9th International Conference for Plant Mitochondrial Biology

WROCŁAW, POLAND

May 17-22, 2015

ABSTRACTS

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Dear Colleagues,

It is our great pleasure to welcome you all to the 9th International Conference for Plant Mitochondrial Biology 2015 (ICPMB 2015) in Wrocław, Poland. The ICPMB is a unique international meeting that brings together researchers from all over the world working in various aspects of plant mitochondrial biology and provides a great opportunity to share views and to unite efforts both to broaden research and increase its quality. We are delighted to host the ICPMB for the first time in Poland. The program of the ICPMB 2015 covers the main topics in plant mitochondrial research with the emphasis on new findings published during the last two years and the new emerging work that has not been published yet.

Wrocław (Wratislavia), the capital of Lower Silesia, is one of the oldest, renowned and most beautiful cities in Europe. It is seated near the Giant Mountains, on the Odra River and, due to its numerous tributaries and canals, it is called "the city of twelve islands and a hundred and twelve bridges". Its face was shaped by numerous nations during past ages that brought Wrocław to the heights of European culture in art and science.

We wish you an inspiring and pleasant time in Wrocław!

Organizing Committee of the ICPMB 2015

ORGANIZERS

The meeting is co-organized by three research groups represented by the following scientists:

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FRAME PROGRAMME

	SUNDAY May 17th	MONDAY May 18th	TUESDAY May 19th	WEDNESDAY May 20th	THURSDAY May 21st	FRIDAY May 22nd
9:00 a.m.		Breakfast	Breakfast	Breakfast	Breakfast	Breakfast
9:15 a.m.		9:00 a.m. – 10:45 a.m. Session: Mitochondrial genome dynamics part II	9:00 a.m. – 10:45 a.m. Session: Interorganellar signalling and interaction	9:00 a.m. – 10:45 a.m. Session: Protein synthesis, modification and quality control	9:00 a.m. – 10:50 a.m. Session: Mitochondrial biochemistry and metabolism	Departures and excursions after the Conference
9:30 a.m.						
9:45 a.m.						
10:00 a.m.						
10:15 a.m.						
10:30 a.m.						
10:45 a.m.		10:45 a.m. – 11:15 a.m. Coffee break	10:45 a.m. – 11:15 a.m. Coffee break	10:45 a.m. – 11:15 a.m. Coffee break	10:50 a.m. – 11:15 a.m. Coffee break	
11:00 a.m.						
11:15 a.m.		11:15 a.m. – 12:55 p.m. Session: RNA transcription, maturation and regulation	11:15 a.m. – 1:00 p.m. Session: Respiratory chain structure, assembly and regulation	11:15 a.m. 12:45 p.m. Session: Mitochondria and plant development	11:15 a.m. – 12:50 p.m. Session: Omics approaches in mitochondrial biology	
11:30 a.m.						
11:45 a.m.						
12:00 p.m.						
12:15 p.m.						
12:30 p.m.						
12:45 p.m.						
1:00 p.m.		1:00 p.m. – 2:30 p.m. Lunch	1:00 p.m. – 2:30 p.m. Lunch	1:00 p.m. – 2:30 p.m. Lunch	1:00 p.m. – 2:30 p.m. Lunch	
1:15 p.m.						
1:30 p.m.						
1:45 p.m.						
2:00 p.m.						
2:15 p.m.						
2:30 p.m.						
2:45 p.m.		2:30 p.m. – 3:00 p.m. Free time	2:30 p.m. – 2:50 p.m. Agrisera's lecture	2:30 p.m. Free afternoon/excursions	2:30 p.m. – 3:00 p.m. Free time	
3:00 p.m.						
3:15 p.m.	3:00 p.m. Registration	3:00 p.m. – 5:00 p.m. Session: Mitochondrial membrane biogenesis, dynamics and transport	3:00 p.m. – 5:00 p.m. Session: Mitochondria and stress response		3:00 p.m. – 4:05 p.m. Session: Breakthrough technologies	
3:30 p.m.						
3:45 p.m.						
4:00 p.m.						
4:15 p.m.					4:05 p.m. – 4:25 p.m. Closing remarks	
4:30 p.m.						
4:45 p.m.					4:25 p.m. – 4:35 p.m. ICPMB 2017	
5:00 p.m.						
5:15 p.m.		5:00 p.m. – 5:30 p.m. Coffee break	5:00 p.m. – 5:30 p.m. Coffee break			
5:30 p.m.						
5:45 p.m.		5:30 p.m. – 7:30 p.m. Poster session I (even numbers)	5:30 p.m. – 7:30 p.m. Poster session (odd numbers)			
6:00 p.m.						
6:15 p.m.	6:00 p.m. – Opening					
6:30 p.m.	6:10 p.m. – 7:25 p.m. Session: Mitochondrial genome dynamics part I					
6:45 p.m.					6:30 p.m. Departure to Miłocin Country Inn	
7:00 p.m.						
7:15 p.m.						
7:30 p.m.	7:30 p.m. – 10:00 p.m. Welcome party – Wine, cheese and pierogi	7:30 p.m. Dinner	7:30 p.m. Dinner			
7:45 p.m.						
8:00 p.m.						
8:15 p.m.						
8:30 p.m.						
8:45 p.m.						
9:00 p.m.		9:00 p.m. Get-together at the Bierhalle	9:00 p.m. Get-together at the Bierhalle			
9:15 p.m.						
9:30 p.m.						
9:45 p.m.						

