigation. Based on these results, we propose new models on triggering mechanisms of water stress recovery in stem.

O6.5
Comparative analysis of the role of the different *Arabidopsis* polyamine oxidases in plant defense responses to environmental stresses

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In plants, the polyamines putrescine, spermidine, spermine and thermospermine are involved in several physiological and defense processes. Polyamine oxidases (PAOs) are FAD-dependent enzymes involved in polyamine catabolism. The five *Arabidopsis* PAOs (AtPAO1–AtPAO5) present important differences among them in subcellular localization, substrate specificity and expression pattern, which suggest distinct physiological roles. In the present study, a comparative analysis of the contribution of the different members of the *AtPAO* gene family to plant defense responses under abiotic stress conditions was performed. This analysis evidenced that the cytosolic AtPAO1 and AtPAO5 are implicated in defense mechanisms to salt stress and drought, the *atpao5* and *atpao1/atpao5* mutants showing increased tolerance and the *35S::AtPAO5* transgenic plants increased susceptibility comparing to the wild-type plants. This study additionally showed that the three peroxisomal PAOs (AtPAO2-AtPAO4) are involved in the abscisic acid-mediated control of stomata movement, the single, double and triple mutants showing reduced response to this process. Studies are in progress to determine the underlying mechanisms.

7- Genetics of Microorganisms

P7.1
Mycobacterium smegmatis katG mutant shows an impaired dormancy behaviour

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Mycobacterium tuberculosis, one of the most powerful human pathogens, has the ability to enter human macrophages and to survive inside them in a 'latent' or 'non-replicating' form for long period of time. It has been hypothesized that mycobacterial latency occurs in response to the immune system injuries induced, for example, by oxygen and nutritional limitations or by the generation of mutagenic and genotoxic agents (Reactive Oxygen and Nitrogen Intermediates). Similarly to *M. tuberculosis*, *M. smegmatis* is able to adapt and persist to conditions that mimic the hostile environment encountered by the pathogen during infection. We used an vitro plate-based dormancy system to screen a *M. smegmatis* mutant library for mutants unable to enter/exit latency. LM-PCR analysis has allowed the identification of a *M. smegmatis katG* mutant with an impaired latency behaviour. *katG* encodes a catalase-peroxidase, an enzyme that function as H_2O_2 scavenger, protecting cells from H_2O_2 toxicity. Real time PCR experiments were used to evaluate the effect of several stress condition (nutrient-/oxygen-deprivation, acid pH, oxidative stress) on *katG* expression.

P7.2
A leaderless mRNA supports the translation of a smaller form of the *Shigella* VirF regulator

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VirF is a transcriptional regulator of the AraC family which plays a central role in controlling virulence gene expression in *Shigella* spp, the etiological agent of bacillary dysentery in humans. VirF expression is activated upon entry into the host and depends on many environmental signals. Here, we show that the *Shigella virF* gene encodes two proteins of different size, $VirF_{30}$ and $VirF_{21}$, that are functionally distinct. The major form, $VirF_{30}$, activates the genes necessary for virulence, whereas the minor $VirF_{21}$ which shares the C-terminal two thirds of $VirF_{30}$, negatively autoregulates *virF* expression itself. Moreover, the expression of $VirF_{30}$ and $VirF_{21}$depends on a differential translation process based on two different forms of *virF* mRNA: a longer mRNA, giving rise to both $VirF_{30}$ and $VirF_{21}$, and a shorter, leaderless mRNA originating only $VirF_{21}$. In addition the leaderless mRNA is transcribed from a newly identified gene-internal promoter. The identification of $VirF_{21}$ as a new player in regulation adds complexity to the regulation of *Shigella*'s invasive process and may help development of new therapies for shigellosis.

P7.3
Distinctive features of the LPS transport system of *Pseudomonas aeruginosa*

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Lipopolysaccharide (LPS) is an essential component of the outer membrane of most Gram-negative bacteria. In the model organism *Escherichia coli*, LPS transport requires a set of seven essential (Lpt) proteins, which have been proposed as promising targets for the design of novel antimicrobials. We have recently demonstrated that LptH, the periplasmic component of the Lpt system, is required for growth and infectivity in the human pathogen *Pseudomonas aeruginosa*. Surprisingly, two transposon mutagenesis studies identified viable insertion mutants in the *P. aeruginosa lptC* and *lptE* genes, suggesting that they might be dispensable in this bacterium. To verify this hypothesis, we generated *P. aeruginosa* conditional mutants in *lptC* and *lptE*. Growth of LptE-depleted cells was only slightly impaired with respect to the wild type, while LptC-depleted cells displayed normal growth after a very long lag phase, which likely involves a still-undefined adaptation mechanism. In contrast, LptE was found to be more important than LptC for cell-envelope stability and antibiotic resistance. Overall, these data suggest that *P. aeruginosa* can tolerate an LptE- and LptC-independent LPS transport system.

P7.4
Expression profile of efflux pumps during the intracellular life of *Shigella*

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In several bacterial pathogens, efflux pumps, besides exporting antimicrobial agents, play a role in bacterial pathogenicity. *Shigella*, the causative agent of bacillary dysentery, shares a high homology with its commensal ancestor *Escherichia coli* but, in contrast to *E. coli, Shigella* is submitted to intense gene decay and has lost many *E. coli* metabolic and housekeeping functions. By genome analysis we observed that 14, out of 20, operons encoding efflux pumps systems have been conserved in *Shigella.* To understand the potential role of efflux pumps in *Shigella* pathogenicity, we monitored their expression during invasion of macrophages and epithelial cells. The data obtained indicate that i) EmrKY efflux pump is notably and specifically induced within macrophages and its loss negatively affects *Shigella*'s fitness; ii) AcrAB, the main efflux pump in *E. coli*, is downregulated during intracellular life. Our observations reveal also that, during infection, *Shigella* modulates expression of specific efflux pumps. Experiments are in progress to investigate the role of these efflux pumps and the environmental stimuli that affect their expression.

P7.5
Fortifying the peptidoglycan layer to avoid cell lysis: a new role for L,D transpeptidases

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The three-layered cell envelope of Gram-negative bacteria composed of an inner membrane (IM) an outer membrane (OM) and a peptidoglycan (PG) layer sandwiched between them, is essential for their survival and is the target of many antibiotics. We recently found a functional connection between the biogenesis of lipopolysaccharide (LPS), the major component of the OM, and the synthesis and turnover of PG. In line with this finding, the analysis the PG composition in mutants in which the LPS transport is blocked, shows a strong increase of non-canonical 3-3 cross-links between *meso*-DAP residues of adjacent stem peptides. In *E. coli* 3-3 cross-links are catalysed by two L,D-transpeptidases (Ldts), YnhG and YcbB that are fully dispensable during growth under laboratory conditions. Deletion of either *ycbB* or *ynhG* in several conditional *lpt* mutants causes cell lysis when growing under non-permissive conditions that is when LPS

transport is impaired. Taken together our results suggest that the increase of 3-3 cross-links is a strategy adopted by *E. coli* cells to strengthen the PG layer to avoid cell lysis.

P7.6
Conjugal transfer of the conjugative and integrative element Tn*5253* of *Streptococcus pneumoniae* is influenced by its integration site

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Integrative and conjugative element Tn*5253* of *Streptococcus pneumoniae*, carrying *cat* and *tet*(M), integrated at a 83-bp specific site (*att*B) in *rbgA*. We constructed an *att*B mutant strain, where a kanamycin resistance cassette replaced the first 63 nts, and 5 point mutations were introduced in the remaining 20 nts belonging to *rbgA* cds. In mutant strain, Tn*5253* was transferred at lower frequency compared to wild type recipient (4.8×10^{-7} vs. 1.7×10^{-5} transconjugants/donor). Analysis of transconjugants showed that (i) 40% had Tn*5253* integrated into mutated *att*B, (ii) 45%, into the original *att*B with loss of mutagenic construct and (iii) 15% in 5 alternative insertion sites. Back transfer of Tn*5253* from the alternative insertion sites occurred at a lower frequency compared to w.t. recipient donor (2×10^{-7} to $< 3.6 \times 10^{-8}$ transconjugants/donor). From a transconjugant harboring 3 copies of Tn*5253* integrated in 3 different sites, the element was transferred at a frequency 100-fold higher than w.t. donor.

O7.1
Temperature-dependent regulation of the *lpxT* gene in *Escherichia coli* and *Pseudomonas aeruginosa*

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The LpxT protein modifies the outer membrane of Gram negative bacteria by phosphorylating the lipid A moiety of the lipopolysaccharide. Recently, we found that the expression of the *lpxT* gene of *Pseudomonas aeruginosa* (Pa) is post-transcriptionally regulated by an RNA thermometer (RNAT). An RNAT is a thermo-labile secondary structure that entraps the mRNA Translation Initiation Region (TIR) at low temperature, thus inhibiting ribosome binding. To assess whether also the *Escherichia coli lpxT* gene was regulated by temperature through a similar strategy, we assayed the expression of different *lpxT*-GFP translational fusions in the BW25113 strain at 28° and 42°C. Moreover, we analysed in the same strains and conditions the transcription profile of the chromosomal *lpxT* locus. We found that i) the *lpxT* transcript starts 29 nt upstream of the ORF start codon. Transcription from the promoter *lpxTp* is modulated both by temperature and growth phase; ii) the presence of the short *lpxT* 5'-UTR, which can form a stem-loop (SL_{lpxT}), confers thermo-dependent expression to a downstream reporter gene; iii) mutations affecting SL_{lpxT} stability impact on the reporter gene expression. On the whole our results suggest that transcriptional and post-transcriptional mechanisms cooperate in the complex regulation of *Ec lpxT* expression.

O7.2
Alkyl-quinolone-dependent quorum sensing controls prophage activation, autolysis and antibiotic resistance in *Pseudomonas aeruginosa* biofilm

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The *pqs* quorum sensing system of *Pseudomonas aeruginosa* produces diverse 2-alkyl-4-quinolones, including 2-heptyl-4(*1H*)-quinolone (HHQ) and 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO). While HHQ influences the expression of multiple genes, the HQNO has no effect on *P. aeruginosa* transcriptome. However, a *P. aeruginosa pqsL* mutant strain, impaired in HQNO synthesis, undergoes autolysis when grown as colony biofilm. Here we show that HHQ accumulation caused by *pqsL* mutation leads to the transition of the Pf4 prophage from the lysogenic to the lytic phase, resulting in cell autolysis. Notably, this phenomenon increases the antibiotic resistance of *P. aeruginosa* PAO1 biofilms. Analyses on *P. aeruginosa* clinical isolates demonstrate that about 40% of these strains show a lytic phenotype when grown as colony biofilm, and that in most clinical isolates autolysis is due to mutations in the *pqsL* gene, hampering PqsL expression or functionality. These data indicate that, although the PqsL-mediated synthesis of HQNO might serve as a sink to limit autolysis, loss of *pqsL* could represent a pathoadaptive mutation increasing antibiotic resistance in *P. aeruginosa* clinical isolates.

O7.3
Gallium-protoporphyrin IX uptake pathways in *Pseudomonas aeruginosa* and growth inhibition by cytochromes targeting

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Pseudomonas aeruginosa represents a challenging pathogen due to its antibiotic resistance, which makes infections very difficult to treat. The importance of iron in bacterial physiology and pathogenicity has made iron-uptake and metabolism very attractive targets to develop new drugs. $Ga(NO_3)_3$ has been shown to successfully inhibit *P. aeruginosa* growth, by acting as an iron mimetic, thus interfering with iron-dependent metabolic pathways. Ga(III) coupled with the heme precursor protoporphyrin (PPIX), has demonstrated a potent antibacterial activity against a wide range of pathogens, although no effect has been reported on *P. aeruginosa.* The aim of our work was to investigate the effect of GaPPIX on *P. aeruginosa*. Here, we demonstrated that GaPPIX inhibits *P. aeruginosa* growth, and that inhibition is reversed by iron-availability. We also demonstrated that GaPPIX enters *P. aeruginosa* cells through heme receptors, mainly through PhuR. Lastly, we have shown that intracellular GaPPIX inhibits the aerobic growth of *P. aeruginosa* by interfering with cellular respiration.

O7.4
Multitargeting antitubercular compounds: a new precious tool in multidrug resistance age

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The emergence of *Mycobacterium tuberculosis* multidrug-resistant strains has emphasized the need of new antitubercular drugs with novel mechanisms of action. The CTP synthetase PyrG was validated as the target of EthA-activated thiophene-carboxamide and of its sulfonic active metabolite (Mori *et al*., 2015). Lately, we identified the panthotenate kinase PanK as a secondary target of these compounds, suggesting that PyrG and PanK could represent a useful tool to identify potential multitargeting hits, through the screening of compound libraries. In this context, we tested the activity of the publically available GSK TB-set library of antitubercular compounds, identifying three pyridine-thiazoles as effective PyrG inhibitors, one of them also active against PanK. In addition, in order to reveal possible cross-reactivity, we produced the human CTPS1 to test these inhibitors. The enzymatic inhibition assays showed that the thiophene-carboxamides were not active against the

human enzyme, differently from the pyridine-thiazoles. In conclusion, we demonstrated that the two targets PyrG and PanK are appropriate tools for identification of new potential multitargeting antitubercular compounds.

O7.5
The small protein SCO2038 modulates tryptophan biosynthesis and morpho-physiological differentiation in *Streptomyces coelicolor*

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In *Streptomyces coelicolor* small open reading frames were identified in several amino acids biosynthetic gene clusters, like SCO2038 (*trpX*) in the tryptophan *trpCXBA locus*. Here, the role of SCO2038, encoding a 63 amino acid protein, was investigated by both phenotypic and molecular analyses. A SCO2038 knockout mutant strain showed a delayed growth on minimal medium (MM), compromised actinorhodin biosynthesis and poor sporulation. The capability of this mutant to grow on MM was restored by tryptophan's and its precursors' supplementation. Pull-down and bacterial two hybrid assays revealed SCO2038 interaction with PepA, which is putatively involved in the metabolism of serine, glycine and cysteine. Moreover, proteomic analysis revealed a SCO2038-dependent regulation of metabolic pathways and cellular processes including tryptophan amino acid biosynthesis and the morpho-physiological differentiation. Finally, a SCO2038 knockin mutant showed an increased actinorhodin production. Altogether, these findings suggest that SCO2038 modulates tryptophan biosynthesis through direct precursor availability and so exerting an effect on *S. coelicolor* morpho-physiological differentiation.

8 - Transcription Mechanisms and Networks

P8.1
Newly identified PHOX2B target genes as drug targets in Congenital Central Hypoventilation Syndrome (CCHS)

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Congenital Central Hypoventilation Syndrome (CCHS, MIM 209880) is a rare neonatal disease characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control. Frameshift mutations (5%) and polyalanine triplet expansions (95%) have been detected in the coding region of the transcription factor PHOX2B, responsible for the proper development and function of the ANS. Consistent with its role as transcriptional regulator, transcriptional dysregulation might be an important mechanism of CCHS pathogenesis. Stemming from the fortuitous observation that progestin Desogestrel can relief some symptoms of the disease, by a not yet identified molecular mechanism, and that it enhanced the expression of some relevant PHOX2B target genes in a promoter specific manner, by acting on the activity of the wild type as well as mutant protein, lead us to identify new PHOX2B target genes, by ChIP-seq analysis, as potential pharmacological targets for alternative molecules without contraceptive effects. The expression of PHOX2B target genes will be selectively validated by comparing wild-type and CRISPR-CAS9 Knocked-down PHOX2B expressing IMR32 cells.

P8.2
Zinc activates GPER, IGF-IR and EGFR transduction network in breast cancer cells and cancer-associated fibroblasts

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Zinc (Zn) is the second most abundant heavy metal in human tissues and contributes to important biological responses, including the progression of breast cancer. Estrogens play a major role in the development of breast tumors activating the classical estrogen receptor (ER) and the G protein-coupled receptor named GPER/GPR30. We found that zinc chloride ($ZnCl_2$) triggers the GPER-mediated signaling in ER-negative SkBr3 breast cancer cells as well as in cancer-associated fibroblasts (CAFs) obtained from breast tumor patients. In particular, we ascertained that GPER is involved in the activation of epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor I (IGF-IR) transduction pathways by $ZnCl_2$ stimulation. We also determined that the interaction of GPER with both EGFR and IGF-IR induced by $ZnCl_2$ leads to gene transcription changes and important biological responses as cell-cycle progression, proliferation and migration of breast cancer cells and CAFs. Our data provide novel insights into the molecular mechanisms through which Zn may elicit stimulatory effects in breast cancer progression, indicating further therapeutic targets in ER-negative breast tumors.

O8.1
Inhibition of Hedgehog-dependent tumors and cancer stem cells by a newly identified naturally occurring chemotype

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Hedgehog (Hh) inhibitors have emerged as valid tools in the treatment of a wide range of cancers. The Smoothened (Smo) receptor is one of the main upstream transducers of the Hh signaling and is a validated target for the development of anticancer compounds, as underlined by the FDA approved Smo antagonist Vismodegib (GDC- 0449/ErivedgeTM). However, Smo mutations that confer constitutive activity and drug resistance have emerged during treatment with Vismodegib. For this reason, the development of new effective Hh inhibitors represents a major challenge for cancer therapy. Here, starting from an *in house* library of natural compounds and their derivatives, we discovered novel chemotypes of Hh inhibitors by mean of virtual screening against the crystallographic structure of Smo. Hh functional based assay showed that the chalcone derivative is the most effective Hh inhibitor capable to binds the Smo receptor in both WT and drug resistant Smo mutant. Our small molecule stands as a promising Smo antagonist able to specifically impair the growth of Hh-dependent tumor cells *in vitro* and *in vivo* and medulloblastoma stem-like cells and potentially overcome the associated drug resistance.

O8.2
Intracellular trafficking of labelled BODIPY-FF-MAS reveals nuclear lipid droplets localization

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FF-MAS (4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol) is a cholesterol biosynthetic intermediate and a biologically active sterol known to interact with the nuclear receptor LXRα. Ligand-activated LXRα promotes specific target genes transcription by the binding to a LXR responsive element in their promoters. In order to investigate the ability of FF-MAS to modulate LXRα transcription activity, we treated HepG2 cells with FF-MAS for 24 h and found the up-regulation of LXRα target genes SREBP-1c and FASN. Since LXRα interaction with FF-MAS implies nuclear localization of the ligand, we synthesized a fluorescence labelled FF-MAS (BODIPY-FF-MAS) by a Steglich-type esterification at the C3β-OH position of FF-MAS with the fluorescent BODIPY-C12 fatty acid. HepG2 cells were treated with BODIPY-FF-MAS and evaluated by fluorescence microscopy to visualize intracellular FF-MAS localization. Interestingly, after 6 h treatment, BODIPY-FF-MAS accumulated not exclusively in cytoplasmic but also in nuclear lipid droplets. Stable fluorescence labelling of FF-MAS could provide a valuable chemical probe for LXR-related interaction studies and for nuclear lipid droplets functional characterization.

O8.3
A new DNA target site for transciption factors from *M. loti*

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Mesorhizobium loti is a nitrose-fixing symbiotic bacterium responsible of nodulation of *Lotus japonicus* (Hayashi M. et al., 2000). Ten putative genes encoding transcription factors are present in *Mesorhizobium loti* genome (Baglivo I., et al., 2009) whose sequence is completely determined (Kelly S. et al., 2014). Five proteins, Ml1, Ml2, Ml3, Ml4, Ml5, encoded by putative genes from *M. loti*, were espressed in E. coli and their ability to bind DNA has been demonstrated by EMSAs using, as target sequence, the DNA binding site of the homologous protein Ros from *Agrobacterium tumefaciens* (Baglivo I. et al., 2009). In this study we show the expression of the five putative genes in *M. loti*

and identified a new DNA target site for the five transcription factors from *M. loti*. The new target sequence is located at -35 bp from the start codon of the *M. loti* gene called *exoy* encoding a crucial enzyme for exopolysaccharide (EPS) production. Biosynthesis of EPS are strictly connected with biofilm formation. Furthermore, we show, for the first time, the biofilm formation of *M. loti* and the attachment and detachment stages.

O8.4
LSD1 mediates MYCN control of epithelial-mesenchymal transition through silencing of metastatic suppressor NDRG1 gene

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Neuroblastoma (NB) with MYCN amplification is a highly metastatic tumor in children, and unraveling the key players involved in MYCN-induced invasion may identify new targets for therapy. Recently, we reported that Lysine Specific Demethylase 1 LSD1/KDM1A can form a tight complex with MYCN and this complex controls transcription of genes involved in MYCN-driven oncogenesis, proposing LSD1 as candidate therapeutic target in NB. RNA sequencing of NB cells treated with pharmacological LSD1 inhibitor (TCP) or LSD1 knockdown indicates that LSD1 affects Epithelial-mesenchymal transition (EMT) pathway, and we identify the *metastatic suppressor N-myc down regulated gene 1* (NDRG1), a potent metastasis suppressor, as a direct LSD1 target. We found that LSD1 co-localizes with MYCN at the promoter region of the NDRG1 gene and inhibits its expression; LSD1 knock down re-activates NDRG1 gene expression, with concomitant block of motility and invasiveness of NB cells. Our data suggest that LSD1 pharmacological targeting by small molecules could modulate invasiveness of cancer cells and knock down the ability of MYCN-amplified Neuroblastomas to metastasize, through NDRG1 de-repression.

O8.5
5-FU targets rpL3 to induce mitochondrial apoptosis via cystathionine-b-synthase in colon cancer cells lacking p53

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Recent findings revealed in cancer cells novel stress response pathways, which in response to many chemotherapeutic drugs causing nucleolar stress, will function independently from tumor protein p53 (p53) and still lead to cell cycle arrest and/or apoptosis. Since it is known that most cancers lack functional p53, it is of great interest to explore these emerging molecular mechanisms. Here, we demonstrate that nucleolar stress induced by 5-fluorouracil (5-FU) in colon cancer cells devoid of p53 leads to the activation of ribosomal protein L3 (rpL3) as proapoptotic factor. rpL3, as ribosome-free form, is a negative regulator of cystathionine-β-synthase (CBS) expression at transcriptional level through a molecular mechanism involving Sp1. The rpL3-CBS association affects CBS stability and, in addition, can trigger CBS translocation into mitochondria. Consequently apoptosis will be induced through the mitochondrial apoptotic cell death pathway characterized by an increased ratio of Bax to Bcl-2, cytochrome c release and subsequent caspase activation. It is noteworthy that silencing of CBS is associated to a strong increase of 5-FU-mediated inhibition of cell migration and proliferation. These data reveal a novel mechanism to accomplish p53-independent apoptosis and suggest a potential therapeutic approach aimed at upregulating rpL3 for treating cancers lacking p53.

O8.6
High-resolution genome profiles of 8-oxodeoxyguanine, gH2AX and NBS1 reveals their co-association at transcribed long genes

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The 8-oxo-7.8-dihydro-2'-deoxyguanine (8-oxodG) is one of the major oxidative modifications occurring to DNA coming from ROS-producing cell activities. There are constitutively several thousand residues of 8-oxodG in the nuclear genome of human tissues and cultured cells, however the genomic distribution has not yet fully characterized. Here, we applied a novel method named OxyDIP-Seq to analyze the genome-wide distribution of 8-oxodG at a single nucleotide level, using next-generation high-throughput sequencing technology. We mapped 8-oxodG distribution in human non-tumorigenic epithelial breast cell line MFC10A and querying 8-oxodG profiles for multiple genomic features. We found a non-stochastic distribution of DNA oxidation in the genome, rather a peculiar correlation between 8-oxodG residues and Polymerase II (Pol-II) coding genes was observed. We determined that spontaneous residues of 8-oxodG occur preferentially at gene body of Pol-II active genes. Moreover, ChIP-Seq analysis of gH2AX and *NBS1* showed a peculiar overlapping between 8-oxodG, gH2AX and *NBS*1. Indeed, we found accumulation of 8-oxodG, gH2AX and *NBS*1 expecially in long, transcribed and late-replicating genes. Our characterization of genome distribution of 8-oxodG reveals a rationale of gene fragility and suggests a potential mechanism linking transcription and DNA breaks.

9 - DNA replication, Repair and Recombination

P9.1
The Nijmegen breakage syndrome protein (NBN) interacts with the heat shock protein 90 (Hsp90) through an ATM-dependent mechanism

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The heat shock protein 90 (Hsp90) is a molecular chaperone that regulates cells proteostasis, gene transcription, and DNA repair. Indeed, diverse proteins of the DNA damage response (DDR) have been described as Hsp90 clients. To dissect the regulatory role of Hsp90 in the DNA double strand break (DSB) response, the Hsp90 17AAG inhibitor has been used in combination with ionizing radiation (IR), a well-known DSB-inducer. Our data indicate that Hsp90 is necessary for ATM and NBN stability as its inhibition induces polyubiquitination and proteosomal degradation of both proteins. Furthermore, ATM regulates the interaction of NBN with Hsp90, as observed in $ATM^{-/-}$ lymphoblastoid cells, in ATM-silenced cells, as well as in cells expressing the NBN mutant protein unable to be phosphorylated by ATM. Notably, the inhibition of Hsp90 causes high levels of basal IR-induced DSBs, as measured by g-H2AX levels, and a defective DSBs signaling and repair (*i.e.*, phosphorylation of DNA-PK, ATM, NBN, CHK2, and H2AX). Overall our data support a regulatory role of Hsp90 in the DDR strictly connected to the ATM kinase activity, and provides a novel insight into the potential of Hsp90 inhibition in cancer therapy.

P9.2
Combined exposure to the G4 ligand RHPS4 and ionizing radiation blocks the growth of glioma xenograft tumors in mice

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RHPS4 is one of the most effective and selective among the molecules capable to bind and stabilize telomeric DNA in G-quadruplex (G4) conformation. Recently, we showed that RHPS4 is capable to induce telomere dysfunction and block of cell proliferation in glioblastoma (GBM) cells and that sub-micromolar concentrations synergistically sensitize cancer cells to ionizing radiation exposure. The present work aim to test the *in vivo* efficacy of RHPS4 and IR combined treatment in tumor xenograft, derived from the U251MG GBM cell line. Mice were randomized to a control (SHAM) group or to 3 treatment groups that received: i) the drug as single agent, ii) X-rays irradiation to a total dose of 10Gy, iii) the combination of the two. Tumor growth curves indicated that combined treatment was able to reduce tumor volume and to control tumor progression whereas single treatments caused only a small delay in tumor growth. These findings provide the first evidence that molecular targeting of telomeres by RHPS4 is able to radiosensitize GBM cells *in vivo,* determining a persistent block of tumor growth in mice. In order to test the hypothesis that combined treatment is efficient to affect cancer stem-like cells fraction, experiments on neurospheres derived from U251MG GBM cells are in progress. These data will help us to clarify the possibility of a future therapeutic application.

P9.3
DNA damage response in colon cancer stem cells

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Colon cancer stem cell lines (cCSC) with high microsatellite instability (MSI) were identified in the ISS biobank and their response to DNA damage was compared with that of cCSC lines without MSI (MSS). Expression profiling showed that cSCC with MSI present frequent silencing of the mismatch repair (MMR) gene MLH1 as well as a significantly lower expression of the stemness gene Id1. Up-regulation of DNA PolB was often detected. cCSC were exposed to two model alkylating agents (N-methyl-nitrosourea, MNU, and methylmethanesulfonate, MMS). cCSC with MSI showed higher resistance to the cytotoxicity of MNU than MSS cCSC while no difference was detected between the two groups upon MMS exposure. This response resembles what observed in MMR defective colon cancer cells. An efficient repair of DNA single-strand breaks in both MSI and MSS cCSC was observed and was comparable with that observed in colon cancer cells. Preliminary analysis of whole exome sequencing data (collaboration with M. Tartaglia) indicates mutations in MMR, BER, recombination and DDR genes but not in NER genes. MSI cCSC present frequent mutations in replicative and trans-lesion synthesis DNA polymerases.

P9.4
$p21^{CDKN1A}$ tunes PCNA binding of NER factors thereby promoting DNA repair efficiency after UVC irradiation

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The cyclin-dependent kinase inhibitor $p21^{CDK1NA}$ is a protein involved in several different pathways, such as cell cycle arrest, transcription regulation, apoptosis, and cell motility. Due to the interaction with Proliferative Cell Nuclear Antigen (PCNA), p21 was found to be involved also in DNA repair processes. We focused our studies in order to investigate whether p21 interacts with NER proteins through PCNA binding after UVC irradiation and whether it could influence the recruitment of PCNA partners, such as DNA pol δ,, XPG, and CAF1. In vivo live-cell imaging confirmed that p21 did not inhibit, rather limited the recruitment of PCNA partner NER factors to avoid their accumulation and retention at the damaged sites. Western blot analysis and immunofluorescence experiments in p21-null fibroblasts reveal a delayed recruitment of NER factors compared with normal human fibroblasts and a lower repair efficiency. All together these findings suggest that p21 appears to play a regulatory role rather than an inhibitory role of the assembly of PCNA partners at DNA damage sites, thereby coordinating in time and space the interaction of PCNA with its multiple partners.

P9.5
Role for the Werner helicase-interacting Protein 1 in response to replication stress

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Replication stress is widely recognized as a major threat for genome stability, a well-known source of human diseases and cancer onset. Mounting evidence suggested that accurate handling of stalled replication forks is crucial to limit genome instability. Among the proteins participating in the maintenance of genome stability, whose function is still poorly characterized, is the human Werner helicase interacting protein 1 (WRNIP1). In this study, we demonstrate that WRNIP1 is directly involved in the protection and restart of stalled replication forks following replication stress. We establish that WRNIP1 avoids uncontrolled MRE11-mediated degradation of stalled forks by promoting RAD51 stabilization of ssDNA. The replication fork protection function seems

to require the ubiquitin-binding zinc finger (UBZ) domain of WRNIP1, but not its ATPase activity, which is instead implicated in the recovery of perturbed replication forks. Interestingly, loss of WRNIP1 or its functional domains causes extensive DNA damage and chromosomal aberrations. Overall, our findings unveil a novel role for WRNIP1 as a replication fork-protective factor in maintaining genome stability.

P9.6
Dna damage response during ribosomal stress

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"Ribosomal Stress" is a cellular condition caused by alterations in ribosome biogenesis as occurrs in Diamond Blackfan Anemia, a disorder caused by mutations in ribosomal protein S19 (*RPS19)*. Since ribosomal stress induces the activation of *p53*, our purpose was to identify a link between Ribosomal Stress condition and DNA damage response (DDR) and identify an extra ribosomal function of RPS19. We performed RPS19 knockdown in MRC-5 human primary fibroblasts, HCT116 and U251 MG tumor cell lines and then exposed cells to Ionizing Radiation (IR). The levels of key proteins involved in DDR and cell cycle control, as p53, $p21^{CIP1}$, MRN complex and γ-H2AX and 53BP1 foci were analyzed. The level of RAD51, member of Homologous Recombination repair, decreased in siRPS19-cells as well as the number of RAD51 IR-induced foci. Finally, we evaluated the level of protein synthesis through phosphorylation of RPS6 and eEF2 factors. peEF2 level increases after IR treatment, meaning a block during protein synthesis process. Experiments are in progress to ascertain the mechanism responsible for the observed effects.