Sunday, May 17th

3:00 p.m.	Registration
6:00 p.m.	Opening
6:10 p.m. – 7:25 p.m.	Session: Mitochondrial genome dynamics part I Chairman: Axel Brennicke (Universität Ulm, Germany)
6:10 p.m. – 6:35 p.m.	Jeffrey Mower (University of Nebraska, USA) – **"Evolution and phylogeny of gymnosperm mitochondrial genomes"**
6:35 p.m. – 7:00 p.m.	Daniel Sloan (Colorado State University, USA) – **"The massive mitochondrial genome of the angiosperm Silene noctiflora is evolving by gain or loss of entire chromosomes"**
7:00 p.m. – 7:25 p.m.	Elizabeth Skippington (Indiana University, Bloomington, USA) – **"The wonderfully unusual mitogenomes of Santalales"**
7:30 p.m. – 10:00 p.m.	Welcome party – Wine, cheese and pierogi

Monday, May 18th

Breakfast

9:00 a.m. – 10:45 a.m.	Session: Mitochondrial genome dynamics part II Chairmen: Kathleen Newton (University of Missouri, USA), Jose Gualberto (IBMP-CNRS, France)
9:00 a.m. – 9:25 a.m.	Wataru Sakamoto (Okayama University, Japan) – **"Regulation of organelle DNA levels by organelle nuclease DPD1"**
9:25 a.m. – 9:50 a.m.	Brent L. Nielsen (Brigham Young University, USA) – **"Non-redundant functions of dual-localized organellar DNA replication proteins"**
9:50 a.m. – 10:10 a.m.	Stephan Greiner (Max Planck Institute of Molecular Plant Physiology, Germany) – **"Structure and inheritance of the Oenothea mitochondrial genome"**
10:10 a.m. – 10:30 a.m.	Clémentine Wallet (IBMP-CNRS, France) – **"A RecG-like DNA helicase is required for the segregation of the Arabidopsis mtDNA"**
10:30 a.m. – 10:45 a.m.	Na Zhao (Zhejiang University, China) – **"MutS HOMOLOG1 mediating substoichiometric shifting of ORF220 causes cytoplasmic male sterility and fertility reversion in Brassica juncea"**
10:45 a.m. – 11:15 a.m.	Coffee break
11:15 a.m. – 12:55 p.m.	Session: RNA transcription, maturation and regulation Chairmen: Maureen Hanson (Cornell University, USA), Daniel Gonzalez (Instituto de Agrobiotecnologia del Litoral, Argentina)
11:15 a.m. – 11:40 a.m.	Laurence Maréchal-Drouard (IBMP-CNRS, France) – **"Organellar plant tRNA fragments as a new source of small non-coding RNA"**
11:40 a.m. – 12:05 p.m.	Stefan Binder (Ulm University, Germany) – **"Processing of 5' transcript termini: PPR proteins and beyond"**
12:05 pm. – 12:30 p.m.	Phillipe Giegé (IBMP-CNRS, Francja) – **"Exploring the mode of action, diversity and evolution of protein-only RNase P"**
12:30 p.m. – 12:55 p.m.	Bentolila Stephane (Cornell Univeristy, USA) – **"New families of proteins required for mitochondrial RNA editing"**
1:00 p.m. – 2:30 p.m.	Lunch

3:00 p.m. – 5:00 p.m.	Session: Mitochondrial membrane biogenesis, dynamics and transport Chairmen: David Logan (Angers University, France), Shin-Ichi Arimura (The University of Tokyo, Japan)
3:00 p.m. – 3:25 p.m.	Monika Murcha (University of Western Australia, Australia) – **"Identification and characterisation of a tRNA Import Receptor into Plant Mitochondria"**
3:25 p.m. – 3:50 p.m.	Jianping Hu (Michigan State University, USA) – **"Post-translational regulation of mitochondrial dynamics in Arabidopsis"**
3:50 p.m. – 4:10 p.m.	Boon Leong Lim (University of Hong Kong, Hong Kong) – **"AtPAP2 and STY kinases regulate the import of certain precursor proteins into chloroplasts and mitochondria"**
4:10 p.m. – 4:30 p.m.	Ute Vothknecht (Ludwig Maximilian University of Munich, Germany) – **"TOM9: a calmodulin-regulated protein involved in TOM complex assembly in Arabidopsis thaliana"**
4:30 p.m. – 4:45 p.m.	Michaud Morgane (CNRS/CEA/INRA/Univ. Grenoble Alpes, France) – **"Identification of a mitochondrial super-complex involved in lipid trafficking in plant mitochondria"**
4:45 p.m. – 5:00 p.m.	Paula Da Fonseca Pereira (Max Planck Partner Group, Universidade Federal de Viçosa, Brazil) – **"Functional characterization of NAD+ transporters in Arabidopsis thaliana"**
5:00 p.m. – 5:30 p.m.	Coffee break
5:30 p.m. – 7:30 p.m.	Poster session I (even numbers)
7:30 p.m.	Dinner
9:00 p.m.	Get-together at the Bierhalle

Tuesday, May 19th

Breakfast	
9:00 a.m. – 10:45 a.m.	Session: Interorganellar signalling and interaction Chairmen: Christine Foyer (University of Leeds, United Kingdom), Kathleen Soole (Flinders University, Australia)
9:00 a.m. – 9:25 a.m.	James Whelan (La Trobe University, Australia) – **"Interorganellar signalling and interaction"**
9:25 a.m. – 9:50 a.m.	Tatjana Kleine (Ludwig Maximilian University of Munich, Germany) – **"Organellar gene expression and retrograde signaling"**
9:50 a.m. – 10:10 a.m.	André Dietrich (CNRS-IBMP, France) – **"Mitochondrial transcriptome control and genetic coordination in plant cells"**
10:10 a.m. – 10:30 a.m.	Agepati Raghavendra (University of Hyderabad, India) – **"Mitochondrial redox as the major signal to mediate the crosstalk with chloroplasts and other organelles in plant cells"**
10:30 a.m. – 10:45 a.m.	Olivier Van Aken (University of Western Australia, Australia) – **"A two-phase stress response to energy organelle inhibition is controlled by WRKY and NAC transcription factors"**
10:45 a.m. – 11:15 a.m.	Coffee break
11:15 a.m. – 1:00 p.m.	Session: Respiratory chain structure, assembly and regulation Chairmen: Allan Rasmusson (Lund University, Sweden), Wieslawa Jarmuszkiewicz (Adam Mickiewicz University in Poznań, Poland)
11:15 a.m. – 11:40 a.m.	Hans-Peter Braun (Leibniz Universität Hannover, Germany) – **"The carbonic anhydrase domain of mitochondrial complex I"**

11:40 a.m. – 12:00 p.m.	Etienne Meyer (Max Planck Institute of Molecular Plant Physiology, Germany) – **"CMS-G of Beta vulgaris ssp maritima contains an unusual cytochrome c oxidase"**
12:00 p.m. – 12:20 p.m.	Oren Ostersetzer (The Hebrew University of Jerusalem, Israel) – **"From gene expression to respiratory complex assembly: the roles of maturases in mitochondria biogenesis in plants"**
12:20 p.m. – 12:40 p.m.	Jennifer Selinski (Osnabrück University, Germany) – **"Multiple AOX Arabidopsis: What makes the difference?"**
12:40 p.m. – 1:00 p.m.	Ivan Radin (Technische Universität Dresden, Germany) – **"A putative auxiliary role of cytochrome c oxidase assembly factor AtCOX11 in ROS scavenging"**
1:00 p.m. – 2:30 p.m.	Lunch
2:30 p.m. – 2:50 p.m.	Agrisera's lecture – **"Tips and tricks of antibody production and validation process - how to obtain good results?"**
3:00 p.m. – 5:00 p.m.	Session: Mitochondria and stress response Chairmen: Miquel Ribas-Carbó (Universitat De Les Illes Balears, Spain), Anna M. Rychter (University of Warsaw, Poland)
3:00 p.m. – 3:25 p.m.	Sally Mackenzie (University of Nebraska, USA) – **"Integration of organellar and epigenomic behavior during reprogramming for abiotic stress response in plants"**
3:25 p.m. – 3:50 p.m.	Greg Vanlerberghe (University of Toronto Scarborough, Canada) – **"Mitochondrial respiration in the light during drought"**
3:50 p.m. – 4:15 p.m.	Markus Schwarzländer (University of Bonn, Germany) – **"Mitochondrial Calcium Dynamics - Checks and Balances of Energy Physiology"**
4:15 p.m. – 4:30 p.m.	Nicolas Taylor (The University of Western Australia, Australia) – **"Unravelling the molecular mechansims of cold and salinity stress/tolerance in wheat mitochondria"**
4:30 p.m. – 4:45 p.m.	Laura Zsigmond (Hungarian Academy of Science, Hungary) – **"The role of mitochondrial proteins in stress responses"**
4:45 p.m. – 5:00 p.m.	Catharina Belt (University of Western Australia, Australia) - **"Investigating succinate dehydrogenase as a central regulator of stress response in plants"**
5:00 p.m. – 5:30 p.m.	Coffee break
5:30 p.m. – 7:30 p.m.	Poster session (odd numbers)
7:30 p.m.	Dinner
9:00 p.m.	Get-together at the Bierhalle

Wednesday, May 20th

Breakfast

9:00 a.m. – 10:45 a.m.	Session: Protein synthesis, modification and quality control Chairmen: Linda Bonen (University of Ottawa, Canada), Hanna Janska (University of Wroclaw; Poland)
9:00 a.m. – 9:25 a.m.	Iris Finkemeier (Max Planck Institute for Plant Breeding Research, Germany) – **"Lysine acetylation and its regulatory roles in Arabidopsis mitochondria"**
9:25 a.m. – 9:45 a.m.	Malgorzata Kwasniak Owczarek (University of Wrocław, Poland) – **"Mitoribosome regulation of protein synthesis in plant mitochondria"**
9:45 a.m. – 10:05 a.m.	Kristina Kühn (Humboldt-Universitaet zu Berlin, Germany) – **"The mitochondrial SHOT1 protein is required for OXPHOS biogenesis"**