P9.7
Cell-cycle phase dependency of BRCA1-induced gene reversion in *Saccharomyces cerevisiae*

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BRCA1 codes for a tumor suppressor protein involved in many cellular pathways, including genomic maintenance by promoting precise DNA repair depending on cell cycle: in S/G2 phases, BRCA1 inhibits the non-homologous end joining (NHEJ), promoting the error-free homologous recombination repair, as supports the NHEJ in G0/G1 phases. Previously, we have shown that cancer associated BRCA1 missense variants increase genome instability in yeast. In the present study, we expressed BRCA1 pathogenic and neutral missense variants in cell cycle arrested yeast cells and determined the effect on gene reversion using the *ilv1-92* system. We characterized the BRCA1 expression and protein level in cell cycle arrested yeast cells through qRT-PCR and Western blot analysis. Our results show that the expression of neutral variants has no effect on reversion, while some pathogenic variants lead to a 2-15 fold increase. This may indicate the involvement of a particular domain of the protein in the function normally performed at that cell cycle stage as well as suggests a possible tumorigenic mechanism of the variant.

P9.8
Biological characterization of three naphthalene diimide derivative G-quadruplex ligands in U251 glioblastoma cells

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G-quadruplex (G4) ligands are able to bind and stabilize secondary structures located in genomic G-rich regions such as telomeres, leading to telomere dysfunction, block of cell growth and radiosensitization. The effect of 3 naphthalene diimide (NDI) derivative G4 ligands was evaluated in glioblastoma cells (U251MG) and in normal primary fibroblasts (AG01522). NDI named C1, C2 and C6, were able to repress cell growth, induce DNA damage in genomic and telomeric regions in glioblastoma cells but not in fibroblasts. Data obtained show a high cytotoxic activity for the 3 ligands partly due to a significant induction of telomere dysfunction, despite the absence of telomere shortening. Chromatin Immunoprecipitation (ChIP) experiments in telomeric regions are ongoing to confirm such result. However, pretreatment with the drugs did not improve the response to ionizing radiation. NDI treatments were able to induce a rapid cell growth reduction also in drug-resistant and -sensitive (MCF-7/DX-WT) human breast adenocarcinoma cells. Despite data indicated that NDI bind and destabilize telomeres, we cannot rule out that the high cytotoxicity observed may be determined by other molecular targets.

P9.9
TLS Polymerases are critical for a proper response to UV lesions and to prevent unscheduled DSBs in non-replicating UV-irradiated cells

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UV light damages DNA by generating CPDs and 6-4PP photoproducts, which are responsible for the pathological effects of sunlight. UV lesions are removed by Nucleotide Excision Repair (NER). Mutations in NER genes cause the onset of severe pathologies, such as xeroderma pigmentosum and Cockayne syndrome. Upon DNA damage, checkpoint activation blocks or delays cell cycle progression to allow repair. In non replicating cells, the checkpoint response to UV light requires prior processing of UV lesions, mediated by NER factors and by the Exo1 nuclease. We proposed that Closely Opposing UV Lesions on the two DNA strands are problematic lesions that cannot promptly repaired by NER and are processed by Exo1. In this scenario, TLS polymerases would be involved in the repair synthesis step. We found that Pol η is recruited at EXO1-positive and EXO1-negative local UV damage sites (LUDs), as expected. Conversly, Pol ι and Pol κ always co-localize with the nuclease. We knocked-out EXO1 and demonstrated a requirement for EXO1 in Pol ι and Pol κ recruitment, consistently with our working model. Silencing TLS polymerases leads to hyper-activation of the UV-induced DNA damage checkpoint, suggesting that EXO1 continues to process UV damaged DNA eventually producing DSBs. TLS polymerases are thus crucial for the proper response to UV damage and to prevent genomic rearrangements in non-replicating cells.

P9.10
Phosphorylation of WRN by CDK1 and ATM is involved in the regulation of end-resection during DSBs repair

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Homology-dependent repair of DNA double strand breaks (DSBs) starts with DNA end-processing driven by WRN/DNA2 and/or EXO1 to form 3'-ssDNA overhang tails. Although CDKs regulate the EXO1-dependent pathway, regulation of the WRN/DNA2 branch is still unexplored.We previously reported that CDKs phosphorylate WRN at S1133, in vitro and in vivo. Here, we analyzed the functional role of this event during end-resection. To this aim, we generated WS-derived cell lines stably expressing WRN phosphomutants that either abrogate or mimic phosphorylation, and analyzed end-resection upon treatment with camptothecin, which induces replication-dependent DSBs. Using cellular and biochemical endpoints of end-resection, we show that CDK-dependent phosphorylation of WRN supports the DNA2-dependent pathway, contributing to replication fork recovery and

limiting chromosome instability. Given that WRN is phosphorylated also by ATM, we next investigated the crosstalk between CDK1 and ATM dependent regulation of WRN on end-resection. Using cells expressing WRN mutants that abrogate or mimic ATM-dependent phosphorylation, we demonstrate that loss of ATM-dependent phosphorylation of WRN affects DNA end-resection more than the absence of phosphorylation by CDK. Thus, our results suggest the existence of a crosstalk between CDK1 and ATM kinases to regulate WRN activity in DSBs processing at replication-dependent DSBs and ensure correct DNA repair.

P9.11
Replication checkpoint kinase CHK2 regulates MUS81 activity in response to replication stress

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MUS81 endonuclease is involved in producing Double Strand Breaks (DSBs) to resolve perturbed replication forks, under persisting replication arrest or checkpoint deficiency, and late recombination intermediates at G2/M transition. MUS81 activation needs to be tightly regulated to mediate the cellular responses to replicative stress in order to maintain genome stability. In yeast MUS81 is negatively regulated by *Cds1* kinase, while little is known about MUS81 regulation in human cells. Our data demonstrate that CHK2 kinase is able to interact with MUS81 *via* its ForkHead-Associated (FHA) domain in human cells. MUS81-CHK2 interaction is stronger in HU-arrested cells where MUS81-mediated DSBs increase, while inhibition of CHK2 activity leads to a decrease in DSBs. Interestingly, CHK2-FHA I157T mutation, identified in Li-Fraumeni syndrome, results in loss of MUS81 interaction and MUS81-depedent DSBs upon replication stress. Furthermore, our MS analyses revealed that T86 and S97 residues on MUS81 are phosphorylated by CHK2 *in vitro*. Therefore, we identify a new mechanism regulating a replication-stress induced activation of MUS81 by CHK2.

O9.1
DNA repair defects in Rubinstein-Taybi syndrome caused by low acetylation levels of base excision repair factors

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The Rubinstein–Taybi syndrome (RSTS) is a genetic disorder associated with growth defects, intellectual disability, and increased risk of tumors. About 60% of RSTS patients carry heterozygous mutation/deletion of *CREBBP* gene, while in a minor percentage (>10%) the *EP300* gene is involved. CREBBP and p300 proteins are acetyl transferases playing a key role in many aspects of DNA metabolism, including DNA repair. However, the efficiency of this process in RSTS is not yet elucidated. Here, we have investigated the DNA damage response and base excision repair (BER) in RSTS lymphoblastoid cell lines. We have observed that expression of cell cycle regulation and DNA repair proteins was not specifically modulated, while acetylation levels of BER factors (such as DNA polymerase b and OGG1) were reduced in RSTS cells. The analysis of BER efficiency evaluated by the comet and DNA incision assays revealed a defect in the repair of oxidative lesions in RSTS cells, due to the persistence of oxidized guanine (8-oxoG) at late recovery time points. These results suggest that reduced acetylation levels may be responsible for the lower OGG1 activity, inducing an impairment of BER in RSTS cells.

O9.2
Balancing DNA double strand break repair pathway choice as trigger for testicular germ cell tumors acquired-resistance to cysplatin

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Testicular germ cell tumors (TGCTs) are the most common cancer type in young men. Are considered a model of a curable neoplasm by cisplatin (CS) treatment. Beside this, patientes refractory to therapies have poor alternative therapeutic options. To understand the biology of TGCT-resistant tumors we analyzed their DSB repair capability in response to different DNA damaging agents. We observed that TGCT CS-resistant cell lines (CR-cells) were cross-resistant to several DNA damage sources, including those routinely used along with CS. However, CR-cells were relatively sensitive to ionizing irradiations. We correlated such behavior with an unbalance in the proficiency of Homologous Recombination (HR) and Non-Homologous End Joing (NHEJ) repair pathways. Specifically, although with different kinetics, CR-cells were more proficient in the assembly of HR factors BRCA1, RPA and RAD51, in response to CS. Conversely CS-sensitive cells showed greater 53BP1 foci accumulation in S/G2 phase of the cell cycle. The latter phenotype correlated with an increased 53BP1 expression in CS-cells. These findings indicate that, targeting HR or NHEJ pathways might improve chemotherapy response of CR TGCTs.

O9.3
Separase prevents genome instability by controlling fork replication speed

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Cohesion between sister chromatid is essential for ensuring that chromosomes are distributed correctly to daughter cells. In eukaryotes, sister chromatid cohesion is mediated by an evolutionary conserved complex called cohesin. Maintenance of genome stability is ensured through the concerted action of many cohesin proteins and in this regard separase plays a key role. Separase dysregulation has been shown to cause mitotic spindle defects, premature sister chromatid separation and lagging chromosomes. Here we describe a novel role for separase in the process of genome safeguarding that involves its ability to interact with replication fork. We provide evidence that separase works together MCM proteins and it is enriched at the origins of replication. Downregulation of separase results in increasing fork replication speed. Separase silencing leads also to chromosome missegregation and, unexpectedly, to structural aberrations in both primary human fibroblasts and HeLa cells. Our data show a novel mechanism for regulation of fork progression, mediated by separase. Loss of this mechanism leads to increase of DNA damage and may contribute to genome instability found in cancer cells.

O9.4
Inaccurate GEN1 DSBs formation promote genome instability in FA-P cells

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Rescue of stalled replication forks is crucial for genome integrity and

cell viability.Mammalian cells have developed several ways to overcome replication stress.One of theme entails the introduction of DSBs by the structure specific endonuclease MUS81 that interacts with SLX4.Lack or mutations in SLX4 causes a new subtype of Fanconi anemia, FA-P. Here we demonstrated that under pathological conditions, as oncogene expression or CHK1 inhibition, the absence of SLX4 in FA-P cells entails GEN1 engagement for DSBs formation. Interestingly, we observe that ectopic expression of the bacterial Holliday junction-binding factor RuvA in FA-P cells prevents GEN1-dependent DSBs during S-phase blocking its association to chromatin.Tuning RuvA expression timing so that its amount decrease approaching mitosis, we demonstrate that, in FA-P cells, premature activation of GEN1 leads to genome instability and cell cycle dealy, even though its presence is necessary in late phases of cell cycle to ensure cell viability.Indeed, GEN1 depletion in FA-P cells leads to high mortality, irrespective of the presence of RuvA during replication stress.Altogether, our results suggest that untimely activation of GEN1 in S-phase could contribute to genomic instability in FA-P cells but its function in mitosis promotes cell viability.

O9.5
Measuring oxidized DNA and RNA precursors by Micro-Raman spectroscopy

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DNA/RNA synthesis precursors are especially vulnerable to damage induced by reactive oxygen species occurring during oxidative stress. 8-oxo-dGTP and 8-oxo-rGTP are the prevalent oxidized nucleotides which can be incorporated into DNA or RNA and lead to mutations and cell death. The MTH1 enzyme protects against these effects by hydrolysing oxidized nucleotides. Since MTH1 inhibition is currently under development as novel target in cancer therapy, measurements of cellular 8-oxo-dGTP or 8-oxo-rGTP concentration provide an important strategy to monitor the enzyme function. Here we present a novel method based on Micro-Raman spectroscopy to reveal oxidative damage in the nucleotide pool. The analysis of d/rGTP and 8-oxo-d/rGTP Raman spectra with the support of *ab initio* calculations allowed us to identify specific and highly sensitive spectroscopic markers of oxidation. We developed a procedure to determine the concentration of 8-oxo-dGTP in dGTP, and their ribo counterpart, down to very low concentration. Present experiments pave the way for employing this procedure to identify the composition and quantitatively determine the oxidatively damaged nucleotide pool present in the cell.

O9.6
Effects of oxidative stress on telomere structure and telomeric epigenetic modifications

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Telomere represents the preferential target of oxidative damage. In our previous study we demonstrated the persistence of telomeric 8-oxoG non repaired and an increase of nucleoplasmic bridges after acute oxidative stress. In this work we wanted to understand how oxidative damage compromised telomere length and integrity in human primary fibroblats. The analysis of γH2AX and 53BP1 Telomere dysfunction-induced foci (TIFs) indicated an higher frequency of γH2AX-TIFs respect to 53BP1-TIFs, leading us to hypothesises a replication fork arrest rather than a DSB at telomere after acute oxidative stress. Results obtained by ChIP evidenced a significant reduction of TRF1 and TRF2 at telomere 48 hrs after hydrogen peroxide treatment. Together these findings lead us to suppose that the persistence of 8-oxoG induces telomere shortening/dysfunction by stalling of the replication fork and telomeric protein detachment, that cause telomeric fusions giving rise to the accumulation of NPBs. Furthermore, results obtained by ChIP assay indicated a significant increase of H3K9me3 48 hrs after hydrogen peroxide treatment. This data could be interpreted as a more condensed status of telomeric chromatin and should be deeper investigated.

10 - Non-coding RNA

P10.1
MicroRNAs as regulators of stress-related behavior: the case of miR-135a

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microRNAs are a class of non coding RNAs with a growing significance in regulatory mechanisms of gene expression related to brain function and plasticity. Recent studies indicate that they potentially orchestrate any complex phenomena sustained by structural and functional plasticity, as learning and memory and neuronal response to homeostatic challenges. We have recently shown that the amygdalar miR-135a is a component of the early stress response (Manironi et al, 2013). In this study, we have examined its role in the context of stress-related behavior. We found that the depletion of miR-135a in the amygdala of adult mice induced an increase in anxiety-like behavior. Furthermore, by *in vitro* studies with neuronal primary cultures, we demonstrated its role in the regulation of synaptic transmission. As direct targets of miR-135a, we characterized complexin-1 and -2 (Cpx1 and Cpx2), fine-tuners of synaptic activity and plasticity. Specific interactions between miR-135a and Cpx1 and Cpx2 mRNAs were demonstrated. Our findings pinpoint to miR-135a as a general modulator of synaptic plasticity, suggesting a physiological role in the modulation of stress-related behavior.

P10.2
RNA-Binding protein HuR and the members of miR-200 family play an unconventional role in the regulation of c-Jun mRNA

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Post-transcriptional gene regulation is a fundamental step for coordinating cellular response in a variety of processes. RNA-Binding protein (RBPs) and microRNAs (miRNAs) are the most important factors responsible for this regulation. Here we report that different components of the miR-200 family are involved in c-Jun mRNA regulation with the opposite effect. While miR-200b inhibits c-Jun protein production, miR-200a tends to increase c-Jun amount through a stabilization of its mRNA. This action is dependent on the presence of the RBP HuR that binds the 3'UTR of c-Jun mRNA in a region comprehending mir-200a binding site. The position of the binding site is fundamental: by mutating this site, we demonstrate that the effect is not micro-RNA specific. These results indicate that miR-200a triggers a microRNA-mediated stabilization of c-Jun mRNA promoting the binding of HuR with c-Jun mRNA, this is the first example of a positive regulation exerted by a microRNA on an important oncogene in proliferating cells.

O10.1
The lncRNA HOTAIR links the repressor Snail to epigenetic modifications of specific genomic sites in Epithelial-to-Mesenchymal Transition

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The transcription factor Snail is a master regulator of cellular identity and Epithelial-to-Mesenchymal Transition (EMT) directly repressing a broad repertoire of epithelial genes. How chromatin modifiers instrumental to its activity are recruited to Snail specific binding sites is unclear. Here we report that the long non-coding (lnc)RNA HOTAIR mediates a physical interaction between Snail and EZH2, enzymatic subunit of the Polycomb Repressive Complex 2 (PRC2) and main writer of chromatin repressive marks. The Snail repressive activity, here monitored on genes with a pivotal function in epithelial and hepatic morphogenesis, differentiation and cell-type identity, depends on the formation of a tripartite Snail/HOTAIR/EZH2 complex. These results demonstrate a lncRNA-mediated mechanism by which a transcriptional factor conveys a general chromatin modifier to specific genes, thereby allowing the execution of hepatocyte transdifferentiation; moreover, they highlight HOTAIR as a crucial player in the Snail-mediated EMT.

O10.2
Linc-NeD125 establishes a ceRNA network in Group 4 Medulloblastoma

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Long noncoding RNAs (lncRNAs) are regarded as crucial regulators of cellular processes in Eukaryotes. About 40% of currently characterized lncRNAs are specifically expressed in central nervous system, where they are involved in critical neural functions. Consistently, lncRNA aberrant expression is associated to neurological disorders. We recently identified a novel human lncRNA, linc-NeD125, that is induced in response to neuronal differentiation stimulus both in tumour cell lines and in embryonic stem cells. Notably, linc-Ned125 is significantly upregulated in a specific and still largely uncharacterized subgroup (Group 4) of Medulloblastoma (MB), the most common malignant paediatric brain tumour. Combining mechanistic and functional studies, we unveiled a novel lncRNA-mediated miRNA sponge regulatory network, in which the cross-talk among linc-NeD125, microRNAs and four Group 4 MB driver gene transcripts may significantly contribute to Group 4 MB cancerogenesis.

O10.3
Newly identified long non-coding RNAs promote proliferation and differentiation of murine myoblasts

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Myogenesis is a tightly regulated process that leads progenitor cells to become myoblasts and to fuse into multi-nucleated fibers with contractile capacity. The repertoire of regulatory factors controlling this process has been recently enlarged with the identification of non-coding transcripts, microRNAs and long non-coding RNAs (lncRNAs). We contribute to this field by the high-throughput identification of lncRNAs differentially expressed during in vitro murine myoblast differentiation (Ballarino et al., 2015). We focused on two lncRNAs, lnc-31 and lnc-049, having a cytoplasmic localization and expressed in proliferating and differentiating myoblasts respectively. We showed that lnc-31 plays a relevant function in controlling cell proliferation both in human and in mouse. In order to molecular dissect the mode of action of the two lncRNAs, we proceeded with the identification of their interactors such as protein factors as well as coding and non-coding RNAs. We found that lnc-31 and lnc-049 interact with specific mRNAs by base pairing and with proteins involved in translation control. These findings together with knock-down and overexpression experiments allow us to suggest that lnc-31 and lnc-049 modulate the expression of specific targets at post-trascriptional level, thus controlling myoblast proliferation and differentiation.

O10.4
Serum miRNAs as novel biomarkers in spinal muscle atrophy

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The identification of biomarkers is a critical issue in several conditions, including spinal muscle atrophy (SMA), a neuromuscular disorder. These tools are necessary for the objective evaluation of patients' function, also in response to different therapeutics. MicroRNAs (miRs) can be stably detected in serum and are considered a promising class of disease biomarkers. In the present study, we determined by Next Generation Sequencing (NGS) the miRNome of muscle biopsies and cultured muscle cells of patients and controls, to identify miRs with a potential pathogenic role in SMA and/or related to the degenerative process of skeletal muscle. To evaluate their potential applicability as biomarkers, deregulated miRs were subsequently quantified by absolute real time PCR in a large cohort of patients and controls. Among the miRs tested so far, three are emerged as novel biomarkers for SMA and their expression correlates with the severity of condition. We report for the first time the miR expression profiling of SMA muscle samples. A specific miR signature distinguishes SMA samples from controls, and may provide novel potential targets for therapeutic strategies.

11 - Environmental and Molecular Mutagenesis

P11.1
Exposure to air pollution and lifestyles of children partecipating in the MAPEC_LIFE (Monitoring Air Pollution Effects on Children for supporting public health policy) study

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The MAPEC_LIFE project is a multicentre cohort study that aims to assess the association between concentrations of certain atmospheric pollutants and early biological effects in children aged 6-8 living in five Italian towns (Brescia, Lecce, Perugia, Pisa and Torino). In order to evaluate the confounding role of other factors to which the subject may be exposed, the parents of the children were asked to fill in in two different seasons an ad hoc questionnaire. It was composed of 148 questions to obtain personal, anthropometric and health information on the children, as well as information on their lifestyles and parental characteristics. The definitive cohort was composed of 1164 children (50.9% boys, 95.4% born in Italy). The frequency of some factors were different between the survey season (physical activity, cooking methods) and among the cities (parents' level of education and rate of employment, sport, perceived traffic near the home, type of heating, passive smoking, cooking methods). Information on environmental exposure and the lifestyles of children will be integrated with other information acquired during the study in order to construct a global model of genotoxic risk.

P11.2
Toxic and genotoxic effects of repeated oral exposure to perfluorinated alkyl substances (PFAS) in C57BL/6 mice

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PFAS represent a class of environmental and water pollutants of high concern, implicated in a variety of adverse effects, including cancer. Studies indicated that PFAS are not genotoxic *per se* in *in vitro* and *in vivo* assays.The consequences on genomic stability of repeated *in vivo* exposure to PFAS, with the ensuing oxidative stress, has not been investigated. In this work perfluorooctanoic acid (PFOA) and perfluorobutanoate (PFBA) were administered for five weeks to C57BL/6 mice. Biochemical and cellular markers of liver toxicity, lipid peroxidation, oxidative stress, genotoxicity in liver, erythropoietic, spleen cells and testis were selected for assessment. A significant increase in liver weight and decrease in epididymis weight was observed at 1 and 5 mg PFOA /kg b.w. Flow cytometry analysis confirmed liver toxicity at the highest PFOA dose, with statistically significant increase of necrosis, S phase cells and polyploidy. A preliminary evaluation of genotoxic biomarkers did not show treatment related effects. Serum biomarkers, gene expression, metabolite profiling in liver, DNA damage in cultured splenocytes will be analysed. This work was partially supported by Regione Veneto

P11.3
A biotechnological approach for the development of new antifungal compounds to protect the environment and the human health

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Aflatoxins are a class of mycotoxins produced principally by *Aspergillus flavus* and *Aspergillus parasiticus* classified in Group 1 by IARC. Aflatoxins can occur naturally in food commodities as a result of fungal contamination in hot and humid environments. Aims of this project are the identification of new compounds that could inhibit *Aspergillus* proliferation and/or aflatoxin production and the set-up of a practical screening procedure to identify the most effective and safe compounds. We have evaluated the biological activity of two different molecules: perillaldehyde thiosemicarbazone and its nickel complex. These molecules once synthesized and characterized, were initially tested on fungal species belonging to the genus *Aspergillus* to determine their effects on fungal germination/growth and aflatoxin biosynthesis. These compounds showed different efficacy on fungal growth and on mycotoxin accumulation. The genotoxicity of these new compounds was assessed through Ames test and Alkaline Comet Assay on normal human cell lines to exclude potential danger to the environment and to human health. Financial support: Fondazione Cariplo-Project N. 2014-0555, http://aflatox.unibs.it/

P11.4
Evaluation of synthetic amorphous silica nanoparticles toxicity on the male reproductive system in rats after sub-chronic oral exposure

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Due to continuous development and widespread use of nanoparticles (NPs), concern has been expressed about their potential harmful effects on human health and their regulatory risk assessment has become mandatory. The potential effects on reproduction and fertility are relevant in this respect. Evidences exist that different types of NPs, after various exposure routes, reach the testis, cross the blood-testis barrier and affect testis cells. Here we report observations on the male reproductive system of rats orally treated for 90 days with low, realistic doses of synthetic amorphous silica, a nanomaterial currently used as a food additive (E551). At sacrifice, testosterone serum levels were evaluated, epididymal sperm were counted, histopathological analyses were conducted on testes, DNA damage was evaluated in testes and sperm, and sperm chromatin alterations were assessed by Sperm Chromatin Structure Assay. Overall results did not show evident induction of toxicity. The results will be presented in relation to literature data about the male reproductive system response to NPs with different physico-chemical characteristics. Supported by EU FP7 project NANoREG, grant 310584

P11.5
Silica nanoparticles and ozone: an evaluation of their *in vitro* cytotoxicity and genotoxicity in a experimental model of indoor air

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Several studies point out the effects on health of indoor air pollutants, i.e. nanoparticles (NPs) containing black carbon, silica and gases like ozone (O_3). The work is a multidisciplinary study of the potential cyto-genotoxicity of silica NPs and O_3 in *in vitro* cell systems.We analyzed cell lines A549 (human lung epithelial cells) and HS 27 (human fibroblasts) exposed under dynamic conditions by a simulator IRC under the stream of ozone and silica NPs (about 40µg/h). A549 do not show a significant difference in viability (MTT test) in the presence of ozone at 48 and 72 h but an increase of 30-40% of cell death in the presence of silica NPs and silica NPs/ozone. HS27 showed a viability reduction of 10-15 % at 48 and 72 h either in the presence of ozone, silica NPs and silica NPs/ozone. Micronuclei show an increase of 45 % in A549 and 35% in HS 27 in the presence of ozone, silica NPs and silica NPs/ozone. The comet test shows a 40% increase of the Tail Moment in both lines in the presence of silica NPs and silica NPs/ozone. Final output will be a picture of the role of silica NPs/ozone in the indoor air quality taking into account the potential simultaneous co-toxicant action.

P11.6
Characterization of telomere length and telomerase activity in iPS cells and the relationship with chromosome instability

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Induced Pluripotent Stem cells (iPSCs) are derived by adult differentiated cells by Yamanaka's retrovirus transduction: after reprogramming, this cells are totally similar to embryonic stem cells (ESCs). Telomeres are heterochromatic region that caps chromosome end and telomerase is responsible to maintain their length. We know that in mouse, telomerase activity is essential to maintain self-renewal ability of iPSCs, telomere and karyotype stability, while its absence does not prevent colony formation but with an increase of chromosome instability (CIN). Therefore, in human, there is the need to better investigate on this cells because if telomerase is not active enough, clones could be more susceptible to CIN. With this aim we test for telomere length, telomerase and CIN in iPSCs derived from human fibroblasts. Our data show that in our iPSCs samples telomeres are elongated respect to the original fibroblasts and they became longer during subsequent passages, until they reach ESCs telomeres length. In fact preliminary results obtained from TRAP assay indicated a telomerase activity in these cells. Due to the role of telomere in chromosome stability, also the CIN will be evaluated.

P11.7
Effects of telomerase inhibitor Epigallocatechingallate on human glioblastoma cells

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Epigallocatechingallate (EGCG) is the major polyphenol in green tea, with known anticancer features, such as anti-oxidative and anti-angiogenetic properties, regulation the molecular pathway of the cell cycle and signal transduction. Moreover, it is also well known as telomerase inhibitor. In this work, we have chronically treated with EGCG glioblastoma cells (U251) for 100 days with low, pharmacologically appropriate concentrations, in order to investigate its effects both on telomeres and on genome integrity. Inhibition of telomerase activity caused telomere shortening, ultimately leading to senescence and telomere damage after 100 days. Interestingly, we have observed DNA damage through an increase of micronuclei, nucleoplasmic bridges and phosphorilation of γ-H2AX histone, also when telomere shortening was not present. Therefore, we concluded that this genotoxic damage was not correlated with telomere shortening and that EGCG chronic treatment induced not only an increase of telomere-shortening-induced senescence, but also genotoxicity. Thus, DNA damage induced by EGCG raises serious concerns for its application in cancer therapy

P11.8
Evaluation of mutagenic/genotoxic effect of $PM_{0.5}$ collected in five italian towns in two seasons: results of the MAPEC_LIFE study (LIFE12 ENV/IT/000614)

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Particulate Matter (PM) is the atmospheric pollutant that mostly affects human health. One aim of the MAPEC_LIFE study is to evaluate children exposure to urban air pollution investigating the $PM_{0.5}$ mutagenic and genotoxic effect. The samples were collected in children school areas in two seasons (winter-spring) using a high-volume air sampler. $PM_{0.5}$ organic extracts were chemically analyzed, assayed on four Salmonella strains by Ames test and on A549 cell line by comet assay and micronucleus test. Results revealed that $PM_{0.5}$ represents a very variable PM_{10} percentage (range 19.6-63% and 9.9-55.9% in winter and spring respectively). In winter all $PM_{0.5}$ extracts showed at least one mutagenic dose with Salmonella TA98 strain suggesting the presence of indirect mutagens, while a lower effect was observed with the TA100 strain. The results with TA98NR and YG1021 strains in both seasons showed the presence of nitroaromatic compounds as confirmed by the chemical analysis. No genotoxic or oxidative effect was observed using the comet assay and micronucleus test in both seasons. The results suggest to investigate the biological effect of the other PM fractions, in particular $PM_{0.5-1}$.

12 - Plant Nutrition

P12.1
The characterization of durum wheat adaptive responses to different Fe availability highlights an optimum Fe requirement threshold

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Durum wheat, as Strategy II plant, copes with iron (Fe) deficiency by increasing the secretion of phytosiderophores (PS). Sulfate assimilatory pathway is known to be induced upon Fe deprivation in several grasses, such as maize, barley and wheat, most likely because PS are produced from nicotianamine, whose precursor is methionine. The physiological plant response - in terms of plant ionome, PS release, thiol content and S pathway-related enzymes - induced by decreasing Fe availability by degrees (from 75 to 0 µM) allowed the identification of three specific limit Fe concentrations: 75 µM, 25 µM and 0 µM Fe, *i.e.* the complete Fe starvation. At each limit, plants begin to induce different and specific adaptive responses to improve Fe acquisition or to reduce the damage resulting from limited Fe availability. The identification of the Fe availability level below which durum wheat plants start an expensive metabolic reorganization of S and several other elements, could be of benefit not only for an effective cultivation of the crop but also for the grain quality.

P12.2
Nitrogen assimilation , yield productivity and quality in mycorrhized tomato plants

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The effects of root colonization by arbuscular mycorrhizal (AM) of mix fungus *Glomus mosseae and Glomus intraradices*, on nitrogen metabolism, fruit yield and environmental sustainability were studied in field-grown tomato plants by drip irrigation and exposed to limiting P soil content 5 µg/gDW (basal soil) with nitrate fertilization (40 µg/gDW), after greenhouse germination. At 140 days after sowing, in the harvesting fruit stage mycorrhizal plants (M) had significantly higher mineral nutrient, organic nitrogen and phosphate compounds in both roots and leaves compared to no mycorrhizal plants (NM). In this contest the enzyme activity as NR and GS involved in nitrogen metabolism was tested in root as in leaf. AM inoculation also significantly increased growth and productivity parameters. The fruit yields of M plants were higher than NM plants by 40% and containing significantly higher quantities of licopene, carotene, mineral nutrients and total amino acids. , than NM plants, suggesting that mycorrhizal colonization affects host plant nutritional status, and growth under P limitant field conditions and modified reproductive behaviour, fruit production and quality. We thank the University of Molise and the Microspore S.p.A. for their support.