10:05 a.m. – 10:25 a.m.	Beata Kmiec (Stockholm University, Sweden) – **"Shredding the signal: Systems for targeting peptides degradation in Mitochondria and Chloroplasts"**
10:25 a.m. – 10:45 a.m.	Chris Carrie (Ludwig-Maximilians Universität München, Germany) – **"Identification of cleavage sites and substrate proteins for two mitochondrial intermediate peptidases in Arabidopsis Thaliana"**
10:45 a.m. – 11:15 a.m.	Coffee break
11:15 a.m. – 12:45 p.m.	Session: Mitochondria and plant development Chairmen: Per Gardeström (Umeå University, Sweden), Bozena Szal (University of Warsaw, Poland)
11:15 a.m. – 11:40 a.m.	Tomohiko Kazama (Tohoku University, Japan) – **"Mitochondrial genotype determines fate of pollen development in cytoplasmic male sterile rice"**
11:40 a.m. – 12:05 p.m.	Elina Welchen (Universidad Nacional del Litoral, Argentina) – **"Cytochrome c connects carbon metabolism with plant growth and developmental transitions"**
12:05 p.m. – 12:25 p.m.	Gaël Paszkiewicz (INRA IRHS, France) – **"Reactivation of mitochondrial bioenergetics and dynamics during germination of Arabidopsis thaliana seed"**
12:25 p.m. – 12:45 p.m.	Jinghua Yang (Zhejiang University, China) – **"Identification of the unique alternative oxidase (ClAOX) in watermelon (Citrullus lanatus)"**
1:00 p.m. – 2:30 p.m.	Lunch
2.30 p.m.	Free afternoon/excursions

Thursday, May 21st

Breakfast	
9:00 a.m. – 10:50 a.m.	Session: Mitochondrial biochemistry and metabolism Chairmen: Jan A. Miernyk (University of Missouri, USA), David Day (Flinders University, Australia)
9:00 a.m. – 9:25 a.m.	Janneke Balk (John Innes Centre, United Kingdom) – **"A smelly business: Persulfide metabolism and trafficking in mitochondria"**
9:25 a.m. – 9:45 a.m.	Toshihiro Obata (Max-Planck-Institute of Molecular Plant Physiology, Germany) – **"Manipulation of cellular metabolism by stabilizing an enzyme-enzyme interaction"**
9:45 a.m. – 10:05 a.m.	Veronica Maurino (Heinrich Heine University, Germany) – **"Plants Possess a Mitochondrial Metabolic Repair Cycle Involving Malate Dehydrogenase and L-2hydroxyglutarate Dehydrogenase"**
10:05 a.m. – 10:25 a.m.	Tatjana Hildebrandt (Leibniz Universität Hannover, Germany) – **"Integration of amino acid catabolic pathways at the sulfur dioxygenase ETHE1"**
10:25 a.m. – 10:50 a.m.	Elke Stroeher (University of Western Australia, Australia) - **"Glutaredoxin S15 is involved in Fe-S biogenesis in mitochondria influencing lipoic acid-dependent enzymes, plant growth and arsenic tolerance in Arabidopsis"**
10:50 a.m. – 11:15 a.m.	Coffee break
11:15 a.m. – 12:50 p.m.	Session: Omics approaches in mitochondrial biology Chairmen: David Macherel (Angers University, France), Michał Rurek (Adam Mickiewicz University in Poznań, Poland)
11:15 a.m. – 11:40 a.m.	Harvey Millar (University of Western Australia, Australia) – **"Protein turnover by progressive 15N labelling for analysis of plant mitochondrial biogenesis and maintenance"**

11:40 a.m. – 12:00 p.m.	Stefanie Dukowic-Schulze (University of Minnesota, USA) – **"Mitochondria connections in plant meiosis"**
12:00 p.m. – 12:20 p.m.	Helena Štorchová (Institute of Experimental Botany AS, Czech Republic) – **"Ever changing mitochondrial genomes from a transcriptomic perspective"**
12:20 p.m. – 12:35 p.m.	Stefanie Mueller (Albert-Ludwigs-Universität Freiburg, Germany) – **"Mitochondria and Plastids in Moss: From Quantitative Proteomics to Dynamics"**
12:35 p.m. – 12:50 p.m.	Zhang Ning (University of Missouri, USA) – **"A Deep-Learning Method for Predicting Mitochondria-Localized Proteins in Plants"**
1:00 p.m. – 2:30 p.m.	Lunch
3:00 p.m. – 4:05 p.m.	Session: Breakthrough technologies Chairmen: Philippe Giegé (IBMP-CNRS, France)
3:00 p.m. – 3:25 p.m.	Elzbieta Glaser (Stockholm University, Sweden) – **"Can plant research provide insight into Alzheimer's disease?"**
3:25 p.m. – 3:50 p.m.	Ian Small (University of Western Australia, Australia) – **"Potential uses of synthetic RNA binding proteins"**
3:50 p.m. – 4:05 p.m.	Catherine Colas des Francs-Small (University of Western Australia, Australia) – **"Redesigning PPR proteins to block the expression of selected organelle genes"**
4:05 p.m. – 4:35 p.m.	Closing remarks & ICPMB 2017
4:05 p.m. – 4:25 p.m.	Ian Max Møller (Aarhus University, Denmark)
4:25 p.m. – 4:35 p.m.	ICPMB 2017
6:30 p.m.	Departure to Miłocin Country Inn