P12.3
Lupinus albus (L.) plants use common mechanisms to overcome either iron (Fe) or phosphorus (P) deficiencies

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White lupin (*Lupinus albus* L.) has developed a highly efficient strategy to acquire sparingly soluble nutrients, like P and Fe, from soils. This strategy is based on modification of the root architecture with the formation of cluster roots that are able to release large amounts of exudates in a small volume of soil. The aim of this work was to unravel the mechanisms involved in these processes via the combination of RNAseq and physiological approaches. In comparison to control roots (+Fe, +P), about 5500 or 2000 genes were modulated in cluster roots of Fe- or P-deficient plants, respectively; more than 1000 genes were commonly modulated. Most of the known genes coding for mechanisms involved in either Fe or P acquisition were upregulated by Fe as well as by P deficiency. The reciprocal activation of Fe and P acquisition systems was also confirmed by uptake and mobilization assays using labelled ^{59}Fe and ^{32}P sources. In conclusion, white lupin plants activate both P and Fe acquisition mechanisms contributing to the overall nutrient efficiency of white lupin plants; this behavior would reflect an adaptation to low pH soils, where Fe and P co-precipitate.

P12.4
Diatom response to variations in nitrogen availability

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Nutrient concentrations, in particular Nitrogen (N), in the ocean show significant temporal and spatial variability, which affects growth and distribution of phytoplankton. Diatoms are unicellular microalgae constituting one of the most important phytoplankton groups in the ocean, their adaptive capacity suggests that they have sophisticated mechanisms to perceive and respond to environmental variations. The molecular mechanisms that allow diatoms to efficiently cope with N availability remain largely unknown. Our analysis of available genomic and transcriptomic data shows that, the presence of multiple NH4+(AMT) and NO3−(NRT2s/NPFs) transporter genes, differentially regulated, is a conserved feature of diatom species living in different ecological niches and we refined the analysis with a phylogenetic assessment (Rogato et al, 2015). In addition, we found that N limitation influence the expression of the light-harvesting protein family LHCXs, potential markers for evaluating the effects of N deficiencies (Taddei et al, 2016). These preliminary studies provide a springboard to shed light on the evolutionary advantage of these N transporters and on the N metabolism in diatoms.

P12.5
Action of protein hydrolysates on maize seedlings roots: a genome-wide transcriptional analysis

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Protein hydrolysates are plant growth-promoting products that contain a mixture of peptides and free amino acids derived from the hydrolysis of plant and animal sources, but also from industrial and agricultural residues. Free amino acids have several beneficial effects, but the role played by short chain peptides could also be relevant. The aim of this work is to study the effects and the mechanism of action of a protein hydrolysate produced by SICIT 2000 S.P.A. on maize roots compared with the effects produced by either free amino acids mixture or inorganic nitrogen. The protein hydrolysate was highly effective in promoting root growth and increasing the concentration of micronutrients. The total surface area of the lateral roots was approximately 1.5 and 7 times higher in seedlings treated with the protein hydrolysate as compared with seedlings treated with the same total N supplied as either free amino acids or inorganic N, respectively. The genome-wide transcriptional analysis allowed to highlight global changes in gene transcription produced

by the different treatments across multiple metabolic pathways and processes such as N metabolism, ions transport, hormonal metabolism.

O12.1
Plant adaptation mechanism to Fe deficiency: the role of metabolism

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Iron (Fe) is an essential element for plant life as it is a fundamental constituent in many metabolic processes, such as photosynthesis and respiration. Low Fe availability affects such processes impairing, therefore, the functionality of chloroplasts and mitochondria respectively. While chloroplasts are leaf-localized, mitochondria are widely present in all cell tissues. Therefore, mitochondria represent the most Fe-requiring compartments in cells and they might be of a central importance to decipher the regulation of Fe deficiency-induced metabolic changes in plants. However, Fe is required also for several enzymes involving in other important processes, such as pathway characterizing the secondary metabolisms. Indeed, several reports showed that Fe deficiency affects also the content of a wide number of metabolites in the plant. Such metabolites have potentially a role in some signalling pathways, representing therefore putative players of the Fe sensing and signalling mechanism in plants. Understanding the regulation of the Fe-containing enzymes in plants under Fe deficiency will provide important findings on the plant metabolic adaptation to this nutritional stress.

O12.2
The urease inhibitor N-(N-butyl) thiophosphoric triamide (NBPT) affects urea acquisition and metabolism in maize seedlings

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To limit nitrogen (N) losses from soil, it has been proposed to provide urea fertilizer in association with the urease inhibitor NBPT. Aim of this work was to study at physiological, metabolic and transcriptomic levels, the effects of NBPT on urea nutrition in hydroponically grown maize seedlings after a short-term exposure to the inhibitor. The presence of NBPT in the urea-containing nutrient solution limited growth, ^{15}N (urea-derived) accumulation and urea uptake rate. Furthermore, the activity of endogenous urease was limited by NBPT treatment determining an accumulation of urea in maize tissues. Transcriptomic analyses provided evidence that NBPT treatment led to a wide reprogramming of plant metabolism in urea-fed maize roots. Genes involved in the cytosolic pathway of ureic-N assimilation and ammonium transport were down-regulated by NBPT. Microarray data also indicate that the inhibitor modulated primary and secondary metabolic pathways (glycolysis, TCA cycle, polyamine and phenylpropanoid synthesis). Data suggest that when NBPT is provided in conjunction with urea, an imbalance between C and N compounds might occur in plant cells, mimicking a condition of N deficiency.

O12.3
Fungal and plant gene expression in the *Tulasnella calospora - Serapias vomeracea* association provides cues on the N pathways in orchid mycorrhiza

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Orchids display a fascinating ecological strategy as mycorrhizal parasites because, especially during early development, they receive all nutrients from their fungal partner without any apparent reward. Most experiments on nutrient exchanges in orchid mycorrhiza (ORM) have focused on organic C transfer from the fungus to the host plant, but far less is known about other nutrients, such as nitrogen. We have investigated N metabolism in a model system developed *in vitro* between the fungus *Tulasnella calospora* and the orchid *Serapias vomeracea*. The genome of *T. calospora* lacks genes for nitrate uptake but features two ammonium transporters that were shown to be functional by heterologous expression in yeast. A low affinity fungal transporter, in particular, was differentially expressed on different N sources and upregulated in symbiosis. We also investigated the expression, in mycorrhizal and non-mycorrhizal conditions, of several other fungal and plant genes that may be potentially involved in N metabolism. Although the results of RNASeq and RT-qPCR require further experimental support, they provide a first working model and suggest that some amino acids may be the main N form transferred to the orchid host.

O12.4
$FePO_4$ nanoparticles ad fertilizers: synthesis and evaluation of their effect on plants

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The expected increase of world population and the limited availability of new cultivable soil suggest the importance of plant nutrition for a sustainable development. In order to increase field production, the use of fertilizers is indispensable, although their negative environmental impact. A strategy to improve nutrient use efficiency (NUE) could be the development of new and more efficient fertilizers. A promising field in order to achieve this goal could be the use of nanotechnology. Nanomaterials are widely used in medical and pharmaceutical fields, but their application in agriculture is at its infancy. Data shown here are about the development of the method for the synthesis of $FePO_4$ nanoparticles, and a preliminary characterization of their features and effects on cucumber plants growth. $FePO_4$ nanoparticles are produced via a continuous co-precipitation method, based on the continuous mixing of a $FeCl_3$ solution with a K_2HPO_4 solution. This method allows producing nanoparticles smaller than 100 nm. Preliminary experiments made on cucumber plants grown hydroponically suggest positive effects of $FePO_4$ nanoparticles in restoring Fe and P deficiencies, comparing to non-nano $FePO_4$.

O12.5
Isolation and characterization of a new *low phytic acid* mutant in the common bean *PvMRP1* gene and study of *PvMRPs* promoters in two different plant systems

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Phytic acid ($InsP_6$) is the main storage form for phosphate in seeds and in the plant it plays an important role in signal transduction in response to environmental stress and hormonal changes. $InsP_6$ is a strong chelator of mono and divalent cations, such as iron, zinc, magnesium and calcium, essential minerals in the diet, reducing their bioavailabilty. We previously isolated the common bean *lpa1-1* mutant, affected in the *PvMRP1* gene, coding for a putative tonoplastic phytic acid transporter. With the aim to isolate new common bean *lpa* mutants we partially screened an EMS population and identified a new *lpa* mutant, hereinafter called *lpa1-2*, as it resulted allelic to *lpa1-1* mutant. The *lpa1-2* mutation consists in a premature stop codon. In this work, we present preliminary data of the phenotypic characterization of *lpa1-2* mutant. In common bean there is also the *PvMRP2* gene, paralog of *PvMRP1*, probably able to complement the *lpa1* phenotype in other tissues than the seed, thus explaining the lack of pleiotropic effects in the bean mutant. In order to analyze promoters of these two genes, constructs harboring portions of 1.5 kb of their putative sequences fused upstream of the *GUS*

reporter were generated and used to transform two species: *Arabidopsis thaliana* and *Medicago truncatula*. Data on reporter expression in both plant systems will be presented.

13 - Cellular Stress, apoptosis and autophagy

P13.1
Different responses to berberine in human cell lines

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The natural alkaloid berberine exhibits different pharmacological properties. We analyzed antitumor effects of berberine in MIA PaCa-2 cells (pancreatic carcinoma) and U343 (glioblastoma). Human dermal fibroblasts (HDF) were used as a non-tumor control. We observed that berberine localizes in the cytoplasm and/or in the nucleus in a dose-dependent manner. Berberine reduced the TMRM signal, a marker of mitochondrial membrane potential, indicating a decay of mitochondrial activity in all cell lines. Since berberine differentially affects viability of these cells, we investigated what type of death is induced by the alkaloid. Our results show that low concentrations of berberine induce CASPASE-3 activity in HDF, whereas a higher concentration is required in MIA PaCa-2 and U343 cells. As shown by β-galactosidase assay, berberine increases senescence in all cell types. Berberine also induces autophagy in the two cancer cell lines, but not in HDF. Wound healing assay indicates that berberine dose-dependently inhibits migration of MIA PaCa-2 cells. Finally, this alkaloid differently affects the expression level of genes involved in carcinogenesis in the analysed cell lines.

P13.2
Development of an ES based system for the analysis of antioxidant or oxidant activity of natural molecules

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The use of stem cells as alternative method to animal testing represents a novel tool for development of specific methods to validate the biological activity of natural plant-derived compounds in a short time. Our group is interested in evaluating the effectiveness of natural extract such as olive fruits and leaves extracts, curcumin and other. Preliminary experiment indicated that these molecules induce apoptosis in caner cells while they show a protective effect against the oxidative stress in normal cells. In our lab is available an ES based system that allow evaluate the protective ability against oxidative stress of different natural molecules. Embryonic stem cells (ES), having the ability to self-renew and to differentiate into a wide range of tissue-specific cells, represent one of the most powerful model system in basic research and technological applications. The system was developed generating a mouse G6PD-null embryonic stem cell line (G6pd delta ES cells) that are extremely sensitive to oxidative stress.

P13.3
DNA damaging agents and autophagy

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Autophagy is a pathway that plays a key role in the degradation and recycling of cellular components. It can be activated in response to DNA-damaging agents and it seems to have a role in DNA damage response (DDR). Recent studies have partly clarified the pathways that induce autophagy activation during DDR, but the real role of autophagy in this cellular response remains unknown. We have selected molecules able to induce or not DNA damage. The toxic and genotoxic effects on U937 cell line were assessed for each compound and for each of them in presence of an inhibitor (chloroquine) or an inducer (rapamycin) of autophagy. Preliminary data show that the co-treatment with rapamycin reduces the genotoxic potential of the assessed compounds. The inhibition of autophagy through chloroquine do not alter the genotoxicity induced by each molecule but it leads to an increase of cytotoxicity. We confirm the cytoprotective role of autophagy during DDR; its inhibition can sensitize cancer cells to DNA-damaging agents. The modulation of this pathway could be an innovative approach able to reduce toxicity of many compounds and to enhance the activity of some others, including anticancer drugs.

P13.4
Involment of PDIA3 in cellular response to cigarette smoke condensate

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PDIA3, a disulfide isomerase protein contributing to the correct folding of newly synthetized glycoproteins, is also involved in oxidative stress response and several human diseases. Cigarette smoke condensate (CSC) has been widely associated to important illness. To relate the involvement of PDIA3 with the stress induced by CSC we followed PDIA3 expression levels, by western blot and RT-PCR analysis, in breast cancer cells subjected to CSC treatment. Results show that PDIA3 do not increase up to 24h following the treatments while there is fivefold increase in PDIA3 mRNA levels at 24h. This discrepancy may be the result of mRNA instability, a protein degradation or secretion. Immunofluorescence analysis on CSC treated cells shows a redistribution of PDIA3, which shift from a manly perinuclear localization to a more periplasmic zone, supporting the ideas of a protein secretion. *In vitro* studies show that punicalagin is a strong PDIA3 inhibitor. Hence, we tested the effects of punicalagin on breast cancer cells treated with CSC. Initial studies show that punicalagin may reduce cytotoxicity induced by CSC treatment as well as the protein redistribution observed by immunofluorescence.

P13.5
Simultaneous mitochondrial retrograde pathway activation and SNF1-dependent relief of glucose repression are responsible for yeast acetic-acid induced programmed cell death evasion in raffinose.

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We use yeast as a model to investigate stress response in different environments, such as different carbon sources. *Saccharomyces cerevisiae* cells grown on glucose undergo programmed cell death (PCD) induced by acetic acid (AA-PCD) similarly to mammalian mitochondrial apoptosis, but evade AA-PCD when grown in raffinose. This is due to glucose catabolite repression (GCR) relief and activation of mitochondrial retrograde (RTG) pathway. The relationships between the RTG and GCR pathways in the modulation of AA-PCD sensitivity under glucose repression or derepression conditions are investigated. Yeast cells lacking the RTG pathway regulator *RTG2* and/or certain factors regulating carbon source utilization, such as *MIG1* and *HXK2,* active during GCR, and *ADR1*, *CAT8*, and *HAP4,* active under derepression conditions, have been analyzed for their survival and RTG-pathway activation after AA treatment. *ADR1* and *CAT8* interact with *RTG2* in the regulation of transcription of retrograde target genes and AA-PCD evasion in raffinose-grown cells. Simultaneous RTG-pathway activation and relief of GCR have a key role in carbon metabolism reprogramming which modulates yeast AA-induced stress response.

P13.6
Fusion or fission: the destiny of mitochondria in different severities of traumatic brain injury

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Mitochondrial dysfunction contributes to the pathophysiology of acute neurological disorders, as traumatic brain injury (TBI); mitochondrial dynamics is fundamental to identify the pathological mechanisms and potential therapeutic targets. The mitochondrial plasticity depends on a balance between antagonistic forces of fission and fusion, determining the fate of mitochondria and consequently of the cells. These complicated mechanisms are regulated by the synergistic effect of GTPase fusion or fission related proteins. Gene and protein expressions and Immunohistochemistry were performed using an experimental TBI (mild and severe) in rats, sacrificed at 6, 24, 48, 120h after insult. Results showed that proteins inducing fusion were upregulated and proteins inducing fission were downregulated in mTBI, whilst in sTBI all proteins propended to fission. In particular, OPA1 played an important role in determining the fusion or fission of mitochondria. We reported how different ratio of long and short OPA1 can induce or inhibit cell death, suggesting the role of OPA1 either to maintain cell functions or as an attractive therapeutic target to counteract mitochondrial dysfunction after injury.

P13.7
Targeting cutaneous melanoma with nutraceutic compounds: the antitumor activity of δ-Tocotrienol

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Cutaneous melanoma is the deadliest form of skin cancer: the prognosis of metastatic disease is still poor, despite the emerging therapies. The interest in several nutraceutic compounds is increasing, because of their antitumor activity and the lesser toxicity with respect to standard therapies. Tocotrienols, vitamin E components, were reported to exert an anticancer activity in different tumors, so we assessed the effects of δ-Tocotrienol (δ-TT) on the viability of human melanoma cell lines, together with the molecular mechanisms associated with its activity. We could demonstrate that δ-TT activates the apoptosis process (e.g. cleavage of caspase-3 and PARP, Bax/Bcl2 increasing ratio and cytochrome c release), and the endoplasmic reticulum (ER) stress pathways (e.g. IRE1α and PERK pathways, expression of ER stress markers such as CHOP). Finally, experiments were conducted on mouse melanoma xenografts, demonstrating the efficacy and the lack of toxicity of this compound, and suggesting that it could be considered to prevent tumor development or improve the current treatment strategies. *(Supported by Fondazione Banca del Monte di Lombardia and Comitato Emme Rouge Onlus).*

P13.8
Retinoic Acid sensitizes acute myeloid leukemia cells to ER stress

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Chemotherapy plus retinoic acid (RA) result in cure rates of acute promyelocytic leukemia (APL) exceeding 80%. Although resistant or relapsed patients are effectively treated with arsenic trioxide (ATO) plus RA, elevated costs limit its use. Moreover non-APL AML do not respond to RA indicating the need for novel therapeutic strategies. We show that RA induces ER stress in APL cells slightly activating the unfolded protein response (UPR). This is sufficient to render human leukemic cell lines and primary blasts very sensitive to doses of ER stress inducing drugs, like tunicamycin (Tm), not toxic for the same cells in the absence of RA or for most cell types. Furthermore, low doses of Tm, even in the absence of RA, are sufficient to strongly increase ATO toxicity. Indeed both RA-sensitive and resistant APL cells resulted sensitive to Tm-ATO combined treatment at doses of ATO ineffective in the absence of ER stress. The use of inhibitors targeting specific UPR branches indicates a main role for the PERK pathway. Finally, we extended our observations in a non-APL model, assessing that RA sensitize the non-APL cell line HL60 to ER stress. Thus, our data indicate ER stress as a possible target for novel combination therapeutic strategies in AML.

P13.9
Role of PLCγ-mediated signaling in the crosstalk between autophagy and phagocytosis induced by FGFR2b

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Signaling of the epithelial splicing variant of the fibroblast growth factor receptor 2 (FGFR2b) induces both autophagy and phagocytosis in human keratinocytes. Here we investigated, in our cell model of HaCaT keratinocytes, if the two processes might be related and the possible involvement of PLCγ signaling in the autophagy triggered by FGFR2b activation. Using fluorescence and electron microscopy we demonstrated that the FGFR2b-induced phagocytosis and autophagy involve converging autophagosomal and phagosomal compartments. Moreover, the forced expression of FGFR2b signaling mutants and the use of specific inhibitors of FGFR2b substrates showed that the receptor-triggered autophagy requires PLCγ signaling, which in turn activates JNK1 via PKCδ. Finally we found that, in primary human keratinocytes derived from light or dark pigmented skin and expressing different levels of FGFR2b, the rate of phagocytosis and autophagy and the convergence of the two intracellular pathways depend on the level of receptor expression. These results suggest that FGFR2b signaling would control in vivo the number of melanosomes in keratinocytes, determining skin pigmentation.

P13.10
Biological features of honeydews honeys

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Honey is a sweet and flavorful natural product, which is consumed for its high nutritive value and for its effects on human health, with antioxidant and antimicrobial properties, as well as wound and tissue healing effects. According to the origin, honey can be classified in different categories as follows: (1) blossom honey, obtained predominantly from the nectar of flowers and (2) honeydew honey, produced by bees after they collect "honeydew" (secretions of insects, which pierce plant cells). Within monofloral honey, manuka honey, a dark honey, has recently attracted the attention for its biological properties. At present time, the relationships between honeydew honeys and their healing properties and tissue repair processes are largely unexplored. Therefore, we started a project to investigate the potential wound healing effects of honeydew honeys on skin cells. By cellular and molecular analysis, applied to *in vitro* scratch wound models, we have shown

an increase of wound closure on both keratinocytes and fibroblasts. These studies would support the increased use of honeydew honeys in skin medicine, and they can also be the basis for the isolation and purification of compounds for the development of bio-pharmaceutical products and to add economic value that can favor also the beekeepers in their production.

P13.11
Cell toxicity by mitochondriotropic environmental contaminants

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In recent years attention has grown about those molecules that in leaving cells are able to directly target mitochondria, modifying their bioenergetic functions. Besides the known ionophores such as valinomycin, nigericin, melittin all depressing the mitochondrial membrane potential, other molecules possess uncoupling properties, disconnecting the redox-coupled electron/H^+ transfer reactions from ATP synthesis. The possibility of measuring the mitochondrial $\Delta\mu H^+$, and its components $\Delta\psi$ and ΔpH, prompted us to verify whether changes in their absolute or relative values are induced by environmental contaminants. One might expect that these mitochondriotropic molecules would a) alter the cell ATP levels, compatible with the glycoytic compensation b) induce the nitro-oxidative stress c) activate cell apoptosis. In this frame, we have observed the bioenergetic changes of HepG2 and HaCaT cells incubated with pesticides like glyphosate or other ubiquitous pollutants used to manufacture antioxidants, household and industrial detergents and lubricating oils.

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O13.1
Citron Kinase deficiency leads to p53-dependent microcephaly and DNA damage accumulation indipendently of its cytokinetic function

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Citron kinase (CITK) is a highly conserved protein, whose inactivation in mammals leads to a severe microcephaly, characterized by cytokinesis failure and apoptosis. This finding led to the hypothesis that the two processes could be causally related. Here we show for the first time that the loss of CITK induces DNA damage accumulation and chromosomal instability both in mammals and in Drosophila. In CITK depleted cells we observe that the accumulation of DNA damage correlates only partially with cytokinesis failure, indicating that maintenance of genomic stability is a primary function of CITK. Moreover, we observe that CITK is capable of forming a complex with RAD51 and AGO2, and that RAD51 recruitment to DNA-damage foci is impaired by CITK depletion, suggesting a direct role of CITK in homologous recombination-dependent DNA repair. Finally, we observe that CITK/TP53 double knockout mice show a remarkable improvement of the clinical and neuroanatomical phenotypes, concomitant with the disappearance of apoptosis despite a strong accumulation of multinucleated cells. Our results underscore a crucial role of CITK in the maintenance of genomic integrity during CNS development.

O13.2
The nucleolar protein Nucleophosmin is rapidly secreted by human cardiac stromal cells in response to genotoxic stress: implications in miRNA mediated cell/cell communication

S. Beji

Doxorubicin (Dox) & Trastuzumab (Trz) are highly effective against breast cancer but linked to cardiotoxicity. Recent evidence shows that chemotherapy targets cardiac progenitor cells decreasing heart tissue homeostasis and repair. We studied the effect of Dox/Trz on human cardiac stromal cells (hCStCs), a highly regenerative subpopulation. Human CStCs expressed HER2 receptor (the molecular target of Trz) both in vivo and in vitro, as assessed by immunofluorescence (IF) and FACS analysis. Dox and Trz provoked hCStCs death within 48 hours and nucleolar stress within 8h of treatment that was associated with delocalization of Nucleophosmin (NPM), a sensor of genotoxic stress and inhibition of rRNA synthesis. Dox/Trz elicited p53 and DNA damage of γH2aX induction. It is noteworthy, Dox/Trz induced NPM secretion in the medium within 8h of treatment in the absence of cell necrosis. Moreover, NPM active secretion was observed also in response to UV induced DNA damage. Finally, recombinant NPM induced a dose-dependent decrease of hCStCs proliferation within 48h of treatment and when associated to mimetic C.Elegans miR-39a it enhanced its uptake in hCStCs. We suggest that NPM could be considered as a new potential signaling molecule regulating cell/cell comunication through modulation of CStCs proliferation and miRNAs uptake following nucleolar stress.

O13.3
RNA oxidation and ageing in mRNA degradation mutants of *S. cerevisiae*

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The management mechanisms of the oxidized cellular mRNA and/or the way in which the oxidized bases influence the process of mRNA translation are not well characterized. During aging, glucose deprivation, oxidative and osmotic stress, the mRNAs are transferred to the P-bodies that, in these conditions, increase in number. We found that in mRNA decapping mutants the mutation rate increases during chronological life span indicating that, in yeast cells, the capability to handle oxidized RNAs drops with aging. In this respect, the Kllsm4Δ1 mutant, which accumulates mRNAs and ages much faster that the wild type strain, represents a useful tool for deciphering the molecular mechanisms of cell response to mRNA oxidation.

O13.4
rpL3 mediates the cell response to nucleolar stress induced by Act D in p53 null cancer cells

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Many chemotherapeutic drugs impair ribosomal biogenesis causing nucleolar stress and p53-independent pathways mediating the nucleolar stress response are emerging. Here, we demonstrate that nucleolar stress induced by Actinomycin D is associated to the up-regulation of ribosomal protein L3 (rpL3) and its dissociation from the ribosome in lung and colon cancer cell lines lacking p53. We show that rpL3 is involved in drug-induced apoptosis and inhibition of cell proliferation and migration by controlling p21 expression both at transcriptional and post-translational levels. Depletion of rpL3 abolishes the cytotoxic effects of Act D while rpL3 overexpression was associated to a strong increase of Act D-mediated inhibition of cell migration. We identified

extracellular-signal-regulated kinases1/2 (ERK1/2) and mouse double minute-2 homolog (MDM2) as new molecular targets involved in rpL3-mediated molecular pathways activated by Act D in cancers lacking of p53. Taking together our results show that the efficacy of Act D chemotherapy depends on rpL3 status. Hence, the development of treatments aimed at upregulating rpL3 may be beneficial for the treatment of these cancers

O13.5
In vitro bioactivity of ruthenium-based multifunctional nucleolipidic liposomes: new promising agents for cancer therapy

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As part of pre-clinical research aimed at testing novel metal-based antiproliferative drugs, we have recently developed amphiphilic high-functionalized nucleolipidic Ru(III) complexes, inspired to NAMI-A but more effective in *in vitro* tests, which *ad hoc* mixed with zwitterionic (POPC) or cationic (DOTAP) lipids provide stable and biocompatible liposome formulations for cancer therapy. Behind an in-depth microstructural characterization, *in vitro* bioactivity profile reveals high antiproliferative effects on cancer cells derived from human solid tumours, as in different tumour clones of mammary origin (MCF-7, MDA MB-231, MDA-MB 436, CG5) on which our Ru-containing liposomes have shown anticancer efficacy similar to that of a reference drug such as cisplatin. In order to explore the molecular mechanisms underlying the anticancer effect of Ru-drugs, as well as ruthenium intracellular trafficking, we have analyzed the main molecular pathways involved in cancer cell survival and death. Following *in vitro* treatments, our results show the activation of the intrinsic pathway of apoptosis coupled to DNA fragmentation induced by a significant accumulation of the active Ru at nuclear level.

14 - Development, Differentiation and Aging

P14.1
Berberine, a natural alkaloid, affects zebrafish cardiovascular development

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The zebrafish larva is increasingly used as a vertebrate animal model for in vivo drug discovery and for toxicology and biosafety assessments, bridging the gap between cell assays and rodent assays. In fact, zebrafish responses to toxic compounds are highly predictive of mammalian responses because of their very similar physiology, development and molecular pathways. In this study we aim at testing the potential toxicity and teratogenity of berberine, a natural alkaloid displaying a variety of therapeutical properties, whose effects on embryo development have been poorly characterized. We determined the LC50 value as 213 mg/L ± 8,30 and 156 mg/L ± 4,965 after 72 hrs and 96 hrs of treatment, respectively. We then analysed the effects observed at a concentration of 100 mg/L. In particular, we focused on cardiovascular development, which we found to be affected in a concentration and time dependent manner by berberine treatment. Treated embryos display cardiac defects, with a critical treatment window from 48hpf to 72hpf. Morphological analysis performed with heart specific markers shows the presence of defects in cardiac looping, while valves morphogenesis does not appear to be perturbed. We are currently further analyzing the cardiac defects as well as evaluating vascular alterations in pigmentless zebrafish larvae specifically expressing GFP in endothelial cells (*casper*/*kdrl:*GFP).

O14.1
Modeling human intellectual disability and autism: role of the chromatin regulator setd5 during zebrafish brain development

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SETD5 loss-of-function (LoF) mutations have been recently associated to intellectual disability (ID) and autistic spectrum disorders. Interestingly, SETD5 gene encodes for a putative histone H3 methyltransferase highly expressed in the brain and it falls within the critical interval deleted in the 3p25.3 microdeletion syndrome, characterized by ID, microcephaly and congenital heart defects. The aim of this study is to generate and characterize zebrafish models in which *setd5* has been knocked down or knocked out. Antisense morpholino oligonucleotides-mediated targeting of *setd5* in zebrafish embryos determined microcephaly, cardiac edema and reduced locomotory response. Compared to embryos injected with a control morpholino, *setd5* LoF brains, despite their reduced size, show an increase of phospho-H3-positive mitotic cells, while neuronal differentiation marker HuC is not changed. We are currently evaluating whether phospho-H3 increase in setd5 morphants could be actually associated with a possible mitotic arrest, responsible of the increased apoptotic rate in developing brain areas. Furthermore, we are establishing stable setd5 knockout zebrafish lines through Crispr/Cas9 strategy. These animal models will be extremely useful to identify the molecular mechanisms underlying SETD5 LoF phenotype and to screen for compounds able to rescue the developmental defects.

O14.2
miR-143/β-DB/synapsin I, new players in Early Stages of Neural Differentiation

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Duchenne Muscular Dystrophy (DMD) is associated to cognitive impairment in about 1/3 of dystrophic patients. The brain dysfunction is independent of the muscular pathology and likely due to defects in the assembly of the Dystrophin-associated Protein Complex (DPC) during embryogenesis. We recently suggested that the DPC member β-dystrobrevin (β-DB) may play a role in neuronal differentiation; since O_2 concentrations and miRNAs appear as well to be involved in this process, we have studied how these factors act on β-DB and the possibility of their functional interplay. We found that β-DB expression is regulated during early stages of retinoic acid-induced neuronal differentiation of NT-2 cells under hypoxia and normoxia, that β-DB pattern is delayed under hypoxia, together with a delay in the differentiation and an increase in the proliferation rate of cells, we identified miR-143 as a direct regulator of β-DB expression, and demonstrated that β-DB is a repressor of synapsin I in live cells. The novel regulatory pathway miR-143/β-DB/synapsin I provides new insights into the functions of β-DB, and for elucidating the molecular mechanisms underlying the neuronal involvement in DMD.

O14.3
AKTIP (Ft1), a protein that interacts with lamins, is required for telomere maintenance and mouse development

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We have identified a human telomeric protein named AKTIP, Ft1 in mouse, an ubiquitin E2 variant enzyme, which interacts with the telomere capping proteins TRF1 and TRF2 and immunoprecipitates telomeric DNA. Loss of Ft1 results in defective telomere replication and AKTIP interacts with the DNA replication factors PCNA and RPA70. A further feature of AKTIP is its interaction with A- and B-type lamins. AKTIP co-localizes with lamins at the nuclear lamina in interphase cells. In mitosis, AKTIP is found in association with the spindle poles and with the midbody. The expression of the premature aging-related mutant lamin A, named progerin, results in AKTIP delocalization from the nuclear rim. Upon AKTIP depletion, primary cells show distorted nuclei and senescence-associated markers, which phenocopies progeroid cells. In vivo, the depletion of the Ft1 causes premature death and severe abnormalities, including the absence of subcutaneous fat, skeletal and muscle alterations and male sterility. Altogether, our results suggest that the telomeric protein AKTIP/Ft1 plays a crucial role in vitro and in vivo, which intercepts that of lamins.