Friday, May 22nd

Breakfast

Departures and excursions after the Conference

SPEAKER ABSTRACTS

LIST OF SCIENTIFIC SESSIONS

SCIENTIFIC SESSION I: MITOCHONDRIAL GENOME DYNAMICS PART I

Chairmen:
Axel Brennicke (Universität Ulm, Germany)

LECTURE 1

Evolution and phylogeny of gymnosperm mitochondrial genomes

Wenhu Guo[1], Andan Zhu[1], Weishu Fan[1], Felix Grewe[1], Gregory Young[2], Monica Polsakiewicz[3], Volker Knoop[3], Robert Adams[4], Jeffrey Palmer[2], Jeffrey Mower[1]

1. University of Nebraska-Lincoln; 2. Indiana University Bloomington; 3. University of Bonn; 4. Baylor University

Keywords: gymnosperms, mitochondrial genomes, intron trans-splicing, RNA editing, phylogenetics

Introduction: Dozens of mitochondrial genomes (mitogenomes) have been sequenced from angiosperms, but only one from gymnosperms. To examine mitogenomic diversity and phylogeny across the five lineages of extant gymnosperms, we sequenced mitochondrial DNA from nine gymnosperms and extracted mitochondrial genes from three nuclear genome assemblies.

Material and Methods: Genomes were sequenced using Illumina technologies, assembled with Velvet software, and annotated and analyzed using standard approaches.

Results: Genome sizes range over 20-fold, from 346 kb in *Ginkgo* to >6.7 Mb in *Pinus*. Pinaceae, *Ginkgo*, and cycads demonstrate extremely slow rates of sequence evolution and the retention of nearly all genes and introns, whereas Gnetales and cupressophytes exhibit highly accelerated substitution rates and the loss of many genes and introns. An unprecedented number of introns have evolved *trans*-spliced arrangements in conifers. Most gymnosperms contain >1,000 sites of mitochondrial C-to-U RNA editing, although *Gnetum* and one cupressophyte have sustained moderate and heavy loss of editing, respectively. Phylogenomic analyses support cycads as sister to the rest of gymnosperms and Gnetales as sister to either cupressophytes or Pinaceae.

Conclusions: These results reveal extensive diversity among gymnosperm mitogenomes in terms of size, substitution rates, gene and intron content, *cis*- versus *trans*-splicing, and RNA editing frequency. The phylogenetic results highlight continuing difficulties in resolving the placement of Gnetales and cycads in relation to the other gymnosperm lineages.

LECTURE 2

The massive mitochondrial genome of the angiosperm Silene noctiflora is evolving by gain or loss of entire chromosomes

Daniel Sloan, Zhiqiang Wu
Colorado State University

Keywords: multichromosomal genomes, recombination, mutation rates

Introduction: Multichromosomal mitochondrial genomes have recently been found in multiple angiosperm species, including *Silene noctiflora*, which harbors an unusually large and complex mitochondrial genome with more than 50 circular-mapping chromosomes totaling ~7 Mb.

Materials and Methods: To determine the extent to which such genomes are stably maintained, we analyzed intraspecific variation in *S. noctiflora* mtDNA, using whole genome sequencing and copy number analysis.

Results: Complete genomes from two populations revealed a high degree of similarity in the sequence, structure, and relative abundance of mitochondrial chromosomes. For example, there are no inversions between the genomes, and there are only 9 SNPs in 25 kb of protein-coding sequence. Remarkably, however, these genomes differ in the presence or absence of 19 entire chromosomes, all of which lack any identifiable genes or contain only duplicate gene copies. Thus, these mitochondrial genomes retain a full gene complement but carry a highly variable set of chromosomes that are filled with presumably dispensable sequence.

Conclusions: In *S. noctiflora*, conventional mechanisms of mitochondrial sequence divergence are being outstripped by an apparently non-adaptive process of whole-chromosome gain/loss, highlighting the inherent challenge in maintaining a fragmented genome. We will discuss the implications of these findings in relation to the structure and inheritance of plant mitochondrial genomes and to the question of why mitochondria, more so than plastids, have been prone to the repeated evolution of multichromosomal genomes across eukaryotic lineages.