O14.4
The human fungal mycobiome composition reflects age, gender, immune function and diet

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The fungal component of the human gut microbiota has been neglected

for long time, and only recently few reports have explored its composition and dynamics in health or disease. Here we investigated the gut mycobiota of a cohort of 111 healthy subjects in order to reduce the gap of knowledge concerning fungal intestinal communities in the healthy status further screening for phenotypical traits that could reflect fungi adaptation to the host. We classified the fungal communities by means of cultivation on fungal selective media and by amplicon-based ITS1 metagenomics analysis. A total of 34 different fungal species were cultured, and studied for phenotypic characteristics potentially important for the adaptation to the intestinal environment such as morphogenesis, tolerance to temperature, to acidic and oxidative stress and bile salts, antifungal resistance. We found a high frequency of azoles resistance in fungal isolates, with potential and significant clinical impact. Combining phenotyping and metagenomics allowed an in-depth understanding of the fungal intestinal community structure revealing its dependence on individuals' life style, diet and age in a gender-related fashion.

O14.5
Regulation of human testicular steroidogenesis during aging

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Is generally accepted that there is a decline of serum concentration of testosterone during aging in men. Lack of knowledge about the mechanisms underlying this event is due to the difficulty of obtaining biopsies and to the lack of a suitable experimental model. Our laboratory has developed an in vitro organ culture model which provides 2-3 mm fresh/cryopreserved human testis fragments obtained from beating heart organ donors. After three hours of culture in the presence of gonadotropins (rhLH, rhCG), response was evaluated determining androgen secreted into the media by young and elderly donors. Androgens were quantified by LC-MS/MS. hCG was found to be stronger than LH in stimulating androgen production in fresh tissues and to act in an age dependent manner. No response was observed from cryopreserved tissues, except in sporadic cases. Basal testosterone and other androgen levels in young donors was significantly higher than in elderly ones. Our experimental model allows us to investigate the regulatory mechanisms of aged Leydig cell. We are now currently analyzing age related differences in gene expression of steroidogenic pathway molecules by PCR and western blot analysis.

15 - Metabolism and its regulation in health and diseases

P15.1
The dark side of HD-GYP phosphodiesterases revealed by the structure of Pa4781 from *Pseudomonas aeruginosa* displaying a bi-nuclear unselective metal binding site

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Cyclic-di-GMP (c-di-GMP) is considered one of the most important second messenger in bacteria. It modulates the activity of a huge number of proteins regulating virulence, motility and biofilm formation. The levels of c-di-GMP are tuned by the opposite action of dedicated enzymes: diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). The first are characterized by a GGDEF domain, while the latter are divided in two unrelated superfamilies: the EAL and the HD-GYP proteins. While GGDEF and EAL proteins have received many attentions in the last years, the role and function of HD-GYP proteins are still discussed. Here, we report the crystal structure of Pa4781 an HD-GYP protein from *Pseudomonas aeruginosa*. The protein shows a bi-metallic centre that is different from precedent resolved structures. Moreover, purified Pa4781 did not contain iron in the active site as for previously reported HD-GYPs, and we show that it binds to a wide range of transition metals with similar affinity. Furthermore, structural features and the lack of appreciable catalytic activity of Pa4781 indicate that is preferentially a pGpG binding protein. Taken together these data suggest that HD-GYP domain may serve as scaffold able to respond to transition metals availability in the complex c-di-GMP-mediated biofilm regulation.

P15.2
Role of Red Blood Cells in vascular dysfunction associated with Alzheimer 's disease

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Alzheimer's disease is the most common form of dementia and its pathogenic mechanisms is still unknown. Here we report a new model based on red blood cell (RBC) interaction with 1-42 beta amyloid peptide (Aβ). RBC are deformable to assist microcirculation and any morphological or functional alterations could lead to brain hypoxia, as well as endothelial dysfunction, with an increase of oxidative stress and inflammation. Firstly, through atomic force microscopy we have studied RBC's membrane properties following to soluble Aβ exposure, in order to characterize specific induced alterations. Secondly, since mechanical properties of RBC membrane are regulated by signalling and regulatory pathways, we focused on the pathway involving antioxidant systems, protein kinase C isoforms, endothelial nitric oxide synthase and caspase 3 that could modulate RBC morphology, deformability and metabolism. Furthermore, because RBC contains, among blood elements, higher acetylcholinesterase (AChE) levels, we hypothesize that AChE could increase Aβ toxicity in blood, with a mechanism similar to the neuronal one.

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P15.3
The effect of Toxoplasmosis disease on plasma serotonin concentration in Sheep

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Toxoplasma gondii is a protozoan parasite able to alter intermediate host behavior. Various studies support the correlation between latent toxoplasmosis and neuropsychiatric disorders (1) in both animals and humans (2). An alteration in neurotransmission has been suggested as one of the factors influencing these mood disorders. Most of the studies is focused on mice (3), without considering other species. Since animal findings might be traced to human species, a better comprehension of these processes in other species might be beneficial not only for veterinary but also for human medicine. The aim of this research was to assess the variation of plasma serotonin concentration among infected and uninfected sheep (control group). Higher serotonin levels in infected animals compared to controls were observed, suggesting an influence of *T. gondii* infection on serotoninergic metabolism. No significant variation in hematological parameters between the two groups was observed. These data are coherent to a status of latent disease in infected sheep.

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P15.4
Physical exercise affects serotoninergic metabolism in horse leukocytes

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Serotonin (5-HT) is a biogenic amine which induces metabolic effects in different cell types, including leukocytes (1). A significant effect of trekking exercise on horse 5-HT plasma levels was observed (2). Immune cells uptake 5-HT because they express 5-HT receptors and intracellular 5-HT transporter (SERT). On this basis, aim of this study was to investigate the effect of hippodrome intensive training on plasma 5-HT levels, SERT, 5-HT2A and 5-HT1B receptor expression. Trotter horses (n.5), aged 6±3 years, were trained on track. Compared to pre-exercise values, 5-HT showed significant higher levels. A negative influence of exercise on SERT and 5-HT_{1B} and a positive on 5-HT_{2A} expression may be related to a lower degree of 5-HT storage and consequently to a higher plasma 5-HT levels.

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P15.5
X-rays induced complete Schiff-base photoreduction at the active site of human alanine:glyoxylate aminotransferase confirms that the geometrical strain of the internal aldimine is crucial for catalysis

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Pyridoxal-5'-phosphate (PLP) dependent enzymes play a major role in

a plethora of metabolic pathways and have long been under study. In the widely accepted catalytic mechanism for aminotransferase family, the PLP binding lysine also acts as a catalyst in the stepwise 1,3-proton transfer, interconverting the external aldimine to ketimine. This step requires that a conserved aspartate protonates the pyridine nitrogen of PLP to enhance the ability of the cofactor to stabilize the carbanionic intermediate. However, it has been recently proposed that this conserved aspartate is also responsible for ground state destabilization, occurring through a geometrical distortion of the internal aldimine, and that this strain is also crucial for catalysis. We observed that for human alanine:glyoxylate aminotransferase the geometrical strain indeed exists, and is responsible for complete X-ray photoreduction of the Schiff-base bond. These results, coupled with the structure of the conserved aspartate mutant, show that the strain is likely necessary not only for ground state destabilization but also to move the external aldimine in the correct position in order for the 1,3-proton transfer to occur.

P15.6
Yeast as a model for identification of short peptides from mitochondrial Leucyl-tRNA synthetase as a new therapeutic tool for mitochondrial tRNA diseases

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Saccharomyces cerevisiae has been used as a model for studying the mitochondrial (mt) tRNA defects: by biolistic procedure we introduced in yeast genes human equivalent pathogenic mutations that impair the mt protein synthesis and OXPHOS ability. We showed that respiratory defects due to point mutations in different mt tRNAs were relieved by overexpression of yeast and human, mt Leucyl-tRNA synthetase (LeuRS). Moreover the non catalytic carboxy-terminal domain of human mt LeuRS (LeuRS-Cterm) has the full suppressing capability. These results were also obtained in human cells bearing the same mt tRNA substitutions. We also showed that the overexpression of two short sequences, encoding 15 amino acids of LeuRS-Cterm, was sufficient to correct the yeast and human mt defects; EMSA shows specific interaction between these peptides and mt tRNAs. Now we propose a new therapeutic approach using the carbon nanotubes (CNT) to deliver short suppressive molecules into mitochondria. Results show that CNT constructs bearing the suppressive peptides are able to target the mitochondria of yeast cells with high efficiency and without toxic effects.

P15.7
Inhibition of human serine racemase by NADH and derivatives

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Serine racemase (SR) catalyzes the synthesis and degradation of D-serine, an obligatory co-agonist of glutamatergic NMDA receptors. An imbalance of D-serine production and degradation in the central nervous system is associated with several neuropathologies, including schizophrenia, Alzheimer and Parkinson diseases. SR activity is allosterically controlled by ATP, divalent cations and at least four interacting proteins. We found that NADH and NADPH, but not NAD^+ and $NADP^+$, inhibited SR at concentrations several fold higher than its intracellular concentrations. However, by dissecting NADH, we discovered that N-substituted 1,4-dihydronicotinamide ring is the inhibitory determinant, with 1,4-dihydronicotinamide mononucleotide exhibiting a partial mixed-type inhibition with a K_i of 18 ± 7 μM. Docking simulations suggested that NADH, as well as 1,4-dihydronicotinamide mononucleotide, binds at the interdimeric interface at a site partially overlapping ATP binding site, and that the 1,4-dihydronicotinamide ring is positioned in an adjacent empty sub-site. This site could be exploited for the design of high affinity serine racemase effectors to finely modulate D-serine homeostasis.

P15.8
Asymmetric dimethylarginine is transported across the inner mitochondrial membrane by SLC25A2

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Asymmetric dimethylarginine (ADMA) is synthesized when arginine residues in proteins are methylated and set free following proteolysis. ADMA has aroused great interest because it inhibits nitric oxide synthases (NOS) and therefore has the potential to regulate nitric oxide generation. Several lines of evidence indicate that ADMA is transported into kidney mitochondria where it is metabolized by alanine:glyoxylate amino-transferase (AGXT2). The transport of metabolites, cofactors and ions across the inner mitochondrial membrane is catalyzed by a family of related transport proteins (the SLC25A family). A few of them had been previously shown to transport basic amino acids with different specificity. Among these we found that only SLC25A2 transports efficiently ADMA. Upon reconstitution into liposomal vesicles, SLC25A2 transports ADMA unidirectionally (uniport mode) or by an exchange mechanism against arginine or lysine (antiport mode). These findings together with its wide expression in tissues, suggest that SLC25A2 plays a role in ADMA homeostasis either by importing it into mitochondria or by exporting it from the mitochondria of cells lacking the mitochondrial enzyme AGXT2.

P15.9
Effect of all-trans retinoic acid on post translational regulation and subcellular distribution of the oncosuppressor GRIM 19

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GRIM-19 is an oncosuppressor which antagonizes the oncogene Stat 3. GRIM19 is partitioned between the nucleus and mitochondria where it appears as a subunit of respiratory complex I. Our group has studied the impact of the chemiotherapic agent all-trans-retinoic acid (ATRA) on the subcellular distribution of GRIM-19. The results show that ATRA treatment of keratinocytes results in increased level of GRIM-19 and other subunits of complex I, in particular of their carbonylated forms, associated with inhibition of its enzymatic activity. In keratinocytes ATRA-promoted phosphatase activity appears to control the proteostasis and activity of complex I. Furthermore, the oncogene STAT-3, besides being localized in the nucleus, has also been found in mitochondria where it interacts with GRIM 19. Thus, perturbation of the interplay of GRIM 19 with factors, associated with complex I, can be involved in the ATRA suppression of keratinocyte growth.

P15.10
What structural/molecular biology and spectroscopic/functional biochemistry tell us on the mechanism of energy transfer in respiratory enzymes

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Utilization of the energy made available by respiration is the biological function essential to maintain thermodynamic order and life from aerobic prokaryotes to humans. Advanced X-ray crystallographic analysis and spectroscopic/ functional studies provide today definite basis to disclose, at molecular/ atomic level, the mechanism by which electron flow in oligomeric respiratory enzymes is converted in the energy of proton (or Na^+) flow. Recent results of these analyses are summarized which show that allosteric cooperative coupling between electron flow at metal centers and electrogenic proton translocation in the apoproteins across the membrane is utilized in combination with membrane anisotropic catalysis, in energy transfer in cytochrome c oxidase, in particular at the level of the low potential heme *a*, in ubiquinone-cytochrome c oxidoreductase (bc_1 complex) involving the Rieske iron-sulphur protein and in NADH ubiquinone oxidoreductase (complex I) in combination with redox interactions of protein-bound quinone(s).

P15.11
Exploiting physiological proteins to boost the Aβ clearance from the brain: implication for Alzheimer's disease therapy

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The accumulation of amyloid-β (Aβ) peptide in the brain, resulting from an imbalance between production and clearance, is an hallmark of Alzheimer's disease (AD). Therefore, strategies to boost Aβ clearance from the brain to the blood, across the blood-brain barrier (BBB), are promising approaches for AD treatment. Starting from the reported ability of some plasma proteins to bind Aβ, we investigated the possibility of ApoAI, ApoE-derived peptides, alpha-2-macroglobulin and albumin, either in solution or attached on liposome surface, to withdraw Aβ from the brain by the so-called "sink effect". Utilizing a transwell *in vitro* model of the BBB, we found that the efflux of Aβ from the 'brain' side was enhanced in the presence of proteins in the 'blood' side, following the order ApoAI>albumin>ApoE-peptides>alpha-2-macroglobulin. When these proteins were absorbed on the liposomes surface, the effect on Aβ efflux was maintained, with except for ApoE-peptide-liposomes where the sink effect was more pronounced, probably due to their ability to cross the BBB. These results suggest the possibility that selected plasma proteins could both participate in Aβ metabolism and exploited to develop new therapeutic strategies for AD.

P15.12
Role of nutrient transport and redox potential in cancer metabolism of *K-ras*-transformed cells

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Reprogrammed metabolism is a hallmark of cancer. Indeed cancer cells show deep changes in bioenergetics, building blocks biosynthesis and redox homeostasis, which allow them to survive and grow under conditions restrictive for normal cells. We analyzed in normal and K-Ras transformed mouse fibroblasts transport and metabolism of essential and non essential amino acids, which may play a key role in anaplerosis or redox homeostasis. Our results demonstrated that K-*ras* oncogene activation correlate with defects in methionine transport and in metabolism of glutathione precursors, that increase the sensitivity to their limitation and deprivation and to oxidative and anti-oxidative stress. Transcriptome analysis of NCI-60 cancer cell lines set suggested that similar defects are present in most of human cancer cells. Our results provide new insights into nutritional perturbations that affects the penetrance of the transformed phenotype in cells expressing Ras oncoproteins, and suggest fragility points to be targeted in innovative and effective anticancer strategies.

P15.13
Role of the citrate in macrophage inflammatory response

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Inflammation is an energetically expensive process involving the shift from oxidative phosphorylation to glycolysis and the switch of Krebs cycle toward the anabolism since TCA cycle enzymes are inhibited. Citrate is a key molecule of cell metabolism. After its synthesis in mitochondria, citrate carrier (CIC) transports citrate into the cytosol where ACLY (ATP citrate lyase) cleaves it into acetyl-CoA - precursor for fatty acid - and oxaloacetate (OAA) that generates NADPH, energy source for lipogenesis. We refer to CIC plus ACLY as "citrate pathway". Unexpectedly, we found that LPS and pro-inflammatory cytokines upregulate CIC and ACLY gene expression as well as they produce an increase in cytosolic citrate concentration in peripheral blood macrophages. By ablation of CIC or ACLY with siRNAs and inhibitors, we proved that acetyl-CoA is essential for prostaglandin E_2 formation. At the same time, ROS and NO production, through NADPH oxidase and iNOS respectively, needs OAA-derived NADPH. These findings provide evidence for a key role for mitochondria-derived citrate in pro-inflammatory mediator synthesis.

P15.14
Connection between hypoxic and glucose regulation of transcription in *Kluyveromyces lactis*

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O_2 limitation induces the transcription of several genes in *K. lactis*. We demonstrated that a mutant strain unable to induce hypoxic transcription of the *KlPDC1* gene was allelic to the glucose sensor *RAG4*. By reverse genetics, we also showed that hypoxic transcription mediated by factor KlMga2 was dependent on glucose signaling. The expression of the glucose transporter gene *RAG1* is regulated by glucose whose signaling proceeds through cascades involving Rag4 and other proteins, including the casein kinase Rag8 and the repressors Sms1 and KlRgt1. Another pathway involves the chromatin remodeler KlSnf2 and Sck1. We have found that transcription of *RAG1* is induced also by hypoxia in the presence of glucose. Analysis of *RAG1* in mutants of glucose regulation allowed to identify Sck1 as the possible element involved also in O_2 signaling. Dependence of Sck1 expression on hypoxia and binding of Sck1 to the *RAG1* promoter has been studied. The level of *RAG1* transcription, but not the hypoxic induction, depended on KlMga2. NuSA analysis of the promoter in different conditions and mutants, indicates the role of chromatin organization in *RAG1* regulation. Work partially funded by MAECI (Direzione Generale per la Promozione del Sistema Paese).

P15.15
Mechanisms of dysregulation of glucose metabolism following graded traumatic brain injury

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In this study, the metabolic, enzymatic and gene changes producing cerebral glucose dysmetabolism following graded diffuse traumatic brain injury (TBI) were evaluated. Experimental TBI (mild and severe) was induced in rats; after 6, 12, 24, 48, 120 h gene expressions and activities of glycolysis and pentose phosphate pathway enzymes, and levels of indexes of mitochondrial phosphorylating capacity, were determined in brain extracts. Results showed that mTBI causes a late increase of glycolytic gene expression and enzymatic activities, and of mitochondrial functional recovery. No changes in lactate and PPP genes and enzymes were accompanied by transient decrease in GSH and nicotinic coenzymes. Severe TBI, showed early increase of same parameters, at anytime post injury, caused by mitochondrial failing (higher lactate, lower GSH, NADPH/NADP+). Both TBIs produced metabolic and gene changes affecting glucose metabolism. Following mTBI, increased glucose flux through glycolysis is coupled to mitochondrial glucose oxidation. "True" hyperglycolysis occurs only after sTBI, where metabolic changes act on genes causing net glycolytic flux increase uncoupled from mitochondrial glucose oxidation.

P15.16
Structural models of the human iron exporter ferroportin in the inward- and outward-open states

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Ferroportin (Fpn) is a membrane protein belonging to the Major Facilitator Superfamily of transporters. It is the only known vertebrate iron exporter. Several Fpn mutations lead to type 4 hemochromatosis, characterized by two distinct iron accumulation phenotypes. No experimental data are available on human Fpn (HsFpn) three-dimensional structure. Recently, the crystal structures of a HsFpn homologue from the bacterium *Bdellovibrio bacteriovorus* (BbFpn), in both the outward- and inward-open states, has been reported. The residues essential for iron binding and transport in HsFpn are conserved in BbFpn. The conservation of functionally relevant residues prompted us to exploit the two BbFpn structures to construct reliable models of HsFPN. Analysis of the models has led to the identification of potential iron binding sites in the inward-open state allowing to propose an iron traslocation mechanism. Further, the outward-open model uncovers details of the interaction site of the peptide hepcidin, a regulator of HsFpn function. Finally, the models provide a mechanistic interpretation for the disease-related mutations that cause HsFpn-disease.

P15.17
Decreased arylesterase activity of paraoxonase-1 (PON-1) might be a common denominator of neuroinflammatory and neurodegenerative disease

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Paraoxonase-1 (PON-1), an enzyme with antioxidant properties, has been advocated as potential pathogenic player for many diseases. In particular, a decline in serum PON-1 activities (arylesterase and paraoxonase), has been associated with several neurological diseases, especially dementia. In this study, we aimed to: (1) measure the PON-1 activities in patients with mild cognitive impairment (MCI, n=232), Alzheimer's disease (LOAD, n=175), vascular dementia (VAD, n=65) and mixed dementia (MD, n=88); (2) explore the association between PON-1 measured in serum and cerebrospinal fluid (CSF) and multiple sclerosis (MS, n=104). Serum arylesterase, but not paraoxonase, activity was lower in patients with MCI, VAD, LOAD, MD and MS than healthy controls. Notably, the most pronounced decline in this activity was shown by MD (−18%, $p<0.01$) and MS (−23%, $p<0.001$). Only arylesterase activity was detectable in the CSF and its levels did not differ between MS and neurological control groups. Our data suggest that a depressed arylesterase activity could be a common denominator of different neurological diseases which appear to be all characterized by an altered systemic redox balance.

P15.18
Tuning cysteine reactivity and sulfenic acid stability by protein microenvironment in glyceraldehyde-3-phosphate dehydrogenases of *Arabidopsis thaliana*

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Cysteines and H_2O_2 are fundamental players in redox signaling. Cysteine-thiol deprotonation favors the reaction with H_2O_2 that generates sulfenic acids. The protein microenvironment surrounding the target cysteine is believed to control whether a sulfenic acid can be reversibly regulated by disulfide formation or irreversibly oxidized to sulfinates/sulfonates. Here, we present experimental oxidation kinetics and a QM/MM investigation to elucidate the reaction of H_2O_2 with glycolytic (AtGAPC1) and photosynthetic GAPDH (AtGAPA) from *Arabidopsis thaliana.* Although AtGAPC1 and AtGAPA have almost identical 3D-structure and similar acidity of their catalytic Cys149, AtGAPC1 is more sensitive to H_2O_2 and prone to irreversible oxidation than AtGAPA. Based on crystallographic structures of AtGAPC1 and AtGAPA, the reaction potential energy surface for Cys149 oxidation was calculated by QM. Activation energies for both oxidation steps were lower in AtGAPC1 than AtGAPA, supporting the higher propensity of AtGAPC1 towards irreversible oxidation. QM/MM calculations coupled to fingerprinting analyses revealed that two Arg of AtGAPA (substituted by Gly and Val in AtGAPC1), located at 8-15 Å distance from Cys149, are the major responsible for sulfenic acid stability, underpinning the importance of long-distance polar interactions in tuning sulfenic acid stability in native protein microenvironments.

O15.1
Modulation of bioenergetics by the F_1F_0-ATPase inhibitor protein in cancer cells

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Energy supply is critical for tumor growth and a fine regulation of F_1F_0-ATPase activity may exert a selective advantage for cancer cell survival and proliferation. On these bases, it has been hypothesized that the master regulator of mitochondrial ATP synthase, IF_1, whose expression is increased in various forms of cancer (Solaini G. et al., 2011; Sánchez-Cenizo et al., 2010), could play a key role in sustain tumor progression. Nonetheless, reported data are controversial (Sánchez-Cenizo et al., 2010; Campanella M. et al., 2008; Barbato S. et al., 2015) and its role in cancer metabolism is yet poorly understood. To address this issue, we explored the bioenergetics of different IF_1-

depleted cancer cell lines exposed to anoxic/hypoxic conditions and found that IF_1 exerts a protective role in cell proliferation and ATP homeostasis when oxygen declines. Collectively, our data suggest that the inhibitor can favor the adaptation of cells in absence of oxygen, by preventing and limiting the dissipation of energy substrates, thereby available to support tumor proliferation.

O15.2
Dexamethasone effects in Ataxia Telangiectasia cell metabolism

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Ataxia Telangiectasia (AT) is a rare neurodegenerative disease still incurable caused by biallelic mutations in *ATM* gene. Oxidative stress is thought to play a crucial role in the complex physiopathology. Recently, steroids were demonstrated to dramatically reduce the neurological features of the disease, but the molecular mechanism of this serendipitous effect is thus far mainly unknown. We showed that Dexamethasone is able to increase up to 30% the content of two of the most abundant antioxidants GSH and NADPH within AT cells. Moreover, we demonstrated that the drug promotes the nuclear shift of the NRF2 to drive the expression of antioxidant pathways. Among these, at least two groups of antioxidant genes were affected, namely those involved in glutathione synthesis and NADPH production. The latter was exerted via G6PD activation and enhancement of pentose phosphate pathway rate. Our evidences indicate that glucocorticoids can act as NRF2 activator being able to potentiate antioxidant defenses and counteract oxidative stress in Ataxia-Telangiectasia and other neurodegenerative diseases
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O15.3
Unraveling the design principles of cancer metabolic rewiring with constraint-based modeling

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A deeper understanding of the metabolic reprogramming (or rewiring) that cancer cells undergo to sustain their enhanced growth is anticipated to boost the efficacy of cancer treatment and to pave the way towards personalized medicine. This goal can be attained by integrating *-omics* data into predictive computational models. Genome-wide reconstructions of human metabolism are today available. Streamlined *core* models extracted from these comprehensive networks can effectively uncover the emerging properties of central carbon metabolism. We applied (Di Filippo et al. *Comput Biol Chem* 62:60-9 (2016)) Flux Balance Analysis (FBA) to three core models associated to breast, liver and lung cancer to estimate the flux distribution that maximizes growth. FBA correctly predicted common and distinguishing metabolic features of the three tumors, as well as the network fragility points. This kind of models are also well suited for other constraint-based approaches, such as the ensemble approach proposed by Damiani et al. (*Natural Computing* 13, 321-331 (2014)) that, by releasing the assumption on optimal growth, may better capture the design principles of cancer metabolic rewiring.

O15.4
Toll like receptors: linking inflammation to carcinogenesis

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A correlation between inflammation and cancer is generally accepted but the mechanisms regulating this interplay are still unresolved . Toll-like Receptors (TLRs), the receptors of innate immunity, are often expressed on cancer cells and play a central role in the cancer/inflammation relationship. We are studying TLR3, known to recognize double strand RNA. While the antitumor function of TLR3 has been demonstrated by us as well as by other groups, we recently discovered another, less known, aspect of TLR3 action. In normoxia TLR3 stimulation activates transcription factor HIF1α in prostate cancer cell lines inducing apoptosis resistance. HIF1α regulates the cancer-specific metabolic alteration called Warburg effect, sustaining the growth of rapidly proliferating cells. We propose a new mechanism of TLR-mediated induction of the Warburg effect in prostate cancer cells clarifying the regulation of the key metabolic enzymes involved in this process. Our work sheds new light on the mechanisms that links inflammation to prostate cancer.

O15.5
Zc3h10 controls mitochondriogenesis and differentiation in skeletal muscle.

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Mitochondria play a crucial role in energy metabolism. Mitochondria have their own genome (mtDNA), whose replication and transcription are mainly regulated by the mitochondrial transcription factor A (Tfam). Recent researches demonstrate how mitochondria participate to a large number of cellular processes like cell cycle and differentiation. Our goal is to identify new mitochondrial regulators to light up the molecular mechanisms underlying mitochondrial function biology. We used a high throughput screening in 293 cells in order to identify positive mitochondrial regulators. By these means, we identified Zinc Finger CCCH-type containing 10 (Zc3h10) as the best hit. Following experiments demonstrated that Zc3h10 knockdown decreased mitochondrial function and differentiation in myotubes. RNA immunoprecipitation assay indicates that Zc3h10 is able to bind >600 transcripts. Several target genes are involved in energy metabolism and iron balance. Notably, Zc3h10 downregulation in C2C12 leads to iron overload while its overexpression restores ferric ion content to control levels. Collectively, our findings annotate Zc3h10 as a new mitochondrial regulator in skeletal muscle.

O15.6
DNA methylation of sirtuin genes in nutrient-deprived cultured cells

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Calorie restriction (CR) is the key factor for extending lifespan in different species, affects both metabolic pathways and epigenetic mechanisms and activates sirtuins (SIRT1-7). These enzymes modify

nuclear, cytosolic and mitochondrial proteins. To investigate if SIRT1, 3, 4, 5 expression were epigenetically regulated during in vitro nutrient restriction, murine Hepa-1 cells were completely or partially deprived of glucose (G) or aminoacids (AA) for 72h. Genomic DNA was either digested with an endonuclease (cyanase) or underwent methylcytosine immunoprecipitation. Then chromatin accessibility, DNA methylation, and gene expression of sirtuins were analyzed by qPCR. For SIRT3, in low G there was a concordance between increased promoter methylation, reduced chromatin accessibility, and reduced expression. SIRT4 did not change its expression but, in absence of AA, the increased chromatin accessibility corresponded to a reduced methylation at its regulatory regions. Instead, for SIRT1 and SIRT5, the increased RNA did not correlate with significant DNA methylation. This is the first evidences that nutrient availability affects SIRT epigenetic regulation.

16 - Human Genetics and Genomic Diversity

P16.1
Overcoming the dichotomy: new insights into the genomic diversity of open and isolated european populations

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Human populations are often dichotomized into "isolated" and "open" using cultural and/or geographical barriers to gene flow as differential criteria. However, these alternative categories could obscure further heterogeneity due to inter-population differences in effective size, growth rate, and timing or amount of gene flow from neighbouring populations. In order to understand to what extent this dichotomy corresponds to the structure of genome diversity, we compared genomic variation measures combining novel and literature data relative to 87,818 autosomal SNPs in 14 open populations and 10 European isolates. Patterns of intra-population diversity were found to vary significantly more among isolates. This heterogeneity, observed even among geographically and culturally close populations, may be due to differential levels of drift and inbreeding. Using a multivariate analysis, we observed a substantial continuity among isolated and open populations concerning their measures of intra-population genomic diversity. We conclude that, dichotomizing human populations into open and isolated fails to capture the continuity and intersection among their genomic features.

P16.2
Mitogenome variation in Ecuador (and Peru): new clues on the first peopling of South America

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South America is the last major geographic area reached by modern humans. Archaeological evidence attests human presence in Central Chile already 14.1-14.6 thousands years ago (kya). Taking into account that the first entry into North America from Beringia occurred ~16 kya, this implies that the human diffusion from North to the Southern Cone was extremely rapid. Only a few South American-specific mitochondrial DNA (mtDNA) haplogroups have been identified so far, but they have been extremely useful to define some of the early steps of the human spread in the sub-continent. In this study, to obtain additional information on the first peopling of South America, we completely sequenced more than 200 mtDNAs from Ecuador and Peru, two geographic areas of particular interest because of their location along the Pacific coastal route. Phylogenetic analyses encompassing these novel mitogenomes as well as those (modern and ancient) previously reported from the same geographic areas allowed the identification of numerous new sub-haplogroups. Most of these branches are South American-specific, but some are also shared with Native Americans from North and Central America, thus increasing the number of founding mtDNA lineages that moved from the North.

P16.3
Is Infertility a matter of taste? the role of taste receptors (TAS) genes polymorphic variants in male infertility

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Male infertility is estimated to affect about 7% of men and the causes are often unknown. About 40% of infertile men have normal reproductive hormone production and do not show the known causes of infertility. Several studies showed the expression of taste receptors in testis and in sperms, suggesting their possible role in infertility. Taste sensitivity varies among individuals and that it is associated with functional polymorphisms in the taste receptor genes. We have investigated the possible role of 24 polymorphisms of 12 taste receptor genes in 452 individuals undergoing spermiogram evaluation during infertility diagnosis at the Centre of Couple Sterility at Siena University Hospital. The patients enrolled were characterized for main sperm parameters: concentration, morphology, progressive and total motility. We found several intriguing association the most convincing of which was the association between the carriers of the rare allele of the TAS2R14-rs3741843 SNP and a decreased total mobility (P value=0.007). In conclusion these results if replicated and functionally validated could be crucial in better understanding why for 40% of men their infertility is idiopathic.

P16.4
Mitogenome insights into the genetic history of Swamp Buffalo populations

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Water buffalo (*Bubalus bubalis*) is one of the most important livestock species in several Asian countries and is used for milk and meat production and draft power in rice cultivation. The domestic water buffalo in Asia is generally classified as two major subspecies, the dairy river buffalo and the draft swamp buffalo, which differ in morphology, behavior and number of chromosomes. The swamp type is typical of Northeast India, China (southern regions and Yangtze Valley) and Southeast Asia. Based on mitochondrial DNA (mtDNA) analyses, the swamp buffalo was proposed to originate from the border region between south China and north Indochina. However, location, time, and mode of domestication are still unclear, because the buffalo mtDNA sequences reported to date are fragmentary, mainly limited to the control region (only four complete mtDNA sequences deposited in GenBank). In this study, we report the complete mitogenome of 107 swamp buffaloes from Southeast Asia to provide a comprehensive phylogeographic overview of the swamp buffalo matrilineal diversity and to allow quantitative inferences of its origin and demographic history.

P16.5
Discrimination power of a forensic Y-STR multiplex (Yfiler Plus) in patrilocal populations from eastern Africa

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Using the recently introduced 25 Y-STR "Yfiler Plus" multiplex, we analyzed 462 males belonging to 20 ethnic groups from four eastern African countries (Eritrea, Ethiopia, Djibouti and Kenya). Sequence analysis, coupled with SNP-defined haplogroup information, allowed to classify micro-variant alleles at four Y-STR loci as monophyletic (DYF387S1 and DYS458) or due to recurrent mutations (DYS449 and DYS627). Diallelic patterns observed at 13 Y-STR were found to be significantly over-represented ($p < 10^{-7}$) among profiles obtained from cell lines, with respect to those from blood and saliva. Most of the diallelic patterns found in cell lines were unbalanced and interested recurrent mutations at 6 rapidly mutating loci included in the multiplex ($p < 10^{-2}$). At a haplotype level, intra-population diversity indexes were found to be among the lowest so far reported, while statistically significant differences among populations from the same country were detected. The strong population subdivision observed is probably due to the practice of patrilocality in eastern Africa, and suggests caution in the use of country-based Y-STR haplotype databases for forensic inferences in this region.

P16.6
Human Y chromosome diversity and the peopling of the "Green Sahara" during the Holocene humid phase

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In Africa, the human population dynamics have been heavily influenced by the Sahara desert, as suggested by the strong Y haplogroup differentiation between northern and sub-Saharan Africa. During the Holocene, the climatic changes of the "African humid period" led to the replacement of the desert with a fertile environment (the "Green Sahara"), which allowed human settlements as indicated by archeological and paleoanthropological evidences. To evaluate the extent and trajectories of human movements across the Sahara, we analyzed by NGS about 3.3 Mb of the X-degenerate portion of the Y chromosome in 104 subjects, focusing on haplogroups currently found both in northern Africa and in the sub-Saharan area. We identified 7,544 polymorphisms, which were used to reconstruct the phylogeny and to estimate the coalescence age of nodes. Informative markers were further analyzed in a wider set of about 5,000 Y chromosomes. Combining phylogeography and age estimates, we found that northern African and sub-Saharan lineages only coalesced within a 5-12 kya time frame, suggesting extensive human movements across the "Green Sahara" and subsequent isolation after the desertification.

P16.7
Investigating the genetic legacy of ancient migrants to Southern Italy by Next Generation Sequencing of the Y chromosome

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The human Y-chromosome lineages found in Southern Italy are a collection of incoming Near-Eastern ones onto a local background. By means of a fine-grained sampling scheme and NGS, we aimed at reconstructing phyletic affinities between Y-chromosomes of the Northern Mediterranean gene pool, to distinguish between two main demic events: the dispersal of early farmers (~8 kya), and the Greek colonization (~3 kya). The Y haplogroup J (Hg J) has long been considered the clearest marker of eastward migrations across the Northern Mediterranean. We selected 59 Hg J chromosomes from the most geographically-informative regions. These were analyzed using an NGS approach, to reach a mean 50x coverage of 4 Mb in the MSY, by using Target Enrichment. We identified >1200 variants. The resulting maximum parsimony tree recapitulated the known Hg J phylogeny, but with a refined branch length for the main sub-clades. We also found several unexpectedly deep lineages. This work produced many novel population-specific SNPs to be tested in larger series. Finally, a reconstruction of the Southern Italian demography in different time-windows will be possible. Grants PRIN-MIUR 2012JA4BTY_004, 003 to FC, AN.

P16.8
Identification of non-LTR retrotransposon insertional polymorphisms between modern and archaic human

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Transposable Elements have generated a large portion of the human genome and have implications in structural, regulatory and functional processes. Throughout recent radiation of the human lineage only some subfamilies of retrotransposons (RTs) have been active, the evolutionary effects of which are still understudied. This research aims at identifying the role of such RTs in the differentiation of the *Homo* genus, contrasting modern and archaic humans. A new *in silico* methodology was developed and tested with Denisovan data. Comparing both the genomes, the putative species-specific insertions 3' portions are isolated. These are confirmed by locating the empty site in the other species' genome, which in turn allows pinpointing the 5' portion of the insertion. Only insertions with both 3' and 5' portions and presenting confirmed empty (pre-insertion) sites in the other species' genome are kept. The precise annotation of the species-specific insertions, the characterization of their genomic surroundings and the comparison of the site's activity and functionality both with and without the inserted elements further clarifies the impact and role of RTs in recent *Homo* evolution.

P16.9
Identification of new molecules with readthrough activity on premature termination codons (PTCs) in cystic fibrosis cells (CFTR deltaF508/W1282X)

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Cystic Fibrosis (CF) patients with nonsense-mutation in the CFTR (Cystic fibrosis transmembrane conductance regulator) gene generally make virtually no CFTR protein and thus often have a more severe form of CF. Recently, Ataluren (PTC124; Translarna) was suggested to induce the readthrough of premature termination codons mainly the UGA codon. However, despite promising results there is not a general consensus on efficacy and mechanism of action. The design of new small molecules (PTC124 related) together with the understanding of their mechanism of action could lead to new pharmacologic approaches for the cure of CF. This work was aimed to identify new molecules (PTC124 analogues) with readthrough activity and to evaluate their efficacy in CF cells. We synthesized 18 analogues of the PTC124 and tested some of them in three different biological models. The IB3.1 cell lines were used to test the new identified products. Three of these new compounds showed high read-through capacity in CF cells. Finally, computational studies were aimed to model the interaction between the synthesized compounds and the possible cellular target, in order to understand the mechanism of action.

P16.10
Short-time evolution and complex neocentromeres in cancer

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Neocentromeres frequently arise on ring/rod-shaped chromosomes, particularly in sarcomas, but the mechanisms behind their genesis are still poorly understood. To investigate this phenomenon, we studied three well-differentiated liposarcomas and a lung-sarcomatoid carcinoma cell line carrying neocentromeres on ring/rod-shaped chromosomes RGM. Whole genome sequencing was carried out to finely define the inner structure of marker chromosomes, while anti-CENP-A ChIP-seq and immunoFISH experiments were performed to characterize the neocentromeric domains. Our results revealed that neocentromeres arose on patchworks of short-sized <100Kb amplified fragments, some from different chromosomes. Intriguingly, two of our liposarcoma cell lines derived from the primary tumour and recurrence of the same patient; this allowed us to disclose a rapid evolution of the neocentromeric domains, which resulted enlarged in the recurrence. The results of our study underlined the strictly epigenetic nature of neocentromeres and suggested the existence of an association between the massive recruitment of CENPA at double strand breaks and the neocentromere seeding. Moreover, the neocentromeric domain expansion from the primary tumour to the recurrence disclosed their crucial role in RGM maintenance, and, consequently, in cancer genesis and progression.

P16.11
Spatially explicit models to investigate geographic patterns in the distribution of forensic STR

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Human forensic STRs are used for individual identification but have been reported to have little power for inter-population analyses. We genotyped at 16 forensic STRs a population sample obtained from many locations in Italy, Greece and Turkey. Using spatial PCA on the full dataset, we detected patterns of population affinities in the area similar to those of NGS studies. Additionally, we devised objective criteria to reduce the overall complexity into reduced datasets. Independent spatially explicit methods applied to these latter datasets converged in showing that the extraction of information on geographical trends and structuring from the overall diversity is possible. All analyses returned the picture of a background clinal variation, with regional discontinuities captured by each of the reduced datasets. These coincided with the main bodies of water. High levels of gene flow were inferred within the main continental areas by coalescent simulations. These results are promising in a microevolutionary perspective. It is foreseeable that this will allow the exploitation of an invaluable genotypic resource to clarify important aspects in the formation of local gene pools

P16.12
Structural and stability studies on phosphoglycerate kinase 1 natural variants

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Phosphoglycerate kinase 1 (PGK-1) is an important enzyme of glycolitic pathway that catalyzes the reversible phosphoryl transfer between 1,3-bisphosphoglycerate and MgADP to form 3- phosphoglycerate and MgATP. Cancer cells acquire an unusual glycolytic behaviour reprogramming their cellular metabolism in a way that confers an evolutionary and thermodynamic advantage. Several mutations in PGK-1 have been identified in humans and they may play an important role in different cancer types. However, while some data has shown that PGK-overexpression is correlated with disseminated cancer, other data shows an inverse correlation with tumor incidence and consider that PGK enhances an anti-tumor effect because of its anti-inflammatory and anti-angiogenic activity. In this study we chose from COSMIC data base (http://cancer.sanger.ac.uk/cosmic) some natural variants of PGK-1 identified in cancer as nonsynonymous single nucleotide polymorphisms (nsSNPs) that occur in the coding region leading to a polypeptide sequence with amino acid substitutions. We investigate the effect of the amino acid substitutions on PGK-1 structure and stability and on the enzymatic function.

P16.13
A genome-wide portrait of Italy

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Surrounded by the sea and bounded by the Alps, Italy extends over more than 1,000 km along a North-South axis and comprises the two largest islands of the Mediterranean, Sicily and Sardinia. The combination of this geographic complexity with a rich set of historical events and cultural dynamics has the potential to shape in a unique way the distribution of genetic variation within the Italian population. Recent investigations have reported substantial stratification in Italy when compared to other European countries, but a fine and exhaustive characterisation of its population structure and admixture history has yet to be conducted. In order to dissect the fine structure and the admixture profile of Italian populations, we genotyped 167 novel samples with the Illumina Infinium Omni2.5 BeadChip and assembled a comprehensive genome-wide SNP dataset which included almost 1,500 individuals representing all of the 20 Italian administrative regions, and data from ~ 300 world-wide reference populations. Preliminary results based on parametric and non-parametric statistical analyses suggest extensive population structure and a complex pattern of admixture episodes over the last few thousand years.

P16.14
An ever-changing tapestry of genes and cultures: insights into the genomic ancestry of Sicily and Southern Italy

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In this study we provide new genome-wide data for a wide set of populations from Sicily, Southern Italy, Albania and Greece, including Greek-speaking Islands. New remarkable samples from Southern Italian ethno-linguistic minorities (Albanian-speaking Arbereshe and Greek-speaking Grecani) are also included. Overall, 511 samples from 23 populations were genotyped with the Illumina GenoChip Array (~150,000 SNPs) and compared with a large collection of modern and ancient Euro-Mediterranean individuals. Autosomal genomic variation reveals Sicily and Southern Italy as belonging to a wide South-Eastern Mediterranean '*genetic continuum'*, extending from Sicily to Cyprus, through Crete and Greek Islands. Besides a predominant Neolithic heritage, these populations show significant Levantine- and Caucasus-related ancestries, particularly during the Bronze-Age. Continental Southern Balkan groups of Greece and Albania show evidences of more recent genetic exchanges with North-Central Balkans and East Europe. Arbereshe ethno-linguistic minorities confirm their pertinence to Southern Balkan genetic landscape, whereas Italian Greeks cluster with the '*continuum'* except for episodes of genetic drift.

P16.15
Natural selection accounts for differences in dizygotic twinning rates at the worldwide scale

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Patterns in the distribution of dizygotic twinning rates have long been recognized, with African and Asian populations falling, respectively, at the top and at the bottom of the range. Various genetic effects have been proposed to account for such population differences, but the evidence for association between twinning rates and specific alleles is, so far, only anecdotal. We assembled a dataset of 20 candidate twinning genes in 26 worldwide-distributed populations, and a Reference dataset of >40 000 SNPs mapping in intergenic genome regions, which we used to derive neutral expectations. We identified association between twinning rates and alleles at seven loci subjected to selection; three such loci, in particular, are involved in folliculogenesis, follicle selection, ovulation, and/or support of multiple implantation (LHCGR, FSHR, and MDM4). Population differences at these candidate genes point to slight, but not negligible, differences for the bioactivity of these enzymes, hormones and receptors, which should in turn have an influence upon the rate of twinning in the populations considered.

P16.16
Genetic diversity at three palindromic sequences of the human Y chromosomes

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One of the most striking feature of the male specific region of the human Y chromosome (MSY) is the presence of eight massive palindromes (P1-P8), composed of two inverted repeats (arms) separated by a spacer sequence. These elements exhibit > 99.9% arm-to-arm sequence identity, probably due to abundant gene conversion (GC) between the arms. Although Y-Y GC has been demonstrated for P1 and P6, its effect on the genetic diversity of MSY remain largely unexplored. To shed light into the dynamics of Y-Y GC, we analysed by NGS (50×) three palindromes (P6, P7 and P8; for a total of ~200 kb) in 104 samples. We identified a peculiar mutational pattern of the palindrome arms respect to the spacer. Moreover, we found a limited number of paralogous sequence variants and a high number of polymorphisms shared by arms, suggesting a marked activity of GC in shaping the nucleotide diversity of palindromes. Because Y chromosomes are clonally inherited, it has been possible to capture their evolutionary relationships in a robust phylogenetic tree with known age of each node. By mapping GC events across this tree, we were able to calculate a precise Y-Y GC rate for each palindrome here analysed.

O16.1
The worldwide spread of the tiger mosquito as revealed by mitogenome diversity

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Over the last 40 years, the Asian tiger mosquito *Aedes albopictus*, indigenous to East Asia, has colonized every continent except Antarctica. Its spread is a major public health concern, given that this species is a competent vector for numerous arboviruses. To determine the most likely ancestral source(s) of adventive populations, we analyzed the

mitogenome variation of samples from representative populations of Asia, the Americas and Europe. Phylogenetic analyses revealed three haplogroups in Asia, but only two were involved in the recent spread. These are differently distributed in Asian populations living in temperate and tropical regions. Moreover, a common lineage in Italy most likely arose in North America and arrived recently from the US. These ancestral genetic sources now coexist in many of the recently colonized areas, thus probably creating novel genomic combinations that might be one of the causes of the apparently growing ability of *Ae. albopictus* to expand its geographical range.

O16.2
Who likes to travel alone? Grammars and genes in the history of Old World migrations

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Linguistics and genetics provide powerful tools to probe human history. If their results eventually converge, stronger conclusions can be reached about reconstructing past. To test Darwin's original prediction of congruence between biological and linguistic variation, we address the possibility of broad-scale parallelism in language and gene transmission through a radical recalculation of linguistic distances, by using universal and discrete syntactic characters to achieve better scope and resolution than previous attempts. This approach uncovers an unprecedented gene/language correlation between gene and language diversity. Our results largely fulfil Darwin's expectation, suggesting that in the Old World grammars and genes have diffused together, with few motivated exceptions.

O16.3
HERV-W presence and evolution within the primates lineage: characterization of the group in non-human primates and identification of highly related elements in New World Monkeys

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The genome of all vertebrates harbor sequences with retroviral origin, acquired million years ago and named Endogenous Retroviruses (ERVs). Among human ERVs (HERVs), the W group is of particular interest due to its role in placenta physiology (Syncytin-1) and the proposed correlation with Multiple Sclerosis. Recently we characterized 213 HERV-W insertions in the human genome. Thus, in order to describe the group evolution within the primates lineage, we i) analyzed the HERV-W loci in 5 Catarrhini primates (1 Old World Monkey and 4 Hominoidea great apes); ii) identified and characterized unreported HERV-W related elements in Marmoset and Squirrel Monkey Platyrrhines (Cebidae family); iii) find supports for the presence of HERV-W related elements also in Atelidae and Pitheciidae Platyrrhines families; and iv) excluded their presence among Prosimians. Taken together data provide an unprecedented detailed picture of the HERV-W group diffusion among primates, defining their evolutionary history that led to the presence in human genome. Moreover, the identification of HERV-W related ancestral elements in New World Monkeys gives unreported insights on the group composition and phylogeny.

O16.4
The phylogeny of Y-chromosome haplogroup Q-L54: new insights on the first peopling of South America.

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The Male Specific region of the human Y chromosome (MSY) has proved to be an important tool in evolutionary and human population genetics studies. As for Native Americans, the identification of the Y-chromosome founding lineages has been complicated by the high rate of historical admixture. At present three main founding lineages have been identified: C-M217, virtually restricted to North America, and Q-L54* and Q-M3, diffused all over the double continent. Despite recent deep re-sequencing efforts, their current phylogenetic resolution is inadequate to investigate the history and demography of Native American populations. To refine the structure of Q-L54, the Native American branch of Hg Q, and its relationships with the other main Hg Q branches, we re-sequenced about 1.5 Mb of the MSY in 34 unrelated males clustering within different Hg Q sub-lineages and from different geographic regions. The new data, added to those available in the literature, allowed us to identify at least two L54* and eight M3 sub-branches whose phylogeographic investigation will be of great help to shed light on the ancient demographic processes that occurred in Central and South America.

O16.5
Reconstructing the autosomal profile of Joseph Smith Jr, founder of Mormonism: a paternity application

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Joseph Smith Jr. (1805-1844) was the founder of Mormonism and, as part of the theology, introduced the practice of polygamy, including a form of polyandry (marrying married women). However, many of the initial unions he was involved with were not publicly known. One of them involved the marriage to Sylvia Sessions, who was already married to Windsor Lyon. Toward the end of her life, Sylvia revealed to her daughter (Josephine; 1844-1924) that she was the child of Joseph Smith. For nearly 150 years, scholars studying Mormon history have been wondering about the truth of this statement to understand the early practice of Mormon polygamy. In the current study, 55 individuals, mostly direct descendants of Joseph Smith and Josephine Lyon, had their autosomal DNA tested to verify Josephine's biological paternity. A few samples were also collected from descendants of Hyrum Smith (Joseph's brother) and from other Lyons, as controls. Nearly 600,000 autosomal SNPs from each subject were typed and detailed genealogical data was compiled. Results were compared to nearly 6,000 pairs of relatives to verify the accuracy of the genealogical data and resolve Josephine's paternity.

O16.6
Evolutionary medicine insights from the depiction of the Italian genomic landscape

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Extensive migrations and a patchy environmental landscape entailing different ecological/cultural selective pressures might have led to peculiar patterns of population structure and local adaptations along the Italian peninsula. A heterogeneous Italian genomic background is thus expected. However, few genome-wide datasets were examined to date to disentangle this complex evolutionary scenario, being often constrained by limited size or by sampling strategies not driven by accurate bioanthropological criteria. To overcome this issue, we generated genome-wide data for 780 subjects selected to be representative of the overall Italian population and we set them into the context of European/Mediterranean genomic diversity. This enabled identification of clusters of genetically homogeneous provinces and of genomic regions underlying their local adaptations. Description of such patterns pointed out evolutionary medicine case studies in which differential susceptibility of Italian subpopulations to certain diseases is mediated by alleles formerly targeted by natural selection, but having become detrimental due to metabolic/immune challenges imposed by recent dietary and lifestyle shifts.

17 - Neurobiology

P17.1
Structural models of huntingtin ordered domains

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An abnormal polyglutamine expansion in the N-terminal fragment of Huntingtin (Htt) leads to the devastating neurodegenerative disorder Huntington's disease. Htt is a protein of 3144 amino acids and its high molecular weight prevents crystals production and X-ray diffraction studies. Further, Htt doesn't display significant sequence similarity with other proteins of known structure. Therefore the main motivation of this study was that of obtaining structural information on Htt in order to provide new insight into its functions and the mechanisms that lead to neurodegeneration. Using different order/disorder prediction methods, Htt sequence has been divided into five regions predicted to form ordered domains. Then structural models have been obtained for each domain and structural homologs have been identified for each of them. This work has led to the identification of a previously undetected HEAT repeats region, predicted to be involved in protein trafficking. To our knowledge, this work represents the first attempt to predict the structural and functional features of all Htt domains and the results obtained may represent a starting point for future experimental studies.

P17.2
Hippocampal neurons of dystrophic *mdx* mice are less responsive than wild type to acute corticosterone treatment *in vitro*

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Duchenne muscular dystrophy (DMD) is a lethal X-linked disease characterized by progressive muscular wasting due to lack of dystrophin (Dp427). As Dp427 is also expressed by hippocampal neurons, DMD patients show neurological disorders. These could be aggravated by the glucocorticoid (GC) chronic treatment of muscular inflammation, since the hippocampus is a major brain target of stress-induced GC release. Here we analyze whether GC treatment affects hippocampal neuron physiology, by studying expression and localization of the GC receptor (GR) in hippocampal cell cultures from E18 wild type and dystrophic *mdx* mice, incubated with either 1 or 10μM corticosterone (CORT). In WT mouse neurons, GR mRNA levels significantly increased after both corticosterone treatments, compared to control (vehicle alone) while, in *mdx* mouse neurons, they increased slightly after 1μM CORT and decreased after 10μM CORT incubation. Protein levels and immunolabeling of GR and *p*GR (phosphorylated) change accordingly to mRNA. These results indicate a different sensitivity of *mdx* mouse neurons to GC compared to WT, possibly derived from neuronal alterations established during development.

P17.3
Cellular adaptation to eustress in models of Parkinson neurodegeneration is mediated by coordinated mitochondrial responses

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Oxidative stress is the outcome of mitochondrial dysfunction that drives aging and the pathogenesis of most neurodegenerative disorders, including Parkinson disease. Correct management of cellular ROS is not well understood. We examined cellular events in response to hydrogen peroxide treatment in PC12 cells, a dopaminergic cell line used as in-vitro model of neurodegeneration, focusing on correlation between oxidative stress and mitochondrial dynamics (fission-fusion) and biogenesis. Protein levels and distribution (cytoplasm, mitochondria, nuclei) were assessed by western blots and immunofluorescence imaging. Time-course studies revealed early (3-6h) differential levels and distribution (cytoplasm/nuclei) of the oxidative-stress sensors (DJ-1 and Nrf-2) and of proteins regulating mitochondrial fusion (Opa1 and Mfn2) and biogenesis (mtTFAM and PGC1a). These changes seem to underlie adaptation mechanisms restoring intracellular ATP levels (at 6h) following their initial depletion after 2h of hydrogen peroxide. These data suggest a cellular adaptation model defying eustress by mitochondrial fusion and biogenesis to compensate ATP depletion and prevent system collapse.

P17.4
Involvement of FUS in cell cycle regulation and ALS pathogenesis

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Fused in sarcoma/traslocated in liposarcoma (FUS/TLS) is an RNA/DNA binding protein; mutations in the coding region of FUS, which cause the retention of the protein in the cytoplasm, have been found associated with ALS. Literature data demonstrated that also mutations in the 3'UTR, which result in overexpressed FUS-WT, are involved in the onset of the disease. In light of its well recognised role in ALS pathology we investigated its function in nervous system development. We derived mouse neural progenitor cells (NPC) from foetal spinal cord in which we overexpressed human wild type (hFUS-WT) or mutated (hFUS-P525L) FUS, which has been found in one of the most aggressive forms of juvenile ALS. The expression of the cell cyle regulators p27 and cdk2 is modulated in cells overexpressing hFUS-WT, suggesting that FUS interferes with cell cycle progression. Accordingly, cytofluorimetric analysis and BrdU immunolocalization show a G1 arrest of cell proliferation between 48 and 72hrs. We are currently investigating the effect of the overexpression of hFUS-P525L mutated form on cell cycle.

O17.1
Crosstalk between insulin and mTOR signaling in Down Syndrome and Alzheimer disease

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Down Syndrome (DS) individuals by the age of 40ys develop a type of dementia that has the same characteristics of Alzheimer disease (AD). Common pathways of neurodegeneration include mitochondrial dysfunction, increased oxidative stress (OS), altered mTOR axis and reduced glucose metabolism. Interestingly, post-mortem analyses of AD subjects demonstrated insulin resistance in the brain proposing a role for cognitive deficits. The present study aims to analyze the crosstalk between insulin and mTOR signaling as possible contributing factors to the neurodegenerative process in Tg mouse model of DS (Ts65Dn) and AD (3xTgAD) as function of aging. Our results show that OS-induced impairment of biliverdin reductase A (BVRA) kinase activity is an early event, prior the accumulation of Aβ and tau pathology, and that this alteration contributes to the onset of brain insulin resistance along the progression of AD pathology in 3xTg-AD and Ts65Dn mice.

We propose a new paradigm for which: OS-induced impairment of BVR-A is responsible for a sustained activation of IRS1, which then causes the stimulation of negative feedback mechanisms aimed to turn-off IRS1 hyper-activity and thus brain insulin resistance.

O17.2
Effects mediated by M2 muscarinic receptors activation in Schwann-like cells induced from adipose mesenchymal stem cells: implication in nerve regeneration

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The peripheral nervous system has an intrinsic regeneration capability; nevertheless, the nerve regeneration is often not satisfactory. Schwann Cells (SCs) play a central role in the nerve regeneration improvement. The clinical use of autologous SCs is limited. The new frontier of the regenerative medicine is focused on stem cells. The adipose tissue contains mesenchymal stem cells (ASC). It has demonstrated that ASC can be differentiated *in vitro* in Schwann-like. Rat SC-like express neuroglial markers and receptors for different neurotrasmitters that can modulate several physiological processes. Acetylcholine (ACh) controls Schwann cell proliferation and differentiation via M2 muscarinic receptors. Schwann-like express all muscarinic receptor subtypes including M2 subtype. In dASC M2 activation causes a decreased cell proliferation, the inhibition of cell migration and modulates expression of neurotrophic factors and P0 protein. Co-cultures of sensory neurons/ dASCs will be used to study the *in vitro* myelination in presence of cholinergic agonists. The data obtained may contribute to identify new therapeutic strategies for peripheral nerve regeneration.

O17.3
Neuronal cell autonomous defects in a mouse model of Down Syndrome: contribution of TTC3 gene

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Down Syndrome (DS) is the most common genetic disorder associated with intellectual disability (ID), characterized by dendritic structure and spine maturation impairment. In this work we explored the contribution of cell autonomous alterations to abnormal cortical phenotype of DS brains. Therefore, we cultured cortical neurons from a DS mouse model, the Ts65Dn (Ts), and evaluated their ability to differentiate in *ex vivo* conditions. Our data indicate that neuronal polarity and dendritogenesis are unaffected, while spines are both reduced and immature, evidencing an intrinsic cell phenotype. Furthermore, we studied TTC3, a negative regulator of neuritogenesis (Berto et al. 2007, 2014), located on HSA21 and overexpressed in DS and Ts brain. Preliminary results obtained modulating TTC3 levels in Ts cortical neurons suggest a role of the gene in Ts abnormal dendritic spine development. These data indicate that defective Ts brain functionality can be attributable not only to circuitry or cellular composition alterations, but also to specific defects within neurons caused by modulation of the expression of genes, such as TTC3.

O17.4
Amino acid substitutions in D-amino acid oxidase related to human pathologies

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In human brain D-serine (D-Ser), a co-agonist of NMDA receptor (NMDAR), is mainly degraded by D-amino acid oxidase (DAAO). Dysregulation of D-Ser metabolism due to an altered DAAO functionality is related to pathological NMDAR dysfunctions: low concentrations of D-Ser are associated with schizophrenia while increased levels with neurodegenerative disorders [1]. With the aim to clarify the role of DAAO on D-Ser concentration under physiological and pathological conditions, we propose a functional classification of DAAO substitutions based on biochemical properties of DAAO variants related to known SNPs: a) DAAO variants involved in neurodegenerative pathologies produce an increase of D-Ser level when expressed in human glioblastoma U87 cells [2]. The R199W (associated with ALS) and R199Q DAAOs show a decreased enzymatic activity and FAD affinity, and the G183R variant is fully inactive. b) hDAAO variants involved in schizophrenia susceptibility produce a decrease of D-Ser level in U87 cells: D31H, W209R and R279A DAAOs show an increased kinetic efficiency and FAD affinity [2].

[1] Sacchi et al., 2012, *Amino acids* 43:1833
[2] Cappelletti et al., 2015, *Biochim Biophys Acta* 1854:1150

O17.5
Peripheral biomarkers of oxidative stress in depressed elderly patients

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Depression in the elderly is a common clinical condition defined by atypical features, recurrent relapses, suicidality and a worsened prognosis of other somatic diseases. The co-morbidity with dementia and Parkinson Disease is also frequent in depressed older adults, suggesting their increased vulnerability to neurodegenerative processes. This study aimed at preliminarily investigating five peripheral biomarkers of oxidative stress in geriatric depression: the plasma levels of advanced oxidation protein products (AOPP), ferric reducing antioxidant power (FRAP), total thiols (TT), and the two anti-reactive oxidant species (ROS) enzymes superoxide dismutase (SOD) and catalase, were measured in 12 depressed patients aged more than 65 years and 12 controls, matched for gender and age. Results showed an increased amount of AOPP and an important reduction of TT ($P<.001$) in patients vs. controls, suggesting unbalanced defense mechanisms against oxidative stress in the aged depressed population. We are currently extending the study to a greater population of subjects, by also evaluating the link between altered anti-oxidant defenses and presence of mild signs of Parkinsonism.