LECTURE 3

The wonderfully unusual mitogenomes of Santalales

Elizabeth Skippington
Department of Biology, Indiana University, Bloomington, USA

Keywords: Mitogenome, Mutation rate, Relaxed selection, Recombination, Sublimons, Genome reduction, Complex I, Mistletoes, Parasitic plants

Whilst there is enormous diversity among parasitic angiosperms in form, structure, life-history strategies, and plastid genomes, little is known about the diversity of their mitogenomes. Most studies of parasitic plant mitochondrial DNAs have dealt primarily with their apparent propensity for uptake of foreign DNA via horizontal gene transfer (HGT). Here I will discuss mitogenomes of the mostly parasitic order Santalales whose 2,000+ parasitic members are either aerial parasites or root parasites. Unlike other parasitic mitochondrial genomes, the mitogenomes I will present show little evidence of HGT, but are wonderfully bizarre in a number of other ways, including genome size, mutation rate, selective pressure, levels of repeat-mediated recombination and sublimons, GC distribution, and the loss of many genes.

SCIENTIFIC SESSION II: MITOCHONDRIAL GENOME DYNAMICS PART II

Chairmen:
Kathleen Newton (University of Missouri, USA), Jose Gualberto (IBMP-CNRS, France)

LECTURE 4

Regulation of organelle DNA levels by organelle nuclease DPD1

Wataru Sakamoto, Tsuneaki Takami
Institute of Plant Science and Resources, Okayama University

Keywords: Arabidopsis, Chloroplast, DNA degradation, Dual targeting, Exonuclease, Leaf senescence, pollen

Organelle DNAs in mitochondria and chloroplasts exist as multiple copies. Their levels in mature leaves appear to vary in species. However, whether they are kept at the constant level or not is under controversy over the past decade. Is there a specific mechanism to regulate organelle DNA copy number, or can we modify it?

Our recent study through the characterization of *dpd* (*defective in pollen organelle DNA degradation*) mutant in *Arabidopsis* led us to identify the organelle exonuclease DPD1 that degrades organelle DNA in a tissue-specific manner. DPD1 is a Mg^{2+}-dependent exonuclease dual targeted to chloroplasts and mitochondria. Interestingly, DPD1 is among two dozens of nucleases reported so far in plants, but it is the only one that belongs to bacterial DnaQ-like exonuclease and degrades dsDNA specifically.

Several lines of evidence implicate that DPD1 plays a role in degrading organelle DNAs tissue-specifically. Particularly, we recently found that DPD1 is dramatically up-regulated during dark-induced leaf senescence, and that it indeed degrades cpDNAs in senescing leaves. We also found that *dpd1* mutant showed a 'stay-green' phenotype, suggesting that increment of cpDNA positively affects chloroplast gene expression in leaves. Based on these results, we propose that DPD1 is an important factor that defines chloroplast functionality, through the control of cpDNA copy number.

LECTURE 5

Non-redundant functions of dual-localized organellar DNA replication proteins

Brent L. Nielsen, Stewart Morley[1], Vikas Shedge[2], Sally A. Mackenzie[2]

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Keywords: DNA replication, mitochondrial genome maintenance

Very little is known about DNA replication and maintenance in plant mitochondria. It is unclear how organelle genome replication is coordinated with plant development. Plants contain two genes for most mitochondrial genome replication proteins. Most are co-localized to mitochondria and chloroplasts and are predicted to have redundant function. However, we have evidence that at least some of these duplicated proteins are not fully redundant. For example, Arabidopsis encodes two DNA polymerase I-like proteins that are dual localized to mitochondria and chloroplasts. Homozygous insertion mutants for each grow at a slower rate compared to wild type and exhibit some other minor phenotypes suggesting they are not fully redundant, and double mutants have intermediate growth phenotypes. No double homozygous mutants have been found, but plants homozygous for the insertion in one DNA polymerase gene but heterozygous for the other gene show different phenotypes. DNA PolIB has a greater effect on mtDNA maintenance and mitochondrial morphology, but also plays a role in ctDNA repair. Both mtDNA and ctDNA levels are affected in DNA PolIA mutants. These mutants have been analyzed for their effect on organelle genome levels as a function of plant developmental stage, analyzed by quantitative PCR. GFP- and RFP-fusion constructs for several mtDNA replication proteins have been analyzed to determine localization in organelles of different tissues and at different stages of growth. Some of the replication proteins contain novel stretches of amino acids compared to orthologous proteins, which may contribute to neofunctionalization.

LECTURE 6

Endogenous factors determine the number of mitochondrial gene copies per cell

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Keywords: Mitochondrial genome organization, gene copy number, genome interaction

The genes of plant mitochondria may be located on different subgenomic molecules rather than residing together on the same master circle, at least in differentiated somatic cells. Therefore, the copy numbers of the individual gene might differ from each other. We further hypothesized that the copy number of mitochondrial genes might not be stable but change under certain conditions.

We have determined the number per cell of mitochondria and of four mitochondrial genes (atp1, cox1 nad6, and rps4) in Arabidopsis and tobacco leaves and in different Arabidopsis organs by quantitative PCR. The number of mitochondria exceeds by far the number of mitochondrial genes per cell. Together with published data on DAPI-stained mitochondrial DNA, our results demonstrate that plant mitochondria do not contain complete genomes and that individual mitochondria differ with respect to their DNA content. The investigated genes have specific copy numbers, which differ from each other. We carried out further experiments to identify factors that affect the mitochondrial gene copy number in plant cells. The following factors were found to have a significant impact on the number of mitochondrial gene copies: species, organ, tissue, cell size, nuclear ploidy, position of the gene within the genome, age, mitochondrial function, and chloroplast/plastid function.