18 - Immunology and Host-Pathogen Interaction

P18.1
Chasing *Burkholderia pseudomallei* antigens/epitopes through a structural vaccinology approach

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We have solved the crystal structures of two potential antigens from the Gram-negative bacterium *Burkholderia pseudomallei*: the putative type 1 fimbrial subunit (BPSL1626), and the putative exported protein (BPSL2520), as targets for structure-based epitope design for melioidosis vaccine development. BPSL1626 was selected as a potential antigen based on Reverse Vaccinology genome sequence screening, whereas BPSL2520 was previously reported to be seroreactive against melioidosis patient antibodies. Two *in silico* epitope-prediction methods were applied to the 3D-structure of BPSL2520, and compared with *in vitro* epitope mapping results. A single antigenic region was revealed that may help guide the design of peptide epitopes/epitope-containing domains endowed with improved immunogenic properties, in comparison with their recombinant antigen counterparts, as previously demonstrated for other antigens. Furthermore, *in vitro* immunological studies showed that both recombinant antigens are cytotoxic to murine macrophages *via* an unknown mechanism. Future efforts will focus on elucidating the function of BPSL2520 and exploring the mechanisms related to the cytotoxic properties of both antigens towards macrophages.

P18.2
Gallium compounds targeting iron metabolism in *Acinetobacter baumannii*

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Acinetobacter baumannii has emerged as leading cause of nosocomial infection. The loss of efficacy of antibiotics calls for the development of novel therapeutic options. Due to the importance of iron for bacterial growth and pathogenicity, recent studies have explored the possibility of using iron mimetics as novel therapeutics targeting bacterial iron metabolism. Gallium is one such mimetics, sharing similar chemical behavior as Fe(III), thus replacing Fe(III) in many biological systems. Different from Fe(III), however, Ga(III) cannot be reduced under physiological conditions and, therefore, it cannot take part in redox reactions, ultimately inhibiting a number of essential cellular functions. We found that Ga(III) inhibits planktonic growth of *A. baumannii* both in iron-poor media and in complement-free human serum. Ga(III) treatment also protected *Galleria mellonella* larvae from lethal *A. baumannii* infection. Lastly, Ga(III) inhibited biofilm formation and disrupted pre-formed *A. baumannii* biofilms. These findings support the idea that the repurposing of Ga(III)-based drugs is a promising strategy for the development of novel non-antibiotic compounds.

P18.3
Esculentin-1a(1-21)NH2 and its diastereomer: antibacterial and immunomodulating activities

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Microbial resistance to conventional antibiotics has become a major challenge. In this context, naturally-occurring antimicrobial peptides (AMPs) hold promise for the development of new drugs against microbial infections, including those caused by the bacterium *Pseudomonas aeruginosa* in the lungs of cystic fibrosis (CF) sufferers. Here we report on the in vitro activities of the frog-skin derived AMP Esculentin-1a(1-21)NH2 [Esc(1-21)], and its diastereomer Esc(1-21)-1c, containing two D-amino acids, on both macrophages and bronchial cells, which express either the functional or the ΔF508 mutant of the CF transmembrane conductance regulator (1). We found that the diastereomer is significantly less toxic; has significantly higher efficacy in killing intracellular *Pseudomonas*; has a higher activity in promoting migration of bronchial cells; disaggregates and detoxifies the bacterial lipopolysaccharide. These results support further studies towards the development of the Esc(1-21)-1c for local treatment of *P. aeruginosa*-induced lung infections.

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P18.4
Eosin topical treatment of psoriasis patients reduces skin secretion of inflammatory cytokines and angiogenic factors and skin leukocyte infiltration

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Psoriasis is a chronic inflammatory skin disease. Treatment options include topical corticosteroids, systemic immunosuppressants, monoclonal antibodies targeting pathogenic cytokines and photo-therapy. Our study aims at investigating the mechanism of action of tetrabromofluorescein, commonly known as eosin, traditionally used as a topical treatment in psoriasis. Eosin is also employed in infantile haemangioma therapy. Immunohistochemical analysis of skin patient biopsies after a 3-day eosin treatment showed a decrease in secretion of the angiogenic factor VEGF-A by epidermal keratinocytes and a reduction of skin inflammatory cell infiltrate. Both a protein array and ELISAs showed that psoriatic cultured keratinocytes treated with eosin for 18 hours secreted significantly lower amounts of inflammatory cytokines such as CXCL10, CCL2, CCL5, and of VEGF-A. Treatment of cultured dermal microvascular endothelial cells with eosin also reduced secretion of CCL5 and IL-6 and membrane expression of the adhesion molecule ICAM-1 involved in leukocyte trafficking. Altogether our data indicate that eosin treatment has an important anti-inflammatory and anti-angiogenic action and represents a valid topical treatment for psoriasis patients.

P18.5
Screening of gram-negative efflux pump inhibitors from natural sources by *in silico/in vitro* approach

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Efflux pumps are involved in the emergence of MultiDrug-Resistant (MDR) strains, due to their ability to extrude different antibiotics. MexAB-OprM and AcrAB-TolC are responsible for the emergence of MDR *Pseudomonas aeruginosa* and *Escherichia coli*. The use of Efflux Pump Inhibitors (EPIs) is a promising strategy to cope with MDR strains.

To identify possible EPIs, an *in silico* high-throughput virtual screening performed by Autodock/Vina was used to test a database of natural compounds able to bind the membrane channels MexB or AcrB, followed by a minimization/focused docking protocol and molecular dynamics simulations. The comparison of their common pharmacophoric features constitutes the basis for future EPIs identification. The effectiveness of the selected compounds was verified by *in vitro* synergy tests using different combinations of EPIs and ciprofloxacin, ceftazidime, meropenem, piperacillin or tobramycin, all extruded by both efflux systems. Two lead compounds (Pregna-20-one and Morelloflavone) showed the ability to decrease (4-8 fold) the MIC of ciprofloxacin against *P. aeruginosa* and one (Krukovine) those of ciprofloxacin and ceftazidime against *E. coli* isolates.

P18.6
Two new cryptic host defence peptides from human apolipoprotein E: ApoE (133-150) and ApoE (133-167)

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Several cationic proteins possess intrinsic antimicrobial properties due to the presence, in their primary structure, of cryptic antimicrobial peptides (AMPs). In order to identify novel cryptic antimicrobial peptides in human proteins, our group developed a informatic tool able to predict antimicrobial propensity of short sequences. The method assigns an antimicrobial score to peptides based on their net charge, hydrophobicity, and length, and two bacterial strain-dependent weight factors. Apolipoprotein E (ApoE) is a glycosylated protein that plays a key role in the transport of cholesterol in the blood and central nervous system and shows immunomodulatory properties. By our method we identified a region with an absolute maximum score corresponding to residues 133-167 and one with a relative maximum score corresponding to residues 133-150. These new ApoE derived AMPs shows a broad antimicrobial activity on Gram negative and positive bacteria, ability to interact to different LPS, no toxicity on human and murine cells and very intriguing immunomodulatory properties, related to their different propensity to adopt amphipatic conformation on human and murine LPS infected monocytes.

P18.7
In vitro and *in vivo* characterization of TonB- and FeoB-dependent iron uptake systems of *Acinetobacter baumannii*

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Acinetobacter baumannii is an emerging bacterial pathogen responsible for a variety of nosocomial infections. Mechanisms that allow *A. baumannii* to infect and persist in humans are still poorly understood. Iron is an essential nutrient for bacterial growth *in vivo*, and the redundancy of iron uptake systems in *A. baumannii* suggests that iron acquisition is a major determinant of its success as a human pathogen. In Gram-negative bacteria, receptor-mediated active iron uptake is dependent upon the TonB-ExbB-ExbD energy-transducing complex. Since *A. baumannii* has three *tonB* orthologs (*tonB1, tonB2, tonB3*) we characterized by reverse genetics the role of individual *tonB* gene and of the ferrous iron uptake system *feoB* in *A. baumannii* ATCC 19606^T. Growth under iron restriction and virulence in both insect and mouse models of infection were primarily dependent on TonB3, but not on TonB1, TonB2 or FeoB. Thus, the TonB3 energy-transducing machinery is essential for *in vivo* growth and represents a promising target for anti-bacterial therapies.

O18.1
Shotgun metagenomic analysis of sputum samples from cystic fibrosis patients revealed distinct metabolic modules and antibiotic resistance genes along with severe lung disease

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Several studies have shown associations between cystic fibrosis (CF) respiratory microbiota and clinical outcomes but it is still not clear if such different microbiota assemblages may underlie a different pattern of bacterial functions. Here we used a full-genome shotgun metagenomics analysis of sputum samples collected from patients with mild and severe CF lung disease to gain new insights into CF microbiome aiming at unraveling the underlying causes of the severe lung disease. Results showed a different core set of metabolic pathways, functions, metabolic modules along with efflux-mediated antibiotic resistance mechanisms and several virulence-related factors between the two groups of patients. In particular, a set of metabolic pathways correlated to a worsening of patient's clinical conditions was found, with two of them of clinical relevance. Furthermore, an imbalanced distribution of virulence factors along with antibiotic resistance genes has been found between patients with mild and severe lung disease, with the latter displaying a massive presence of both gene categories. Funded by Italian Cystic Fibrosis Research Foundation (FFC#10/2014 and FFC#14/2015).

O18.2
Alteration of gut microbiota profiles in juvenile idiopathic arthritis. Associations with HLA-B27 status and disease activity

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Alteration of gut microbiota is involved in several chronic inflammatory and autoimmune diseases, including rheumatoid arthritis, and gut microbial "pro-arthritogenic" profiles have been hypothesized. Intestinal inflammation may be involved in spondyloarthropathies and in a subset of patients affected by Juvenile Idiopathic Arthritis (JIA). We compared the gut microbiota composition of JIA patients with healthy subjects (HS), evaluating differences in microbial profiles between sub-categories of JIA, such as enthesitis-related arthritis (JIA-ERA) and polyarticular JIA (JIA-nERA). Through taxon-level analysis, we discovered alteration of gut microbiota components that could be involved in subclinical gut inflammation, and promotion of joint inflammation. Among the more relevant genera, we found an increase in *Clostridium* cluster XIVb, involved in colitis and arthritis, in JIA-ERA patients compared with HS, and a decrease in *Faecalibacterium,* known for anti-inflammatory properties, in JIA-nERA compared with JIA-ERA and HS. Differential abundant taxa identified JIA patients for the HLA-B27 allele, such as *Bilophila*, *Parvimonas* and *Oscillibacter*. We also discovered intra-group variability between active disease and remission. The observed

differences between JIA and HS, similarly to other chronic autoimmune and inflammatory diseases, suggest insights on the possible involvement of gut microbiota as a co-player in JIA pathogenesis.

O18.3
Novel human antimicrobial peptides from ApoB are endowed with promising anti-inflammatory properties

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By using a bioinformatic method recently developed by our group, we identified in human ApoB (region 914-950) a putative antimicrobial peptide (AMP), and produced two variants of this AMP, (P)$ApoB_L$ and (P)$ApoB_S$, 38- and 26-residue long, respectively. Both AMPs were successfully produced in bacterial cells by using a fermentative process based on an auto-induction system, and found to be endowed with a broad-spectrum antimicrobial activity while they show no toxicity towards normal human cells. Interestingly, in the case of $ApoB_S$ peptide, a significant anti-biofilm activity was also observed. Circular dichroism studies showed that SDS, TFE and LPS significantly alter $ApoB_L$ conformation, whereas no significant effects were detected in the presence of alginate. Interestingly, ApoB derived peptides elicit anti-inflammatory effects, since they are able to mitigate the production of some pro-inflammatory cytokines while determining an increase of anti-inflammatory cytokines levels in eukaryotic cells under LPS stimulation. No hemolytic side effects were detected when peptides were tested on murine erythrocytes. This opens interesting perspectives on the therapeutic use of these AMPs.

O18.4
Human serum albumin acts as a self-defense protein towards *Clostridium difficile* infection

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Clostridium difficile is a toxins-producing bacterium causing from mild diarrhea to fulminant colitis. Low levels of human serum albumin (HSA), one of the most abundant proteins in plasma acting as depot and carrier for many endogenous and exogenous compounds, represent a risk factor for acquiring and developing severe *C.difficile* infection (CDI) and for causing recurrent or fatal disease course. To date, the mechanism by which hypoalbuminemia predisposes to a more severe form of CDI is not yet understood. The aim of this work was to dissect the molecular mechanisms underpinning the protective role of HSA towards *C.difficile* cytotoxicity. Results showed that HSA protects intestinal CaCo-2 cells from toxins-induced cytotoxicity by sequestering toxins outside the host cells. Thermodynamic parameters of HSA-toxins binding were determined and docking analyses predicted that the interaction involves the domain responsible for toxins internalization and the subdomain II of HSA. In turn, HSA binding to *C.difficile* toxins induces their autoproteolytic cleavage with the consequent impairment of their uptake. Experiments with zebrafish are in progress to validate *in vivo* results obtained. Overall, our data represent the first evidence of a self-defense mechanism mediated by HSA towards a bacterial infection, and provides a rationale to explain the clinical correlation between CDI course severity and hypoalbuminemia.

O18.5
Identification and analysis of candidate genes involved in the oligogalacturonide-induced responses in *Arabidopsis thaliana*

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The plant cell wall has key roles in plant growth and development and in defence. Oligogalacturonides (OGs) are pectin fragments that act as elicitors of defence responses and can also modulate developmental processes through their antagonism with the plant hormone auxin. This work aims at elucidating early events after OG perception and characterize the functional role of elements activated by these elicitors. Phosphoproteomic studies identified *Arabidopsis thaliana* membrane proteins that exhibit increased or decreased phosphorylation early after OG perception. Two candidates were chosen for a functional validation of their role in immunity: IAA11 and PCAP1. IAA11 is an AUX/IAA protein that mediates auxin-dependent gene expression; we isolated a null-mutant to investigate the possible role of IAA11 in the OG-induced expression of early defence genes and in the IAA/OG antagonism. PCaP1 is a plasma membrane protein involved in intracellular trafficking. The characterization of null mutants indicated that PCaP1 is required for the full response to OGs. In addition, nuclear proteins interacting with the promoter of IAA5 were identified by DNA affinity capture and proteomic identification.

19 - Protein Synthesis, Degradation and Homeostasis

P19.1
RsgA from Pseudomonas aeruginosa: a structural and functional investigation

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Ribosome small subunit-dependent GTPase (RsgA), is a circularly permutated GTPase involved in ribosome maturation; this protein associates with the 30S ribosomal subunit in a GTP-dependent manner. The *rsgA* gene is required for survival and virulence of different bacterial species since its deletion strongly affects cell growth. Interestingly, RsgA has been recently identified as one of the target of (p)ppGpp, a second messenger responsible for the bacterial switching to stringent response during stress such as nutrient deprivation. RsgA from the pathogenic bacterium *P. aeruginosa* (PaRsgA) was cloned, expressed in *E. coli* and purified in the GDP-bound form. A protocol to obtain the apo form of the protein was also set up, followed by the characterization of both the apo and holo forms stability. PaRsgA ability to bind GTP and GDP was investigated by fluorescence spectroscopy and its GTPase activity was also assessed. Finally, PaRsgA has been crystallized and preliminary diffraction data have been collected. Structural and functional characterization of RsgA will be instrumental for the screening of specific inhibitors aimed at the development of new antibacterial drugs.

O19.1
Schizophrenia susceptibility genes: on the characterization of two variants of human pLG72

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pLG72, a small protein present in primates only, interacts in the human brain with the enzyme D-amino acid oxidase (hDAAO), which main role is the modulation of the levels of D-serine, an endogenous modulator of the NMDAR. Alteration in D-serine-dependent NMDAR activation has been reported in various neurological diseases, e.g. decreased D-serine levels have been detected in schizophrenia. Here, we investigated the effect of two point mutations in pLG72: the R30K (SNP rs2391191) related to schizophrenia susceptibility and the K62E (SNP rs9558562). The substitutions did not affect the protein conformation, the dimeric state and the binding of hydrophobic ligands. For the R30K pLG72 a tighter hDAAO binding was observed as well as a faster inactivation at low pLG72 ratio compared to the wild-type protein, while at increasing ratios the K62E variant most quickly affected the hDAAO activity. Accordingly, an increase in cellular (D/D+L)-serine levels in human glioblastoma U87 cells stably expressing the different pLG72 variants was apparent. Notably, the R30K substitution also strongly affected pLG72 half-life in U87 cells: the effect on the flavoenzyme half-life is under investigation.

O19.2
A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis

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The mechanisms underlying transthyretin-related amyloidosis in vivo remain unclear. The abundance of the 49–127 transthyretin fragment in ex vivo deposits suggests that a proteolytic cleavage has a crucial role in destabilizing the tetramer and releasing the highly amyloidogenic 49–127 truncated protomer. Here, we investigate the mechanism of cleavage and release of the 49–127 fragment from the prototypic S52P variant, and we show that the proteolysis/fibrillogenesis pathway is common to several amyloidogenic variants of transthyretin and requires the action of biomechanical forces provided by the shear stress of physiological fluid flow. Crucially, the non-amyloidogenic and protective T119M variant is neither cleaved nor generates fibrils under these conditions. We propose that a mechano-enzymatic mechanism mediates transthyretin amyloid fibrillogenesis in vivo. This may be particularly important in the heart where shear stress is greatest; indeed, the 49–127 transthyretin fragment is particularly abundant in cardiac amyloid. Finally, we show that existing transthyretin stabilizers, including tafamidis, inhibit proteolysis-mediated transthyretin fibrillogenesis with different efficiency in different variants; however, inhibition is complete only when both binding sites are occupied.

O19.3
The double life of a disordered protein between solubility and aggregation propensity

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Protein aggregation is involved in a wide variety of diseases and represents an important hurdle to produce recombinant proteins in bacterial cells. A better understanding of underlying phenomena can have multifaceted returns in helping to prevent or to exploit aggregation. Just consider the design of in/solubility tags, valuable to recover a target protein by *in-vitro* aggregation or, *viceversa*, to prevent its undesired aggregation. Our study is aimed at understanding the role of electrostatic charges on aggregation propensity of an intrinsically disordered protein. Our model is the N-terminal moiety of a viral phosphoprotein (PNT). The experimental strategy consists in comparing aggregation propensity of wild-type PNT with its negatively and positively "supercharged" variants. We produced the three PNT variants as recombinant proteins *per se* and as GFP-tagged fusions. GFP is a well-known, structured protein that can be induced to aggregate and whose fluorescence easily reports its functional and conformational status. An array of biophysical and biochemical techniques indicates that PNT variants, and PNT-GFP fusions strongly react to pH becoming pretty insoluble at their own pI. Paucity of charges emerges as a leading force of aggregation overwhelming the entropic effects of conformational flexibility on solubility.

O19.4
Integration of cAMP and the ubiquitin system at primary cilium

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The primary cilium is an organelle that emanates from cell surface of growth arrested cells that plays a major role in vertebrate development and tissue homeostasis. Defective cilia are often cause of genetic disorders and proliferative diseases. The mechanisms that control ciliogenesis have been largely explored. However, the intersection between GPCR•cAMP signaling and the ubiquitin pathway in the control of cilium stability are largely unknown. We have identified a multimeric complex nucleated at centriolar satellites that controls primary cilium formation and stability. Activation of the cAMP cascade induces ubiquitination and proteolysis of the mitotic kinase NEK10. Disappearance of NEK10 culminates in cilia resorption. The effects of cAMP on cilium are blunted in human fibroblasts from SCAR16 patients carrying inactivating mutations of components of the ubiquitin system. Conversely, activation of the ubiquitin pathway in high-grade tumors is linked to downregulation of NEK10 and loss of primary cilia. Altogether, these findings unveiled the existence of a pericentriolar multivalent complex that efficiently couples GPCR•cAMP cascade to the ubiquitin-proteasome system, controlling essential aspects of ciliogenesis.

20 - Stem Cells, iPS, Cancer Stem Cells

P20.1
M2 receptor activation counteracts the Glioblastoma Cancer Stem Cells responses to anoxia conditions.

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Glioblastoma multiforme (GBM) is the most malignant brain tumor. Anoxia/hypoxia condition is a predominant feature of the GBM contributing to tumor growth and resistance to conventional therapies. Therefore, the identification of drugs able to impair the malignancy and aggressiveness of the GBM is considered of great clinical relevance. Recently, we demonstrated that the activation of M2 muscarinic receptors, through the agonist arecaidine propargyl ester (APE), arrests cell proliferation in glioblastoma cancer stem cells (GSCs). In the present work, we have characterized the response of GSCs to anoxic condition (95%N 2 , 5%CO 2) demonstrating an up-regulation of HIF1α, Glut1, VEGF, EGFR, pro-granulin and stemness markers expression (CD133 and Nestin). The APE treatment is able to decrease the cell number and inhibits cell cycle progression in anoxia conditions. qRT-PCR and immunolocalization have also indicated that APE down-regulates the expression of stemness markers and Glut-1 expression in anoxia. Our data suggest that APE impairs the ability of GSC to adapt themselves to anoxia, making the GSCs less able to proliferate and develop resistance characters to hypoxic stress.

P20.2
The role of oncogenic RAS in human skin tumorigenesis depends on clonogenic potential of the founding keratinocytes

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The role of Ras in human skin tumorigenesis induction is still ambiguous. Overexpression of oncogenic Ras causes premature senescence in cultured human cells and hyperplasia in transgenic mice. We investigate whether the oncogenic insult outcome may depend on the nature of the founding keratinocyte. We demonstrate that Ras-V12-overexpression induces senescence in primary human keratinocyte cultures, but some cells escape senescence and proliferate indefinitely. Ras-overexpression in transient-amplifying (TA)- or stem cell (SC)- enriched cultures shows that p16 levels are critical for the final result. Indeed, TA-keratinocytes expressing high levels of p16 are sensitive to Ras-V12-induced senescence whereas cells with high proliferative potential, which do not display p16, are resistant. The subpopulation, which sustains the indefinite culture growth, exhibits SC features. Bypass of senescence correlates with pRb pathway inhibition and TERT resumption. Immortalization is also sustained by activation of ERK1/2 and Akt pathways. Moreover, only transduced cultures, originating from cultures bearing SCs, induce tumors in nude mice. Our findings demonstrate that Ras-overexpression outcome depends on the clonogenic potential of the recipient keratinocyte and only the SC compartment is competent to initiate tumorigenesis.

P20.3
Dose-dependent response of neural stem/progenitor cells to ionizing radiation

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Ionizing Radiation (IR) is a genotoxic agent that can cause oncogenic transformation of irradiated cells, but can also be employed in a clinical setting for cancer radiotherapy. Several forms of cancer are thought to arise from transformation of stem/progenitor cells (SPCs). Since radiotherapy may cause exposure of healthy SPCs to IR, the response of tissue-resident SPCs to IR needs to be thoroughly investigated. We have analyzed the response of mouse neural SPCs to low (0.2 Gy), moderate (1 Gy) and high (10 Gy) doses of X-ray IR during the first 24 hours post-irradiation. We found that 10 Gy IR significantly increased neural SPC mortality, although the majority of irradiated cells were still viable. Furthermore, we detected dose-dependent delay of cell cycle progression and dose-dependent up-regulation of cell cycle inhibitors, apoptotic markers and differentiation markers in irradiated cells. These results suggest that neural SPCs are resistant to IR-dependent toxicity, at least in the short term, but their self-renewal may be compromised by IR in a dose-dependent fashion. We are presently investigating the long-term effects of IR on neural SPCs.

P20.4
Autologous adipose tissue transplantation: regenerative potential of lipoaspirates obtained by different processing systems

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The aim of this study was to compare the effect of three common fat-processing techniques (decantation, centrifugation, and washing through a close system) on the *in vitro* regenerative potential of the collected tissues. Liposuction was performed in the lower abdomen and three specimens were collected from each patient to obtain three 10 ml samples of purified fat. The number of blood cells, mature adipocytes and stromal vascular fraction (SVF) cells were assessed together with the growth rate and colony unit formation (CFU) of SVF cells. The greatest number of mature adipocytes and blood cells was observed in the decanted aspirates ($p < 0.05$). Both the amount and growth rate of SVF cells, as well as CFU formation, were the highest in washed samples ($p < 0.05$). Finally, osteoblast and adipocyte commitment of SVF cells obtained from samples of all three procedures confirmed the proneness of the contained AMSC subpopulation to differentiate toward mesenchymal lineages. In conclusion, washing seems to be the best processing technique for adipose tissue graft as it maintains adipocytes integrity, clears the fat from most blood contaminants and provides the greatest number of AMSCs.

P20.5
Immunomodulatory properties of aminiotic stem cells obtained through a newly established isolation protocol

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Human amnion-derived stem cells (hASCs) are considered as a novel and

convenient source in cell-based applications due to their great plasticity and demonstrated differential capacity. The aim of this study was to optimize protocols for the isolation of a homogenous ASCs population and to investigate their immunomodulatory properties in view of their potential use in cell therapy. A new strategy to improve the efficiency of hASCs isolation was established by combining amnion fragmentation, prolonged digestion treatment and filtration compared to previously reported protocols. Isolated cells expressed multi- and pluripotent-associated markers, and had great proliferation rate. Functional studies showed that the soluble factors released by hAMSCs (i.e IL-10, TGF-β, PGE-2 and COX-2) were able to reduce the expression levels of pro-inflammatory markers (i.e. IL-1β, iNOS) of activated immune cells (U937) and to inhibit lymphocytes proliferation. These findings make hASC suitable candidates for the treatment of chronic disorders, as in the case of endometriosis where the immune system has been suggested to play an important role.

P20.6
Molecular and phenotypic characterization of cancer stem cells from human tumor cell lines

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The cancer stem cell theory states that a subset of tumor cells, termed cancer stem cells (CSCs), is responsible for tumor initiation, metastasis and recurrence. In this work, a culture system was setup to enrich CSCs from Hep-2, T24, MG63, CaCo-2 and A549 cancer cell lines, through sphere formation. Molecular and phenotypic characterization of CSC-enriched populations was performed by exploring the expression of stem markers and *in vivo* evaluating the tumorigenic potential. The expression of nicotinamide N-methyltransferase (NNMT) was also investigated. Results obtained showed the upregulation of stem markers in CSC-enriched populations compared with controls. After subcutaneous injection of Hep-2 cells into athymic mice, CSC-enriched population yielded tumors of a much larger size compared with those generated by parental cells. NNMT expression levels were markedly higher in CSC-enriched populations compared to parental cells. This study provides a useful methodology to enrich CSCs from tumor cell lines, allowing their molecular characterization. Moreover, NNMT overexpression in CSC-enriched populations seems to suggest a pivotal role of this enzyme in cancer growth and metastasis.

P20.7
Exploiting telomere and telomerase status for targeting colorectal cancer stem cells

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Cancer stem cells (CSCs) are the cell subpopulations presenting self-renewal and long-term repopulation potential that are believed to drive cancer initiation, propagation and spreading. CSCs have been linked to tumor recurrence and resistance to anticancer therapy in most neoplasms, including colorectal cancer (CRC). In this study, with the aim of discovering candidate therapeutics to be clinically investigated, we have characterized telomeres length, telomerase activity (or the presence of alternative telomeres lengthening mechanisms), and telomere damage on a large panel of patient-derived colorectal CSCs (CRC-SCs). Preliminary results indicate that most CRC-SCs have short telomeres, increased telomerase activity and high levels of telomere damage as compared to human fibroblasts and colorectal carcinoma cell lines. This evidence suggests the existence, in CSCs, of specific mechanisms for regulating telomere length and functionality, whose discovery can lead to the development of more effective anti-cancer therapies.

P20.8
Evaluation of the effect of Multistage vectors on stem cell behaviour for their potential use in Stem Cell Therapy

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Nanotechnology and stem cells have been recently combined to enhance the outcome of several regenerative medicine approaches. The purpose of the work is to shed the light on the potential use of multistage vectors (MSV), constituted by silicon nanostructured microparticles currently exploited as cancer therapeutics, to improve mesenchymal stem cells (MSC)-based therapies. MSC were incubated overnight with different concentrations of MSV (1:2 and 1:10, cell/particles) and MSV effect was assessed in terms of metabolic activity, MSC-specific markers expression, differentiative and immunosuppressive potential. MTT assay showed no alterations in MSC metabolism overtime, no differences were observed in the MSC phenotype (assessed by flow cytometry and qPCR), and MSV-treated cells retained the capability to differentiate into osteoblasts as revealed by Von Kossa staining and the expression of osteogenic-associated genes. MSV uptake induced a transient overexpression of stress related molecules (i.e nitric oxide synthase, prostaglandin E2). When the immunosuppressive potential of MSV-treated MSC was assessed, high levels of immunomodulatory markers (*PGE-2*, *TNF*-a, *iNOS*) were revealed following the stimulation with pro-inflammatory cytokines (TNF-a and IFN-g). Our preliminary findings show that MSV are able to improve MSC therapeutic properties and make them suitable for the development of innovative cell-based therapy.

O20.1
Stearoyl-CoA-Desaturase (SCD1) regulates lung cancer stemness via stabilization and nuclear localization of YAP/TAZ

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One of the most common traits of cancer is the radical change of cellular metabolism. Recent evidences suggest that the enzyme involved in Monounsaturated Fatty Acids synthesis, SCD1 plays a role in several cancers and we previously showed that SCD1 is important in lung cancer stem cells survival and propagation. We report here, that the effectors of the Hippo pathway, YAP and TAZ, are required for the generation of human lung cancer 3D cultures. We demonstrate that SCD1 inhibition determines a decrease in the expression and nuclear localization of both YAP and TAZ and a decrease in their transcriptional activity. Regulation of YAP/TAZ by SCD1 is in part dependent upon β-catenin pathway activity as SCD1 activation of nuclear YAP/TAZ is dependent upon inactivation of the β-catenin destruction complex. Finally, interrogation of gene expression datasets from stage I-II adenocarcinoma of the lung

showed that high co-expression levels of SCD1 with either YAP or TAZ or β-catenin itself have a negative prognostic value. Overall our data demonstrate for the first time the involvement of SCD1 in the regulation of the Hippo and β-catenin pathways in lung cancer stem cells.

O20.2
CHK1 as a target to kill colorectal cancer stem cells

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Cancer stem cells (CSCs) are cell subpopulations responsible for tumor formation also contributing to therapeutic resistance, cancer dissemination and intratumor heterogeneity. CSCs limit the efficacy of antineoplastic regimens and their targeting is required for tumor eradication. To discover potential anti-CSC therapeutics to be clinically investigated as single agent, we performed a drug-library screening on patient-derived colorectal tumorspheres enriched for CSCs (CRC-SCs). This screening led to the identification of LY2606368 as a potent anti-CSC agent acting *in vitro* and *in vivo* in tumor cells from a considerable number of patients (~36%). We provided evidence that, by inhibiting CHK1, LY2606368 affected DNA replication and abrogated the intra-S and G2-M checkpoint in most CRC-SCs. This pushed DNA-damaged or incorrectly DNA-replicated CRC-CSs through premature, lethal mitoses. Given that a fraction of CSCs displayed resistance to LY2606368, we are investigating biomarkers predicting CSC sensitivity to this agent. This will give insights on the true potential of this anti-CSC approach also allowing for patient selection that more likely will benefit from LY2606368 therapy.