LECTURE 7

Structure and inheritance of the Oenothea mitochondrial genome

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Keywords: mitochondrial genome organization; cytoplasmic incompatibility; Programmed Cell Death

The genus *Oenothera* (evening primrose) is a classical model for chloroplast mediated speciation barriers, but there are incompatibilities described which are independent from the chloroplast and the nuclear genome. This indicates the involvement of a further cytoplasmatic component.

One of these incompatibles is the "*falcifolia*-sydrome", leading to malformation of organs. It arises in reciprocal crosses between *O. glazioviana* and *O. biennis*, two species forming natural hybrids. Depending on the crossing direction, the phenotype occurs with different frequency, pointing to a biparental inheritance of an extra-nuclear determinant, likely the mitochondrion.

To address question, we initiated comparative mitochondrial genome sequencing, based on highly pure mitochondrial DNA, obtained from leaf material. NGS data from 454 and Illumina HiSeq were assembled de-novo employing various assemblers (CLC, MIRA, Newbler, IDBA-UD). A three-dimensional mitochondrial genome could be accomplished with IDBA-UD. In contrast to the classical "circular" master- and mini-circle model, the suggested branched sphere-like structure dramatically broadens our understanding of plant mitochondrial genome organization in terms of complexity and sub-genome organization. The bioinformatic data could be confirmed by PCR and Southern analysis.

Although genetic mapping of *falcifolia* population could not link the *falcifolia*-syndrome to the mitochondria so far, histological analysis strongly suggests Programmed Cell Death as underlying mechanism causing organ maldevelopment in the hybrids.

LECTURE 8

A RecG-like DNA helicase is required for the segregation of the Arabidopsis mtDNA

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Keywords: RecG, mtDNA, recombination, repair, stoichiometric shifting

The structure of the mtDNA evolves rapidly through recombination mechanisms involving repeated sequences, but the processes controlling the segregation of the alternative mitotypes generated by recombination are still not understood.

We identified an Arabidopsis gene (*RECG1*) coding for a homologue of bacterial DNA helicase RecG, a DNA translocase that in bacteria has multiple roles in DNA repair, control of stoichiometric genome replication and suppression of ectopic recombination. The plant RECG1 is dually targeted to mitochondria and plastids, and can complement bacterial *recG* deficient strains for repair and replication control. Arabidopsis *recG1* mutants have increased ectopic recombination between intermediate size repeats in mitochondria, and are deficient in homologous recombination dependent repair of double strand breaks induced by a genotoxic stress.

In addition we found that RECG1 has roles in the segregation of the mtDNA. In a *recG1* mutant an alternative mtDNA sequence generated by recombination is stably maintained as an independent replisome. Reintegration of the wild type *RECG1* allele produces plants inheriting different mtDNA versions: i) the wild type mtDNA, ii) deleted forms that affect the plant phenotype, or iii) a reorganized new version of the mtDNA that is compatible with normal plant development. The characterization of the recombination steps involved allowed us to build a model accounting for the control of stoichiometric shifting of the plant mtDNA by RECG1.

LECTURE 9

MutS HOMOLOG1 mediating substoichiometric shifting of ORF220 causes cytoplasmic male sterility and fertility reversion in Brassica juncea

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Keywords: cytoplasmic male sterility, mitochondrial genome rearrangemtn, MSH1, revertant, substoichiometric shifting

Cytoplasmic male sterility (CMS) has consistently been associated with expression of mitochondrial open reading frames (ORFs) that arise from mitochondrial genome rearrangements. We have previously identified a CMS-associated *ORF220 in CMS Brassica juncea*. Here, we show evidence of mitochondrial genomic rearrangement and *ORF220* substoichiometric shifting (SSS) causing CMS and fertility reversion. We identified spontaneous revertant lines for this CMS cytoplasm, of which mitochondrial genomic rearrangement has undergone SSS to suppress copy number of the *ORF220* gene to revert sterility to fertility. We placed ORF220, with or without a mitochondrial targeting pre-sequence, under the control of the CaMV 35S and AP3 promoters in *Arabidopsis* to confirm that ORF220 causes male sterility when mitochondrially-localized. The expression of *MSH1,* a nuclear gene that is involved in the suppression of illegitimate recombination in plant mitochondria, was reduced in the CMS line and recovered in the revertant line. When expression of *MSH1* was suppressed, SSS of the *ORF220* was observed in *MSH1*-RNAi lines of *B. juncea*. CMS-associated mitochondrial genomic rearrangement has undergone SSS to suppress copy number of the ORF220 gene, providing a useful system for studying the reversibility of SSS and interplay of MSH1 in cytoplasmic male sterility induction and fertility reversion.

SCIENTIFIC SESSION III:
RNA TRANSCRIPTION, MATURATION AND REGULATION

Chairmen:
Maureen Hanson (Cornell University, USA), Daniel Gonzalez (Instituto de Agrobiotecnologia del Litoral, Argentina)

LECTURE 10

Organellar plant tRNA Fragments as a new source of small non-coding RNAs

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Keywords: tRNA cleavage, endonucleases, plant development, mitophagy

In the expanding repertoire of small noncoding RNAs (sncRNAs), tRNA-derived RNA fragments (tRFs) have been identified in all domains of life. Initially discarded as RNA turnover by-products, accumulating evidence suggests that some of them are not just random degradation fragments but rather stable entities with major biological functions.