O20.3
Biomimetic coatings to improve MSC homing toward the site of inflammation

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A major challenge in mesenchymal stem cell (MSC) therapy is to deliver therapeutic cells to a target location efficiently and with minimal invasion. The aim of this work was to promote the transient expression of CD44, the hyaluronic acid (HA) receptor, on MSC membranes, which mediates cell migration toward the site of inflammation. Tissue culture plates were coated with different concentrations of HA (1-0.01 mg/ml) and CD44 expression was evaluated on murine MSC at 1 and 3 days, showing a significant increase in cells treated with the highest concentration of HA. An *in vitro* migration assay revealed a 2.87-increase in the migratory potential of compared to untreated cells. The efficacy of the system was finally evaluated *in vivo*, using LPS-induced inflamed ear murine model to determine whether HA-treated MSC displayed enhanced homing to other distant sites of inflammation following systemic administration. 24 hours after HA-cell injection, an IVIS *system* demonstrated a 2-fold increase in the number of homing MSC compared to the controls. These results suggest that the HA-coating represent a simple and versatile method to transiently overexpress CD44 on the MSC surface and potentially target systemically administered cells to the site of inflammation.

O20.4
Induced Pluripotent Stem Cells (iPSCs) as a disease model system to identify altered pathways in Amyotrophic Lateral Sclerosis

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The RNA-binding protein FUS has been recently associated to Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disease caused by loss of motoneurons (MNs). Cytoplasmic inclusions containing mutated FUS represent a hallmark of the pathology, however little is known about molecular mechanisms leading to MNs death. We have derived a collection of iPSCs carrying ALS mutations in FUS by reprogramming from patients or gene editing. We show that aberrant cytoplasmic localization of mutated FUS and its recruitment into stress granules (SGs) were recapitulated in iPSC-derived MNs. This is in line with the evidence that FUS cytoplasmic inclusions contain SGs markers. To fully exploit the potential of ALS-iPSCs, we coupled a rapid and high yield differentiation protocol with a fluorescent reporter that allows FACS isolation of MNs for molecular characterization. miRNAs profiling revealed several miRNAs deregulated in FUS ALS MNs. The biological consequence of miRNAs deregulation could provide insight into the altered pathways in ALS. Therefore, the iPSC system presented here represents a suitable model for investigating ALS etiopathogenesis.

O20.5
Induced Pluripotent Stem Cells (iPSC) as a model of Huntington disease

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Huntington disease patients' cell lines may offer the opportunity to study longitudinal changes due to the mutated protein in its own physiological context. We are therefore exploring whether human induced pluripotent stem cells (iPSCs) from HD subjects represent a model to investigate the polyQ effects in vitro. Aims: Creation of an iPS collection from HD subjects; Analysis of neural functions in reprogrammed cell lines, according to the different repeat sizes.Patient's skin fibroblasts were reprogrammed into iPSCs by virus-free protocol. All iPS clones that show an uniform flat morphology were characterized for their stemness and pluripotency, both in vitro and in vivo. A new protocol was optimized for differentiation of iPCs in neurospheres of Neuronal Precursor Cells (NPCs). We may obtain a neural population of astrocytes, oligodendrocytes and neuron cells by spontaneous differentiation of neurospheres in particular conditions.We have obtained skin biopsies from positive subjects carrying borderline and very high repeats and control subjects without the mutation. We expect this model will allow us to highlight polyQ dependent changes in neural function and differentiation, in vitro.

21 - Nutrition Biochemistry

P21.1
Pigmented grains as a source of immunomodulating bioactives

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Phenolics and anthocyanins present in the outermost fractions of the pigmented varieties of common grains were collected by de-branning and used for preparing nutritionally enriched pasta. Analytical profiling of the phenolics in bran components/ fractions separated by these physical techniques was carried out by advanced LC methods. The immunomodulating effects of selected fractions from the separation procedures were assessed by using cytokine-stimulated biosynthesis of a luciferase-labeled reporter of gene activation in properly transformed Caco-2 cells. The outermost fraction from debranning of pigmented grains was found to be a very rich and convenient source of both phenolics and fiber. The Immunosuppressive activity of the fractions used for pasta making was found to be dose-dependent, and was much higher than that of reference antocyanins. Enriched pasta was produced from wheat flour and from purple wheat bran, with fiber content in excess of 10%, and a content in anthocyanins in the 100 mg/kg range. Enrichment in specific bioactive compounds of staple foods through physical methods will allow further studies on the effects of their consumption on the gut microbiota and on stress markers.

P21.2
Antioxidant properties and *in vitro* gastric digestion of *Lathyrus sativus*

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Grass pea (*Lathyrus sativus)* is an orphan legume, whose importance is increasing day-by-day since it is characterized by several advantageous biological and agronomic characters such as nitrogen fixation, resistance to insects and pests, high gran-yielding capacity and high protein content of its seeds. In this study a grass pea variety present in the market as a Slow Food Recommended Product, was characterized according to phenolic content, antioxidant activity and digestibility. The total phenolic content of grass pea flour yielded average value of 0.511±0.02 mg/g, expressed as gallic acid equivalents, evidencing a relative abundance of these molecules in the grass pea flour. The antioxidant activity measured by DPPH assay by dissolving flour in methanol for 1 h, 2 h, 3 h and 24 h, increased by increasing the incubation time with methanol, and reaching, after 24 h, a activity equal to 34.4%. In this work we also simulated the digestibility of grass pea flour proteins following both the adult and elderly model, in order to study the digestion of the flour proteins by the human gut. Our results demonstrated that the grass pea proteins were easily digested and no evident differences were recorded between both models, suggesting a potential application of this legume to develop age-tailored oral formulations.

P21.3
17β-estradiol modulates sulforaphane protection against oxidative stress in cardiomyocytes

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It has been shown that hormones can influence the response to cardiovascular medications in males and females, but no studies have been carried out on hormone-related response to nutraceuticals. This study investigated the protective effect of sulforaphane (SF) in the presence/absence of 17β-estradiol (E2) against oxidative stress in primary rat cardiomyocytes. Cells were treated with SF and/or E2 and oxidative stress induced by H_2O_2. SF and E2 co-treatment led to a higher level of protection against H_2O_2 and reduced intracellular ROS levels in respect to SF or E2 alone. Co-treatment increased GSH level and the expression of several antioxidant enzymes in respect to SF or E2. The co-treatment induced a higher activation of Akt and ERK1/2 signaling pathways in respect to SF and E2. Transmission electron microscopy analysis revealed that co-treatment was more effective in counteracting H_2O_2-induced alteration of cell organelles than E2 or SF. Our results demonstrate for the first time that estrogens could enhance SF protective effects, suggesting that nutraceutical efficacy might be different in males and females. This work was supported by Fondazione del Monte di Bologna e Ravenna

P21.4
Castanea Sativa Mill. bark extract exerts chemopreventive properties triggering extrinsic apoptotic pathway in Jurkat cells

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Chemoprevention represents the possibility to prevent, stop or reverse the cancerogenetic process. In this context the interest towards natural extracts has grown due to their phytochemical content. *Castanea Sativa Mill.* (CSM) bark extracts showed to exert positive effect in the counteraction of chronic/degenerative diseases, therefore, we evaluated its potential chemopreventive effect. Flow cytometry (FCM) analyses of Jurkat cells treated with CSM bark extract 0-500 µg/mL for 24-72h allowed to evaluate its cytotoxicity and ability to induce apoptosis. Moreover, to define if CSM bark extract was selective towards cancer cells, its cytotoxic and proapoptotic effect was evaluated in human lymphocytes (PBL) from healthy donors. CSM bark extract induced apoptosis in Jurkat cells in a dose- and time-dependent manner by activating the extrinsic pathways as evidenced by the activation of caspae 8. Moreover, at 24h treatment IC_{50} resulted 304 and 128 µg/mL in PBL and Jurkat cells respectively. CSM bark extract resulted a partially selective chemopreventive agent thanks to its ability to induce apoptosis in cancer cells at concentrations lower than in non-transformed cells.

P21.5
Characterization of peptide fraction from sourdoughs

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Sourdough fermentation represents one of the oldest biotechnologies to ferment cereals, thanks to its effect on the properties of leavened baked goods such as the positive effect on glycaemic index and antioxidant and anti-inflammatory properties. In fact, microbial metabolism during fermentation produce bioactive compounds, such as peptides, beta-glucans, and amino acid derivatives with various functionalities. The present study was funding by Regione Toscana and it deals with the nutraceutical properties of peptides from sourdoughs produced in Tuscany. 20 Lactobacillus strains (LABs) have been used to produce sourdough and assessed for proteolytic activity. Peptides from water extraction were fractionated on RP-HPLC and the low-molecular weight fractions were assayed for anti-oxidant and anti-inflammatory activity on RAW-264.7 cells. All the samples showed a slight ability to increase the cellular vitality, and some of them are able to reduce the ROS formation in LPS-treated cells. Peptides with the higher anti-oxidant activity have been further analysed for anti-inflammatory properties (i.e. the ability

to decrease pNF-kB and to increase the expression of its inhibitor Ikb).

P21.6
Insulin-mimetic and antioxidant properties of the natural sweetener *Stevia rebaudiana* Bertoni

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Stevia rebaudiana Bertoni has been used not only as sweetener but also for its beneficial and therapeutic properties. Several studies suggest that *Stevia* is able to modulate glycemia, facilitating glucose uptake into the cells. Nevertheless, mechanisms underlying these effects are still unclear. Therefore, the aim of this study was to deepen the knowledge about insulin–mimetic effect exerted by *Stevia* glycosides in a cell model system. Phosphorylation levels of enzymes involved in the two mechanisms of insulin signal transduction were investigated in the presence of *Stevia* extracts. Results show an increase in phosphorylation levels of IGF-1R, PI3K, and Akt but not of AMPK. Moreover, cell treatment with the extracts increased GLUT4 translocation to plasma membrane. The potential antioxidant activity of *Stevia* extracts was also assessed: a direct antioxidant activity was not detected, although an increase of catalase and superoxide dismutase activities occurred. The obtained results suggest that *Stevia* glycosides can be considered as potential nutraceuticals, useful in the co-treatment of diseases (e.g. diabetes, obesity, metabolic syndrome) and for health maintenance.

P21.7
Bioavailability of tomato cystine-knot miniproteins with anti-angiogenic properties

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The cystine-knot miniproteins (TCMPs) present in tomato fruit and tomato-based products have been shown to exert anti-angiogenic effects by inhibiting *in vitro* endothelial cell migration. The bioavailability and the potential anti-angiogenic effects of TCMPs were investigated *in vivo* using the zebrafish as animal model. TCMP purified from tomato fruit was administered to zebrafish embryos assaying changes in vessel development. TCMP at 500 nM inhibited the formation of subintestinal vessels in zebrafish, a process susceptible to the activity of antiangiogenic compounds. To explore the potential of TCMPs for oral administration, we investigated the effects of *in vitro* gastrointestinal digestion of TCMPs extracted from tomato fruit and tomato paste demonstrating that are resistant to proteolysis. To quantify the intestinal transport of TCMPs we used the differentiated Caco-2 cells model. After 24h incubation, 37.73 ± 9.34% of TCMPs crossed the epithelium, without altering the integrity of the cell layer. In conclusion, we show that the gastrointestinal process does not alter the *in vivo* biological activities of TCMPs, making them a good candidates for further drug development.

O21.1
Evaluation of the potential protective effect on vascular endothelium exerted by the peptide nsLTP2 identified in wheat

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The maintenance of the endothelial function, challenged by oxidative stress and inflammation, is a fundamental target of preventive dietary strategies. The aim of this study was to investigate the protective activities of a wheat peptide belonging to the family of the "non-specific lipid transfer protein type 2" (nsLTP2). Results showed that HUVECs pretreatment with nsLTP2 significantly decreased both oxidative stress and cell damage induced by inflammation, and significantly counteracted the increased expression of adhesion molecules (VCAM-1, ICAM-1, E-selectin). Moreover, nsLTP2 increased the expression of HO-1, similarly to statins. As HO-1 has been shown to ameliorate inflammation in part through its ability to inhibit expression of endothelial adhesion molecules, experiments were also conducted using two HO-1 inhibitors, showing that the induced expression of HO-1 contributes to the anti-inflammatory activity exerted by nsLTP2. Taken together, obtained results suggest a nutraceutical role played by nsLTP2 in maintaining vascular health. This work was supported by BACCHUS project (FP7 European Commission Grant Agreement 312090)

O21.2
Strawberry tannins exhibit anti-inflammatory activities in vitro by inhibiting the NF-κB pathway and by other mechanisms

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Strawberries (*Fragaria* x *ananassa* Duch.) are among the most consumed berries in the world and an attractive functional food because of several preventive and therapeutic health benefits, mainly versus chronic diseases. Gastritis represents one of the most common diseases characterized by chronic inflammation. Strawberries contain different classes of compounds with potential anti-inflammatory activities and we decided to investigate in more depth the chemical and biological features of the tannin fraction. Strawberry tannins inhibit IL-8 secretion in TNF-α-treated gastric (AGS) cells by dampening the NF-κB signaling. In vitro simulated gastric digestion slightly affected the chemical composition and the biological properties of strawberry tannins. By using pure compounds we found that casuarictin is more selective for the NF-κB pathway while agrimoniin inhibits IL-8 secretion also acting on other biological targets; in our system procyanidin B1 prevents the TNF-a-induced effects without interfering with the NF-kB pathway. In conclusion, strawberry tannins, even after in vitro simulated gastric digestion, exert anti-inflammatory activities at nutritionally relevant concentrations.

O21.3
Methionine metabolism imbalance in AMPK-deficient yeast models

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AMPK is an evolutionary conserved regulator of energy homeostasis in eukaryotes (Hardie, 2007). Yeast strains with altered Snf1/AMPK kinase activity have been used as excellent models for energy metabolism studies in response to nutrient changes (Nicastro et al, 2015). Since methionine and S-adenosylmethionine (SAM) represent critical barometers of cellular metabolic state and elevated level of homocysteine has been associated with various disorders, methionine metabolism was investigated as a function of Snf1 activity. Specifically, the metabolic rewiring features occurring in cells lacking Snf1 catalytic activity

indicated that fermentative metabolism was reduced in favour of a higher respiration, intracellular methionine and homocysteine were consistently accumulated and *SAM1* and *SAM2* mRNAs (coding for SAM synthetases) were down-regulated, more strongly in the presence of methionine. Moreover, methionine and SAM induced lipid droplets accumulation. Qualitative and quantitative results from the different analytical approaches will be discussed to better elucidate the molecular mechanisms of metabolic dysfunction diseases involving AMPK in proliferating cells.

O21.4
Polystyrene nanoparticles to mimic a complex matrix: functional and structural features of a hypoglycaemic lupin protein

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In vitro simulation of *in vivo* conditions is usually achieved by reproducing the typical molecular crowding occurring in a food system or in a cell. Proteins and peptides often exert their function when inserted in high-order macromolecular structure such as protein complexes or membranes, or, in food systems, when associated to disperse phases. Stable adhesion of proteins to hydrophobic or hydrophilic structures, such as nanoparticles or nanoemulsions, could be considered a model to mimic these complex systems. In this study we explore the effects of adhesion to polystyrene nanoparticles (NPs) on the functional bioactivity of gamma-conglutin (Cg), a lupin seed protein able to reduce glycemia, and to elicit allergenic reponses. The molecular features at the basis of such activities are still not completely elucidated. The nature of the reversible interaction between Cg and NPs was characterized in terms of: i) amount of protein bound, ii) stability of the interaction, iii) protein structural modification. The biological effects of the protein absorbed to NPs has been studied by using transfected Caco-2 cells transformed to express markers evidencing triggering of immune response.

O21.5
Protective effect of inulin on LPS-induced intestinal smooth muscle impairment: a redox and proteomic approach.

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Inulin is a dietary fiber able to stimulate gastro-intestinal function acting as prebiotics. We recently demonstrated its protective effect on LPS-induced oxidative damage of colonic smooth muscle in an *ex vivo* experimental model. In the present study, this protective role of inulin has been further evaluated using a proteomic approach. Inulin exposure of human colonic mucosa was able to restore the LPS-dependent alteration of some proteins involved in the host response and in the intestinal smooth muscle contraction (ZG16, CALM1/MLCK/MYL signaling pathway), to reduce the up-regulation of two proteins involved in the radical-mediated oxidative stress induced by LPS (APEX1, CCT7) and to increase the level of some detoxification enzymes (MT2A, GSTK1 and UGT2B4) with respect to LPS treatment. Consistently inulin exposure to colonic mucosa and submucosa was able to restore the LPS-induced alteration of intestinal smooth muscle contraction and to prevent the oxidative damages of LPS-exposed tissues.

22 - Evolution

P22.1
Candidate genes involved in the evolution of viviparity: a RAD sequencing experiment in the lizard *Zootoca vivipara*

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Viviparity evolved from oviparity at least 150 independent times in vertebrates. More than 80% of these transitions occurred in squamate reptiles, where both reproductive modes are rarely seen in different populations of the same species. This condition (bimodal reproduction) is ideal to study the physiological and morphological changes underpinning the evolution of reproductive mode, and their genetic determinants. Here we analysed the genomes of *Zootoca vivipara* populations with either oviparous or viviparous reproduction using a RAD sequencing approach. No signature of interbreeding between oviparous and viviparous individuals was found at more than 80,000 SNPs. When the analysis was reduced to approximately 5,000 SNPs with low levels of missing data, we identified 37 annotated genes potentially associated with parity mode transition. Ten of these genes are transcription regulators that are also expressed in reproductive tissues of mammals and reptiles, suggesting that changes in gene expression are important for the evolution of viviparity. Four additional candidate genes encode protease enzymes, and we propose that the evolution of these genes support uterine remodelling during pregnancy.

P22.2
High resolution populations genomics reveals an extremely complex dynamic of early speciation in Afrotropical malaria vectors

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Genomes of natural populations are continually exposed to a number of evolutionary forces. Our ability to detect genomic signatures left by such processes depends upon the level of resolution available in terms of loci, individuals and populations sequenced. The *Anopheles gambiae* 1000 Genomes Consortium produced an extraordinary genomic data resource of 765 genomes sequenced at high coverage from 8 countries spanning Sub-Saharan Africa of the main malaria vectors *An. gambiae* and *An. coluzzii*. We report complex dynamics of early speciation, leading to various levels of reproductive isolation and to the establishment of two hybrid forms. We virtually described all possible directions a non-homogenised early speciation process can take: i) local genomic divergence surrounded by remarkable homogeneity, ii) adaptive introgression events, iii) reinforcement of reproductive barriers in sympatry, iv) origins of hybrid forms with local complete replacement of pure individuals. Moreover, we identified candidate genes under divergent selection giving a molecular support to previous hypothesis on mechanisms for the maintenance of reproductive isolation necessary to species formation.

O22.1
Evolution of vitellogenin gene family in basal sarcopterygians

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The genes of vitellogenin, from which the main egg-yolk proteins of oviparous animal species arise, are an excellent example to understand how a gene family origins and has been changing during the evolutionary history. The coelacanth *Latimeria menadoensis* and the lungfish *Protopterus annectens*, considered living fossils, occupy a key phylogenetic position to investigate changes that have affected the genomes of the aquatic vertebrates that colonized dry land. In this study, transcripts of several vitellogenin genes in the two species of interest were isolated and their inferred amino acid sequences were compared to those of other vertebrates. The phylogenetic data suggest that the evolutionary history of this gene family was characterized by different duplication events that occurred in teleosts, aquatic sarcopterygians, amniotes and amphibians. The sequences obtained in the two sarcopterygians, compared to those of other oviparous vertebrates, present conserved domains but also some sites similar to those of teleosts and others to those of tetrapods emphasizing the intermediate characteristics of these living fossils.

O22.2
Tropical rainforests that persisted: histories from the Quaternary in the Guiana shield

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The long lifespan of forest trees often makes us forget that most terrestrial forests have also a long and eventful history. In temperate Europe and North America, sudden range expansions from refugia soon after the Last Glacial Maximum (LGM), associated to bottlenecks and founder events are now well recognised. For Mesoamerica, recent bottlenecks in four rain forest species have also been shown, whose dates are compatible with the end of the LGM, whereas in Equatorial Africa tree populations appear to have gently contracted / expanded without dramatic range changes. In South America several climatic changes during the Tertiary and Quaternary have likely shaped the current composition of Amazonian rainforests. However, the past distribution and the demographical history of South American rain forest communities is still relatively unknown. The goal of this study is to make inferences on the historical demography since the Pleistocene of eight Neotropical rainforest tree species. The French Guiana is one of the most easily accessible continuous large swaths of pristine Amazonian forest: trees were sampled in an area of 80,000 km^2 and characterised by a combination of cpDNA sequences and nuclear microsatellites. The data obtained was submitted to coalescent modelling and Approximate Bayesian Computation (ABC) to infer demographic parameters. Our results show that different species have undergone different demographic patterns, linked to each species' ecological properties: light-demanding and pioneer species show a trend to expansion, while shade-tolerant species tend to be stable or to contract. This permits to make inferences about processes having occurred during and after the last glaciation, and provides a test for currently accepted hypotheses about past compositional transitions in Neotropical rainforests – something that classical paleobiological approaches forbid almost entirely, due to the extreme paucity of reliable fossil record.

O22.3
DNA methylation diversity in human populations

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Epigenetics is a reversible molecular mechanism that is supposed to play a role in many adaptive processes, along with the classical ways to adapt represented by genetic and/or, in the case of humans, cultural changes (Giuliani et al. 2015). Population epigenetic studies represent a tool for the study of environmental dynamics that shape DNA methylation variability across human groups in recent time. Moreover it is known that changes of DNA methylation profiles could represent both a consequence of the genetic architecture and of the chromatin conformation (Cedar & Bergman 2009), but also a more complex mechanism of variability in response to environmental stimuli (Flores et al. 2013). We show an analysis of public epigenome-wide datasets according to their geographical origin with the aim of identifying genes and pathways whose DNA methylation profiles change across human populations. We applied a new pipeline in order to identify the regions that more likely exert a phenotypic effect. The analysis of enrichment of differentially methylated regions (DMRs) revealed differences between populations that could be crucial also for human health.

O22.4
The role of promoter in horizontal transposon transfer: the case of *Bari* transposons

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Transposable elements (TEs) are ubiquitous components of eukaryotic and prokaryotic genomes. TEs are able to propagate both in vertical way and between species, in a process known as Horizontal Transposon Transfer (HTT). Tc1-mariner elements are eukaryotic TEs, particularly prone to HTT events. A successful HTT event relies on several theoretical bottlenecks, including the promoter ability to support transcription in a new genomic context. With the aim to gain insights into the role of TEs promoters in HTT events we have tested two promoters from the Tc1-like transposons *Bari1* and *Bari3*, for which HTT events have been also predicted. Promoter-luciferase assay, performed in a wide range of cellular systems, unexpectedly showed that Bari promoters drive the reporter expression in very different cell types (insects, human, bacteria and yeast). Our results challenge the idea that the promoter is a limiting factor in HTT, suggesting that a background promoter activity could grant early transposon gene expression after a new genome is invaded. Our results could also open to new possible applications of transposon-derived promoters in the development of laboratory gene expression systems.

O22.5
Complete mitochondrial sequences from Mesolithic Sardinians suggest genetic discontinuity within the island

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Little is known about the genetic prehistory of Sardinia. The scarcity of Pre- and Early Neolithic human remains has made it difficult to properly study the first colonization of the island,as well as the Neolithic transition and the advent of the agriculture. The only ancient genetic data available so far regarded a small portion of the mitochondrial DNA of Nuragic people, and allowed us to identify a genealogical continuity between Bronze-Age and some (but not all) isolated Sardinian communities; however how far the genealogical continuity extends and how it originated was impossible to test. We present the first and oldest complete mitochondrial sequences from Sardinia, dated back to about 10,000 ya. These two sequences belong to rare mtDNA lineages and carry newly-described haplotypes, more similar to those present in European pre-LGM populations, than to those found in coeval sequences. ABC analysis allowed us to gather insight into the Paleolithic contribution to the present-day Sardinian genetic pool and to quantify the genetic impact of the Neolithic transition within the island. The most supported model suggests that the genetic diversity of present-day Sardinians derives from a massive migration from continental Europe during the time of the spread of agriculture, and that the contribution of the first colonizers of the island to the mtDNA of modern Sardinians has been negligible.

23 - Cell Communication, Cell Adhesion and Membrane Trafficking

P23.1
The RNA binding protein SYNCRIP controls microRNAs sorting in exosomes

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While recent evidence clearly showed that exosomal microRNAs (miRNAs) are able to modulate the cellular microenvironment and that exosomal RNA cargo selection is deregulated in pathological conditions, the mechanisms controlling RNAs sorting into extracellular vesicles are still poorly understood. We identified in hepatocytes the RNA-binding protein SYNCRIP (Synaptotagmin-binding Cytoplasmic RNA-Interacting Protein, hnRNP-Q or NSAP1) as a component of the exosomal machinery controlling microRNA sorting: SYNCRIP knock-down was found to impair sorting of miRNAs in exosomes. Furthermore, SYNCRIP directly binds to a subset of miRNAs enriched in exosomes sharing a common extra-seed sequence (hEXO motif). Notably, the hEXO motif was proven to have a role in the regulation of miRNA localization, since embedment of this motif into a poorly-exported miRNA enhances its loading into exosomes. This evidence provides new insights into the mechanisms of miRNA exosomal sorting process. Moreover, these findings open the way for the possible selective modification of the miRNAs exosomal cargo.

P23.2
Telomerase-independent effects of dyskerin silencing in U2OS cells

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hDKC1, the gene causative of the X-linked dyskeratosis congenita disease, encodes dyskerin, a multifunctional nucleolar protein involved in multiple functions related to cell growth, proliferation, and telomere maintenance. In a previous study, we generated a stable and inducible colon carcinoma cellular model to silence the *DKC1* gene and found that dyskerin depletion causes alteration of cell-cell and cell-substratum adhesion. These results suggested that dyskerin might participate in tumorigenesis by regulating trafficking to and from cell membrane. With the aim to firmly establish whether these alterations were telomerase independent and/or tissue-specific, we generated a stable and inducible cell line expressing a shRNA able to trigger inducible silencing of the *DKC1* gene in U2OS cells. These cells do not express either TERT or TERC and do not exhibit any telomerase activity, thus allowing to define the effects of dyskerin depletion that are unrelated to telomerase complex function. Intriguingly, we found that dyskerin silencing in these cells causes deregulation of Rab11-mediated exocytic and recycling processes, thus impairing vesicular trafficking to membrane. These results further confirm the generality of these new and unpredicted roles of dyskerin.

P23.3
Ultrasound stimulation affects cellular activities: examples on gene transfection, drug delivery and antibiotic production

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Ultrasound (US) stimulation has been shown to have an increasingly important role in biotechnology, in gene and drug delivery methodologies and in the production of microbial molecules. One of the most difficult tasks to achieve with instrumentation used to study the interaction between US and cellular model systems is to design an experiment where the effects of one physical parameter is evaluated, while all the others are kept constant. We introduce a bench-top US apparatus to be used for prokaryotic and eukaryotic *in vitro* experiments showing a good reproducibility using standard multiwell plates. Three examples of US stimulation realized with our apparatus are described: sonoporation of oxaliplatin in colon cancer stem cells, transfection of pEGFP-N1 DNA in 293T cells and actinorhodin production in *S. coelicolor* M145. It is shown that with our apparatus it is possible to obtain reproducible results on cellular experiments, using the culture supports that are commonly available in life science labs. The improvement on the side of reproducibility and portability of the experiments allows a straightforward comparison between our results and those obtained with other techniques.

O23.1
Does SOD1 mediate the cellular communication between mitochondria and nucleus in stress condition?

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The Voltage-Dependent Anion Channel isoform 1 (VDAC1) is the most abundant eukaryotic porin of Mitochondrial Outer Membrane (MOM), directly involved in metabolism and apoptosis regulation. The yeast *S. cerevisiae* lacking VDAC1 (Δ*por1*) shows a strong growth impairment in any non-fermentable condition. However, the overexpression of the human anti-oxidant enzyme Superoxide Dismutase 1 (SOD1) in Δ*por1* mutant completely restores the yeast growth defect, promoting a recovery of mitochondrial functionality [1]. These results indicate that, hSOD1 in yeast cells has an additional effect and not only a detoxifying one. To deepen this aspect, we analyzed the expression level of genes encoding for other putative MOM pore-forming proteins. Our results indicate clearly that level of the putative porin VDAC2 (a VDAC1 paralog), as well as, levels of the main components of TOM and SAM complexes, Tom40 and Sam50, are significantly increased upon hSOD1 expression. Overall, our data suggest that, in stress condition, hSOD1 mediates the communication between mitochondria and nucleus, acting as modulator of expression of stress relief genes. [1] Magrì A et al (2016) Biochim Biophys Acta, 1857: 789-798.

O23.2
Cysteine mutagenesis in VDAC3 reveals a functional role of these residues in the intracellular communication

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VDACs are multifunctional channels located in the mitochondrial outer membrane (MOM) that have central role in controlling cellular metabolism. In mammals, the VDAC family consists of three isoforms

called VDAC1, VDAC2 and VDAC3 that despite a high sequence conservation play different roles within the cell. Only recently, it has been demonstrated that the isoform 3 is capable of forming channels in artificial membranes, though with a much lower conductance than VDAC1 and VDAC2. Since the three isoforms differ notably for the number and the distribution of cysteine residues, analysis of VDAC3 mutants deprived of selected cysteines revealed significant changes in the protein activity both *in vivo* and *in vitro*. The cysteine mutations that allowed VDAC3 to form larger channels in a phospholipid bilayer were also those that led to complement the absence of porin1 in yeast cells [1]. Overall, our results suggest an involvement of VDAC3 in intracellular communication by keeping track of the redox level in the inter-membrane space and eventually signaling it through conformational changes. [1] Reina S., et al. *Oncotarget* 7 (2016) 2249-2268.

O23.3
Tetra-branched peptide theranostics: preclinical development for cancer targeting

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The NT4 peptide is a potential cancer theranostic, which selectively binds to human cancer tissues in different malignancies and can efficiently and selectively deliver drugs or liposomes for cancer cell imaging or therapy, *in vitro* and *in vivo*. We reported that conjugation of paclitaxel to NT4 increase therapeutic activity of the drug in an orthotopic mouse model of breast cancer and produces tumor regression which is not achieved with unconjugated paclitaxel. We demonstrated that NT4 specifically binds to sulfated glycosaminoglycans (GAGs) and LRP receptors on cancer cells and tissues. Considering the role of sulfated GAGs in cancer cell interaction with the extracellular matrix, we analyzed the effect of NT4 in cancer cell adhesion and migration on different supports, observing that NT4 inhibits adhesion and migration of different human cancer cell lines. We have also constructed and validated novel theranostics nanodevices, by conjugation of NT4 to quantum dots, for selective diagnosis and imaging of different human carcinomas.

O23.4
Biochemical and functional characterization of cancer-associated p27^{Kip1} mutants

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p27^{Kip1} (hereinafter p27) is a cyclin-dependent kinase inhibitor belonging to the Cip/Kip family. When localized in the nuclear compartment, it controls G1/S and S/G2 transitions as well as the progression along M phase. Conversely, the cytosolic p27 functions appear more complex and mainly associated to the modulation of cytoskeleton structure and cell motility. It has been proposed that nuclear p27 exerts an antiproliferative activity. Accordingly, low nuclear p27 content has been associated to an aggressive phenotype in several human tumors. Initially, p27 was not categorized as a classical oncosuppressor, since mutations of *CDKN1B* (the gene encoding p27) were rarely found in human tumors. Then, more thorough genetic studies have evidenced that *CDKN1B* alterations occur frequently in human cancers and, in some cases (i.e. MEN4 and breast cancer), represent driver mutations. Aim of our project is the characterization of the mutation effects on p27 structure/function. We report the characterization of cancer-associated G9Rp27 mutant. Our data demonstrated that the genetic change significantly alters the protein phosphorylation pattern, resulting in loss of CDK inhibitor activity.

O23.5
S-Palmitoylation Modulates Type 1 Cannabinoid Receptor Localization, Trafficking and Biological Activity

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We have previously demonstrated that type 1 cannabinoid receptor (CB_1) is palmitoylated at cysteine 415, and that such a post-translational modification affects biological activity of CB_1. To assess the molecular mechanisms responsible for this modulation by *S*-palmitoylation, here biochemical and morphological studies were paralleled by computational analysis of CB_1. Molecular dynamics simulations suggested that the palmitoyl chain stabilizes helix 8 (H8) of CB_1, as well as receptor interaction with specific membrane cholesterol molecules. In addition to *in silico* data, experimental results showed that non-palmitoylated CB_1 was unable to effectively interact with caveolin 1, whatever the activation state of the receptor. Moreover, unlike wild-type receptor the mutant CB_1 lacking *S*-palmitoylation in H8 was completely unresponsive to agonist-induced effects, in terms of both lipid raft partitioning and receptor internalization. Overall, our results support the notion that *S*-palmitoylation of cysteine 415 modulates H8 conformation and influences CB_1 interaction with cholesterol and caveolin 1, pointing to the palmitoyl chain as a functional interface for CB_1 localization, trafficking and biological activity.

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P24.1
HD-Zip II transcription factors control columella stem cell maintenance in the Arabidopsis root

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Plants' lifelong growth and organ formation are dependent on the activity of pluripotent stem cells. In the root apical meristem (RAM) of Arabidopsis, stem cells surround a small group of less active cells, the Quiescent Center (QC). The QC, together with its surrounding stem cells, forms the root stem cell niche. The regulation of columella stem cell (CSC) activity has been intensively studied and a key role of auxin and several transcription factors has been identified. However, more insights are needed to unravel the network of transcription factors controlling CSC activity. Here we show that ATHB2, ATHB4 and HAT3 HD-Zip II transcription factors - known to play a crucial role in apical embryo development - are required for CSC maintenance in the Arabidopsis RAM, as demonstrated by promotion and inhibition of CSC differentiation in the *hat3 athb4 athb2* triple loss-of-function mutant and in ATHB2 and ATHB4 gain-of-function mutants, respectively. Genetic, molecular and phenotypic analysis will be presented to discuss how ATHB2, ATHB4 and HAT3 interact with known regulatory molecules involved in the control of CSC activity.

P24.2
Effects of HDAC (histone deacetylase) inhibitors on the seed pre-germinative metabolism in *Medicago truncatula*: molecular profiles of chromatin remodeling, DNA repair and antioxidant genes

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The term 'vigour' defines a combination of physiological processes that contribute to seed performance in terms of storability, germination rate, seedling growth and ability to withstand stresses. DNA repair pathways are activated during the early phase of seed imbibition ('pre-germinative metabolism') to maintain genome integrity, thus preserving seed vigour. The pre-germinative metabolism also plays a crucial role in the seed response to priming treatments currently used by seed technologists to enhance the commercial value of these products. Up-regulation of DNA repair genes as components of the DNA damage response (DDR) relies on proper chromatin remodeling ruled by several histone acetyltransferases (HATs) and histone deacetylases (HDACs). The role of chromatin remodeling factors in the DDR context is currently an hot issue in plants. Our work focuses on the molecular characterisation of chromatin remodeling genes which encode components of HAT and HDAC complexes in the model legume *Medicago truncatula*. The effects of HDAC inhibitors on the DNA damage response activated during seed imbibition is evaluated to disclose key players in genome stability conferring high vigour.

P24.3
Arabidopsis pectin methylesterase inhibitors participate to immunity against *Botrytis cinerea*

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Infection by necrotrophic fungi is a complex process that starts with the breakdown of the cell wall (CW) matrix initiated by CW degrading enzymes and results in an extensive tissue maceration. Plant can exploit induced defense mechanisms based on biochemical modification of the CW components to protect themselves from the pathogen. We found that *Arabidopsis* activates CW remodeling mechanisms based on matrix strengthening, callose deposition and synthesis of structural defense proteins to resist to CW degradation upon *Botrytis cinerea* infection. In particular, pectin methylesterification was altered in response to the necrotroph. Pectin is secreted in the CW in highly esterified form and de-esterified by pectin methylesterases (PMEs). The PME activity is strictly controlled by proteinaceous inhibitors (PMEIs). AtPMEI10, AtPMEI11 and AtPMEI12 were identified as Botrytis-induced inhibitors. The decrease of methylesterification during infection was higher and the immunity to Botrytis was compromised in T-DNA insertion PMEIs mutants respect to the control plants. Our findings point to AtPMEI10, AtPMEI11 and AtPMEI12 as mediators of CW integrity maintenance in plant immunity.

P24.4
Proteomic profiling of wheat roots and leaves reveals long-distance effects during infection by two rhizobacteria *Burkolderia graminis and Azopsirillum brasilensis*

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The creation of artificial symbiosis between optimized multi strain consortium of plant growth-promoting rhizobacteria (PGPR) and crops represents an original new avenue to enhance productivity and to significantly reduce the chemical input. However, wheat molecular responses to beneficial microbes have never been examined, in contrast to many other crop plants. The aim of this work was to study the common or specific molecular features of Triticum aestivum responses to either Azospirillum brasilensis or Burkolderia graminis, two mutualistic bacteria, both in below- and above-ground compartments. Moreover, we analysed the molecular effects induced by each one of these PGPRs in combination with Xanthomans translucens foliar infection. We performed a proteomic profiling by shotgun nanoflow scale LC/MS/MS on roots and leaves of colonized and control wheat plants. Results show that the two PGPR affect differently both root and leaves proteomes of host plant. Furthermore, the two different root colonisations elicit different defence responses to the bacterial foliar infection.

P24.5
Cell wall traits as resource to improve resistance to *Fusarium graminearum* in durum wheat

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Fusarium graminearum, one of the causal agents of Fusarium Head Blight (FHB, scab), leads to severe losses in grain yield and mycotoxins contamination. Differently from common wheat, showing a well characterized FHB resistance , the tetraploid durum wheat is highly

susceptible to FHB. We have previously characterized cell wall traits differing between a FHB resistant common wheat accession (02-5B-318) and a susceptible durum wheat cultivar (Saragolla) (Lionetti et al., 2015). Recombinant inbreed lines (RILs) have been generated by crossing 02-5B-318 with Saragolla. A RIL population of durum wheat was obtained and the evaluation of resistance to FHB was determined in different field trials (Giancaspro et al., Submitted). A sub-population of the durum wheat RILs showing high FHB resistance was selected. A cell wall detailed analysis was carried out in spikes at anthesis and the correlation analysis between cell wall biochemical traits and the level of Fusarium resistance was performed. Overall, our results indicate that cell wall traits linked to FHB resistance can be transferred from common wheat to durum wheat.

Lionetti V., et al. *BMC Plant Biology*, 2015; 15 (1)

P24.6
A chimera between the EF-Tu receptor and Cf-9 confers resistance to bacterial infection

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Microbe-associated molecular patterns (MAMPs), such as the bacterial elongation factor EF-Tu, can trigger an immune response in plants, but pathogens have evolved effectors able to block this layer of defense. On the other hand, plants can mount an effector-triggered immunity is mediated by Resistance (*R*) genes, and usually accompanied by an hypersensitive response (HR). For instance, the tomato Cf-9 *R* gene product confers resistance to strains of *Cladosporium fulvum* carrying the *Avr9* avirulence gene. Here we show that a chimeric protein, obtained by fusing the ectodomain of the Arabidopsis Ef-Tu receptor EFR and the intracellular domain of Cf-9, confers to tobacco plants the ability to perceive Ef-Tu and mount a strong HR. Notably, transgenic plants expressing the chimera show increased resistance to the bacterial pathogen *Pseudomoans syringae*. These data suggest that an effective and durable resistance to microbial pathogens can be obtained using chimeras between MAMP receptors and *R* genes. Furthermore, such chimeras may be used to investigate the biochemical basis of *R* gene-mediated resistance.

P24.7
Signaling pathways in oligogalacturonide-triggered immunity

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The plant immune system relies on the capability of surface pattern recognition receptors (PRRs) of recognizing microbe-associated molecular patterns (MAMPs) and/or damage-associated molecular patterns (DAMPs) to mount an effective immune response. Pathogens produce effectors that block PRR-triggered immunity. The *Pseudomonas syringae* effector AvrPto abolishes immunity triggered by the peptide MAMPs flg22 and elf18, by inhibiting the kinase function of the corresponding receptors (FLS2 and EFR) and/or their co-receptors (BAK1/SERK3 and BKK1/SERK4). The DAMPs oligogalacturonides (OGs) are oligomers of α-1,4-linked galacturonosyl residues released upon partial degradation of the plant cell wall homogalacturonan. We show here that AvrPto affects only a subset of the OG-triggered immune responses and that, among these, only a subset is affected by the concomitant loss of BAK1 and BKK1. Preliminary results also indicate that another member of SERK family, i.e. SERK2, but not SERK1, is required for defense responses by OGs. Notably, the antagonistic effect of OGs on auxin-related responses is not affected by either AvrPto or the loss of SERKs. These observations reveal an unprecedented complexity of the OG signaling pathway. OGs may be sensed by multiple and partially redundant perception/transduction complexes, among which only some are targeted by AvrPto and do not necessarily comprise SERKs.

P24.8
Role of an OG oxidase enzyme in *Arabidopsis thaliana* Immunity

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Plants possess an innate immune system capable of counteracting pathogen attacks. Recognition of microbe-associated molecular patterns (MAMPs) and/or damage-associated molecular patterns (DAMPs) by pattern-recognition receptors (PRRs) represents the first event for immunity activation. Oligogalacturonides (OGs), released upon partial degradation of the plant cell wall homogalacturonan, are a well-known class of DAMPs. A mechanism which produce elicitor-inactive oxidized OGs has been recently discovered that relies on specific OG oxidases (OGOX1 and OGOX2). We will show here the characterization of the role of OGOX1 in the Arabidopsis defense against pathogens as well as in the OG-activated immunity by reverse genetic approach. Our results indicate that OGOX1 plays a role in plant defense against the necrotrophic fungus *Botrytis cinerea* and the hemibiotrophic bacterium *Pseudomonas syringe*, but not the necrotrophic *Pectobacterium carotovorum*. Moreover, OGOX1 is required for some of the OG-induced defense responses.

P24.9
A single amino acid mutation affects biological activity of cerato-platanin a non-catalytic fungal protein

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Cerato-platanin is the first member of the cerato-platanin family. It is a mono-domain protein with a double Ψ/β barrel fold resembling the D1 domain of plant and bacterial expansins. CP shows a cell wall-loosening activity on cellulose in spite it lacks the D2 domain of plant expansins. The weakening activity on cellulose could facilitate the host interaction, laying the basis to understand its role in defence. In fact CP is an elicitor of defences acting as a PAMP. At molecular level, only the bacterial BsEXLX1 expansin was characterized. Now we present a cloning and purification method of CP in *E. coli* SHuffle which proved to be suitable to obtain the properly folded and biological active protein. The method also enabled the production of a site-specific mutant D77A. Either the wild-type and mutated CP were characterized for cellulose weakening and for PAMP activity. Results clearly show that the carboxyl group of D77 is crucial either for expansin-like and PAMP activity thus correlating the ability to degrade cellulose with the capacity to induce defense responses. Works are in progress to set up an engineered protein with improved cellulose weakening activity.

P24.10
Exposure of *Medicago truncatula* cells to a human tyrosyl-DNA phosphodiesterase 1 (hTdp1)-specific inhibitor triggers genotoxic effects resembling the *Tdp1α* gene depletion

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Tyrosyl-DNA phosphodiesterase 1 (Tdp1) catalyzes the hydrolysis of the covalent linkage between the catalytic tyrosine residue of DNA Topoisomerase I (Topo I) and the 3'-end of a DNA phosphodiester bond. Tdp1 allows cells to remove the stabilized Topo I/DNA cleavage complexes, preventing severe cytotoxic effects. The combined use of inhibitors of human (h) Topo I and Tdp1 is currently envisaged as a promising strategy to enhance the efficacy of chemotherapy. The hTdp1 inhibitor NSC120686 tested in the present work was used as a pharmacophoric model to find new compounds able to inhibit hTdp1 activity. The originality of our study consists in using the hTdp1 inhibitor in *Medicago truncatula* cells which contain two Tdp1 isoforms, unlike the human cells where only one gene is present. When DNA diffusion assay was used to evaluate the nuclear morphology, indicative of cellular viability, the analysis revealed that the NSC120686-treated *M. truncatula* cells and untreated *MtTdp1α*-depleted calli shared similar programmed cell death and necrosis levels. Furthermore, the 4-days old cell suspension cultures treated with 300 μM of NSC120686, presented accumulation of double strand breaks, associated with the down-regulation of essential DNA repair genes. The results are indicative for the use of hTdp1 inhibitor as a tool for future studies aiming to decipher the peculiar roles of the plant *Tdp1* genes.

P24.11
Effect of the *Fusarium*-mycotoxin deoxynivalenol on host innate immunity: modulation of the expression of defensins

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Mycotoxins are secondary metabolites produced by fungi. Among them, deoxynivalenol (DON) is of particular importance due to its prevalence and toxicity. DON is produced by *Fusarium* ssp. during plant infection, particularly in wheat, acting as a virulence factor essential for disease development. Persistence of DON in wheat based products causes deleterious effects in humans and animals, including suppression of their innate immunity as demonstrated by the inhibition of the expression by enterocytes of antimicrobial peptides called defensins. Since these peptides are also part of the plant innate immunity, we investigated if DON could modulate their expression by analyzing *Arabidopsis thaliana* T87 cells as model. Our results demonstrate that DON by it-self causes a time and dose-dependent modulation of the major plant defensins. Moreover, we studied the effects of DON on the induction of plant defensin expression by *Fusarium* cell extracts and the immunity inducers jasmonic acid and salicilic acid. Collectively, our results demonstrate that DON is able to alter the innate plant immunity, revealing additional targets for wheat resistance against *Fusarium*.

P24.12
Analysis of the interaction of trichodiene synthase 5 (TRI5) with natural and natural-like inhibitors of trichothecene biosynthesis

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The fungal pathogen *Fusarium culmorum* causes Fusarium head blight (FHB) on cereals, resulting in yield loss and contamination of grain with type B trichothecene mycotoxins. A key step in the synthesis of trichothecenes is catalyzed by the trichodiene synthase 5 (TRI5) that converts farnesyl pyrophosphate to trichodiene. Ferulic acid proved an efficient inhibitor of type B trichothecene biosynthesis and *TRI5* gene expression in Fusarium liquid cultures. In this work several natural and natural-like compounds belonging to phenol and hydroxylated biphenyl structural classes were tested *in vitro* to determine their inhibitory activity towards TRI5. The recombinant TRI5 was expressed in *E. coli*, and the interaction with different inhibitors was analyzed by Surface Plasmon Resonance (SPR). The screening of inhibitors was performed in both direct and competitive binding format to determine which inhibitors could compete with the binding of farnesyl pyrophosphate to the enzyme active site. A combination of inhibition kinetics and computational modeling of interacting-structures may facilitate the testing of novel potential TRI5 inhibitors and the prediction of their inhibitory mechanism.

P24.13
The role of different AUXIN RESPONSE FACTORS in *Arabidopsis* late stamen development

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In Arabidopsis, late stamen development consists of three processes: stamen filament elongation, pollen maturation and anther dehiscence. In a previous work (Cecchetti et al. 2008) we showed that auxin positively regulates filament elongation while negatively regulating anther dehiscence and pollen maturation. In anther dehiscence auxin has a negative effect on an early event, endothecium lignification, and on the final event of stomium opening, which is regulated by jasmonic acid (JA). Auxin acts by negatively regulating the expression of the transcription factor MYB26, required for endothecium lignification, and that of JA biosynthetic genes DAD1 and OPR3, at specific late stages of flower development (Cecchetti et al. 2013). To determine which genes mediate the control of auxin on the expression of MYB26 and on the synthesis of JA we are currently analysing the effects of the candidate auxin response factors ARF8, ARF6 (Nagpal et al. 2005) and ARF5 (Garrett et al. 2012). By means of arf8 and arf6 single and doub mutants, ARF8ox lines overexpressing different ARF8 splice variants and ARF6ox line, we established the role of different ARF8 isoforms in regulating endothecium lignification and stamen filament elongation. In addition, by means of ARF5-RNAi and ARF5ox overexpressing lines, we also obtained preliminary results on the role of this ARF in regulating anther dehiscence.

P24.14
Effects of chemical priming and physical invigouration on seed performance in *Medicago truncatula*: molecular profiles of the DNA damage response

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Seed enhancement is a technological sector of the agri-seed industry which includes a range of pre-sowing chemical, physical and biological treatments carried out on commercial seed lots. A wide range of chemicals (e.g. osmotics, phytohormones) can be used as priming agents to improve low-quality seeds while physical methods (e.g. magnetic fields, ionizing radiations) can be also exploited. Despite the potential of this technology, the current protocols are still too empiricals and they need urgent implementation. A major weakness point is the gap of knowledge concerning the molecular processes involved in the seed response to treatments. The availability of molecular hallmarks able to predict in short-time the genotype- and treatment-dependent profiles of seed vigour will bring innovation at the industrial level. In the present

investigation, we focus on the effects of chemical priming and gamma-ray-mediated invigouration on the DNA damage response (DDR) activated during seed imbibition in *Medicago truncatula*. The expression profiles of key DDR genes are used as molecular indicators of the seed ability to preserve genome integrity and enhance quality.

P24.15
An *Arabidopsis* adenyl cyclase with a role in plant defense responses against a biotrophic fungus

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The second messengercyclic adenosine 3',5'-monophosphate (cAMP) is increasingly recognized as having many different roles in plant responses including cation transport via the modulation of cyclic nucleotide gated channels. However, in plants, adenyl cyclases (ACs), molecules that can catalyze the reaction from ATP to cAMP and pyrophosphate have remained elusive. In order to discover plant ACs, we have developed a rational search term based on conserved amino acid residues in catalytic centers of annotated AC-[R]X{5,20}[RKS]X[DE]X{9,11}[KR]X{1,3}[ED]. Here we report that one of the *A. thaliana* AC candidates (At3g14460) is a LRR and NB-ARC domain-containing disease resistance and defense response protein. This AC has catalytic activity *in vitro* as determined by mass spectrometry. To determine its biological role, two AC loss-of-function mutants were obtained from public collections of Arabidopsis T-DNA insertion lines and examined for responses to the biotrophic fungus *Hyaloperonospora parasitica (Hpa)*. Two isolates of *Hpa* have been used, Waco9 and Emoy2, that induce compatible and incompatible interactions, respectively, in Col-0. The pathophenotype of AC mutants was quantified and a role of cAMP in host defense reactions is proposed.

P24.16
Visualizing the relevance of bacterial blue- and red-light receptors during plant–pathogen interaction

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The foliar pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) leads to consistent losses in tomato crops, urging to multiply investigations on the physiological bases for its infectiveness. As other *P. syringae* pathovars, *Pst* is equipped with photoreceptors for blue and red light, mimicking the photosensing ability of host plants. Recently, we have investigated *Pst* strains lacking the genes for a blue-light sensing protein (*Pst*LOV), for a bacteriophytochrome (*Pst*Bph1) or for hemeoxygenase-1. When grown in culturing medium, all deletion mutants presented a larger growth than wild-type (WT) *Pst* under all other light conditions, with the exception of blue light which, under our experimental conditions, completely suppressed the growth of the deletion mutants. Each of the knockout mutants shows stronger virulence towards the model plant *Arabidopsis thaliana* than *Pst*WT, as evidenced by macroscopic damages in the host tissues of infected leaves. These results underscore the importance of *Pst* photoreceptors in responding to environmental light inputs. Here we present also some preliminary data about the infectiveness of wild-type and mutated bacterial strains towards tomato plants.

P24.17
The HD-Zip II transcription factor HAT3 acts via recruitment of a chromatin remodelling complex

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Transcriptional repression mediated by the EAR motif is emerging as one of the principal mechanisms of plant gene regulation. The discovery that the EAR motif interacts with TPL together with the demonstration of genetic interaction between TPL and HDA19 support a model where EAR repressors, via recruitment of chromatin remodelling factors, facilitate epigenetic regulation of gene expression. Among the Arabidopsis transcription factors containing an EAR motif are the HD-Zip II proteins, involved in embryonic apical patterning, shoot apical meristem function and organ polarity. There are several evidence that the HD-Zip II proteins act as negative regulators of gene expression, and recent work demonstrated that HAT3/ATHB4 directly repress the expression of the *ATHB2* gene. The presence of the EAR motif in these proteins led to hypothesize a repression mechanism acting via TPL. Consistent with this hypothesis, it has bee recently demonstrated that HAT3 and TPL physically interact and that the HAT3 EAR motif is essential for this interaction. Molecular and phenotypic analysis will be presented to discuss the centrality of the HAT3/TPL complex to HAT3 function.

P24.18
Tissue-specific expression of PvPGIP2 to improve wheat resistance against *Fusarium graminearum*

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Fusarium Head Blight (FHB) is one of the most important wheat diseases caused by *Fusarium* spp.. The pathogen infects the spike at flowering time and causes severe yield losses and deterioration of grain quality due to the secretion of mycotoxins during infection. The understanding of the precise mode of pathogen entering and the subsequent floral tissue colonize is a crucial point to control FHB. Polygalacturonase inhibiting proteins (PGIPs) are cell wall proteins that inhibit the pectin-depolymerizing activity of polygalacturonases (PGs) secreted by pathogens. The constitutive expression of the bean PvPGIP2 limits FHB symptoms and reduces mycotoxin accumulation in wheat. To better understand which spike tissues plays a role in limiting *Fusarium* infection, we have produced transgenic wheat plants expressing PvPGIP2 in the endosperm or simultaneously in lemma, palea, anthers and rachis. This latter approach reduced FHB symptoms, whereas the expression of PvPGIP2 only in the endosperm did not affect FHB development, indicating that when the pathogen has reached the endosperm, inhibition of pathogen PGs ineffective to prevent fungal spread.

P24.19
Role of the *Arabidopsis* HD-Zip II transcription factors HAT3 and ATHB4 in flower development

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HD-Zip II transcription factors have a role in several plant developmental

processes, ranging from embryogenesis to flower development. The flower in angiosperms is formed by four concentric whorls of defined organs: from outside inwards, sepals, petals, stamens, gynoecium. Interestingly, simultaneous loss-of-function of both HAT3 and ATHB4 HD-Zip II proteins causes severe defects in gynoecium development. In the *hat3 athb4* double mutant the valves appear severely splitted, instead of being tightly fused. In the wild type the apical style region undergoes a transition from a bilaterally symmetric stage to a radially symmetric structure during gynoecium development. Two transcription factors, IND and SPT, are both necessary and sufficient for the radialization process and control style symmetry by directly regulating auxin distribution. We are evaluating the genetic interactions between HAT3/ATHB4 and SPT by crossing the respective mutant lines and by expression analysis of HAT3 and SPT marker lines. Moreover, we are studying auxin dynamics in *hat3 athb4* to evaluate if auxin distribution is also regulated by HAT3 and ATHB4.

O24.1
The apoplastic copper amine oxidase AtCuAOβ plays a role in stomatal closure induced by wounding, jasmonate or Microbe Associated Molecular Patterns (MAMPS)

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Copper amine oxidases (CuAOs) convert polyamines to aminoaldehydes producing ammonia and hydrogen peroxide and are involved in key development processes and stress responses. It is known that *AtCuAOβ* (At4g14940), encoding an apoplastic CuAO, is expressed in root protoxylem and in guard cells. In this study we have investigated *AtCuAOβ* role in stomatal responses to wounding, Methyl-Jasmonate (MeJA) and bacterial elicitor treatments. *AtCuAOβ* is induced by leaf or root wounding, consistent with previous data showing *AtCuAOβ* induction of expression upon treatment with the wound-signal, MeJA. Moreover, *AtCuAOβ* gene expression is induced by MAMPS such as the 22-amino acid peptide flg22, present in bacterial flagellin, and the 18-amino acid peptide elf18 present in bacterial elongation factor EF-Tu. Wounding/MeJA/elicitor-induced stomatal closure was analyzed in *atcuaoβ* T-DNA insertional mutant lines. Under physiological conditions no differences between WT and mutants were observed, while mutants were not responsive to the different treatments inducing stomatal closure. These data suggest that AtCuAOβ could be involved in stomatal responses to pathogen infection and mechanical damage.

O24.2
Role of LYSIN MOTIF-CONTAINING RECEPTOR-LIKE KINASE2 in Arabidopsis immunity

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Receptor-like kinases (RLK) characterized by the presence of one or more lysin motifs, or LysM (LYK proteins) mediate the recognition of microbial symbionts and pathogens in plants. The *Arabidopsis thaliana* genome encodes five LYKs (AtCERK1/AtLYK1 and AtLYK2–5); among them, AtCERK1, AtLYK4 and AtLYK5 are involved in the perception of fungal chitin, whereas AtLYK3 negatively regulates defense responses. We have characterized the function of AtLYK2, using a reverse genetic approach. Knock-down *atlyk2* mutants for this gene show unaltered basal resistance to the fungal pathogen *B. cinerea*, but fail to display an increased resistance after pre-treatments with different elicitors. Elicitor pre-treatments also increase the expression of defense-related genes upon subsequent fungal infections or treatments with chitin. This increased expression is largely compromised in the *atlyk2* mutants, suggesting that AtLYK2 contributes to prime defense responses triggered by elicitor pre-treatment. Analysis of plants expressing a RFP-tagged version of AtLYK2 indicates that this protein is localized at the plasma membrane, suggesting a possible interaction with other LYK proteins.

O24.3
Meristem activity is controlled via extensive regulation of brassinosteroid pathway by class 1 homeobox transcription factors family

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The shoot apical meristem (SAM) is a group of stem cells at the shoot apex that gives rise to the plant aerial tissues. The activity of the cells within the SAM is finely controlled through a complex interplay between multiple hormonal pathways and transcription factors. However, despite significant progress towards understanding meristem functionality, the extent to which different hormones contribute to proper meristem activity requires additional understanding. By combining an *in silico* approach with transcript and metabolite profiling, we have identified that, in Arabidopsis, the meristematic Class 1 homeobox *KNOX* genes *SHOOTMERISTEMLESS* (*STM*) and *BREVIPEDICELLUS* (*BP*) are antagonistic regulators of the entire brassinosteroid (BR) pathway. The different *KNOX* patterns of expression in the different domain of the SAM combined with their antagonistic action on BR pathway suggests that different meristem zones have diverse BR levels. Moreover, the formation of leaf-like structures in *stm*-1 null seedling after brassinazole (BR inhibitor) treatment suggests that STM is not needed required for initial shoot meristem formation but it is essential for the subsequent maintenance of the SAM.

O24.4
Proline modulates root meristem size and root growth in *Arabidopsis*

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Beside its role in protein synthesis, proline plays a special role in plants both under stress and non-stress conditions. In most plant species this amino acid accumulates in response to a number of abiotic and biotic stresses, probably as an adaptive mechanism to improve stress tolerance. Under non-stress conditions proline accumulates in reproductive organs and is involved in developmental processes related to reproduction, such as flowering, pollen development and embryogenesis. Recently proline has been shown to modulate root meristem size in Arabidopsis by controlling the ratio between cell division and cell differentiation. The effects of proline on meristem size are parallel to, and independent from, hormonal pathways, and do not involve the expression of genes controlling cell differentiation at the transition zone, such as *ARR1, ARR12* and *SHY2*. On the contrary, proline appears to control specifically cell division in early stages of postembryonic root development, as shown by the expression of the G2/M-specific *CYCB1;1* gene. We are currently investigating the molecular and genetic mechanisms through which proline exerts its effects on root development.

O24.5
Homeostasis of oligogalacturonides and their activity as Damage-Associated Molecular Patterns (DAMPs)

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Transgenic Arabidopsis plants expressing a chimeric protein constituted

by a fungal PG (FpPG) and a PGIP from common bean (PvPGIP2), named "OG-machine" (OGM plants), accumulate oligogalacturonides (OGs) in their tissue and exhibit enhanced resistance to a variety of pathogens, thereby providing direct evidence for the function of OGs as *in vivo* elicitors of the plant defense responses (DAMPs). We have found that OG preparations, obtained from leaf strips of OGM plants by incubation in a chelating agent solution, contain both typical OGs and atypical OG-like fragments. Further analyses showed that the oligomers corresponding to modified OGs are characterized by a galactaric acid residue at the reducing end, leading to the conclusion they are oxidized OGs (oxOGs). Ox-OGs were tested for their ability to induce the defense responses and antagonize auxin responses. In all experiments, oxidized OGs are inactive as compared to the corresponding typical OGs. We have characterized from OGM plants an enzyme activity capable of oxidizing standard OG preparations. We believe that this activity is a key element for regulating OG homeostasis and avoid an exaggerated activation of plant defences.

O24.6
The glutamate receptor AtGLR3.7 as a regulator of growth and development in *Arabidopsis thaliana*

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The genome of *Arabidopsis thaliana* contains 20 genes encoding glutamate-receptor-like receptors (AtGLR) which can be grouped into three clades. Active receptor complexes are supposed to be comprised of four single subunits which form an ion channel similar to their animal homologues. Based on their pore-forming domains, these proteins are nowadays considered as essential elements in plant signal transduction by mediating Ca^{2+} signals across various cellular membranes. Regulations of plant germination, growth and development as well as physiological processes such as plant stress and defence reactions seem to be achieved by amino acid-triggered variations in cytosolic calcium concentrations. AtGLR3.7 seems to be the only AtGLR in *Arabidopsis thaliana* which is expressed in every cell in the plant body. Other members of the same clade have already been reported to function in the root meristem. Our findings indicate also for AtGLR3.7 an activity in meristematic tissues not only in the root but also within the shoot. On the basis of growth experiments conducted on knockout and over-expression lines of this particular receptor, we hypothesize a positive regulation of root and shoot growth as well as of rosette expansion by AtGLR3.7. Furthermore, minor deviations in life cycle progressions are caused by reduced or elevated mRNA transcript levels of AtGLR3.7.

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