So far, only few data report on the existence of plant tRFs. Neither in-depth analysis of their identity nor their biogenesis and roles have been studied. Using high-throughput sequencing technology, tRFs were retrieved from 30 *Arabidopsis thaliana* sncRNA libraries. Among the remarkable observations, numerous tRFs originate not only from nuclear-encoded tRNAs but also from plastidial and mitochondrial tRNAs and with very specific cleavage sites. A few tRFs are either enhanced or repressed depending on stress or plant tissue. Examining the AGO1-associated tRFs also reveals strong bias in term of identity and size and shows the presence of several organellar tRFs in immunoprecipitates. In addition, we now have evidence that organellar tRFs are generated outside the organelles and a family of *A. thaliana* endonucleases able to cleave tRNAs not only in the anticodon but also in the D-loop has been identified. These endonucleases present very specific expression profile and their characterization is currently under way. Our data strongly suggest that some tRFs play important regulatory functions within the plant cell, including tRFs of organellar origin. Beyond translation, this opens new perspectives for nucleus- and organelle-encoded tRNAs as major actors of gene expression in plants.

LECTURE 11

Processing of 5‘ transcript termini: PPR proteins and beyond

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Keywords: RNA maturation, 5’ processing, RNA processing factors, putative ribonucleases

Plant mitochondrial transcripts undergo maturation processes at both extremities. To gain insights into the mode of action and the components involved in 5’ processing, we analyzed transcripts derived from the mitochondrial *ccmC* gene, coding for a component of the cytochrome *c* maturation system. In *Arabidopsis*, there are two mitochondrial *ccmC* gene configurations, discriminated by a short sequence segment located approximately 500 bp upstream of the *ccmC* reading frame. In about 50 ecotypes, these divergent mitochondrial genotypes correlate with the generation of two different *ccmC* mRNAs with distinct 5’ termini. Two different PPR proteins, i.e. RNA PROCESSING FACTOR 3 (RPF3) and RNA PROCESSING FACTOR 6 (RPF6), were found to be required for the generation of the different 5’ ends. These proteins are predicted to bind to RNA recognition sites located within the *ccmC* configuration defining sequences thereby recruiting a potential endonuclease to the cleavage site. This observation further substantiates the hypothesis that upstream located sequences are crucial for the endonucleolytic generation of mature 5’ transcript termini in mitochondria and discriminates this process from the 5’ exonuclease based process in chloroplasts. To get a deeper insight into the composition of the 5’ processing machinery we analyzed putative ribonucleases identified by an in silico screen for proteins containing conserved nuclease and RNA binding domains. In the corresponding mutants, abnormal transcript pattern revealed the involvement of two nucleus-encoded RNases in post-transcriptional processing of distinct mitochondrial transcripts.

LECTURE 12

Exploring the mode of action, diversity and evolution of protein-only RNase P

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Keywords: tRNA maturation, RNase P, pentatricopeptide repeat proteins, evolution

The dogma of the universal conservation of RNase P as a ribonucleoprotein (RNP) has been challenged with the discovery of protein-only RNase P enzymes called PRORP. We have shown that these enzymes are responsible for RNase P activity in both organelles and the nucleus in plants. Although plant PRORP act as single subunit enzymes, we provide evidence to show their interaction with a number of factors *in vivo*, thus showing the integration of PRORP functions among other gene expression processes.

Beyond plants, it becomes evident that PRORP proteins are prominent in many distantly related eukaryote lineages. We thus explore the diversity of PRORP function and mode of action in a number of representative eukaryote model species.

Finally, mechanistic data show that PRORP proteins have evolved a mode of tRNA recognition reminiscent from the one used by RNP RNase P.

The diversity of PRORP and RNP enzymes is compared and should give clues to understand the evolutive history of RNase P.

LECTURE 13

New families of proteins required for mitochondrial RNA editing

Stephane Bentolila, Xiaowen Shi, Tao Sun, Giulia Friso, Klaa Van Wijk, Maureen R Hanson
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Keywords: RNA editing, editosome, PPR, RIP/MORF

Introduction: Transcripts of mitochondrial proteins are profoundly altered by editing of hundreds of Cs to U, changing the encoded amino acids and creating stop and start codons. These extensive alterations are critical for production of mitochondrial proteins that can function properly. One mystery was solved when pentatricopeptide (PPR) proteins were identified as the site-specificity factors that inform the editing complex as to which C should be edited. The major remaining mystery is the composition of the editosomes, which are small RNA/protein complexes 300-400 kD in size. We have previously identified a family of proteins whose members are present in editosomes: the RIP family (also known as the MORF family).

Materials and Methods: We used homology searching and co-immunoprecipitation with another editing factor, followed by gene silencing and mutant analysis of candidate genes, in order to identify additional RNA editing factors.

Results: We have now identified two more gene families whose members are required for editing of particular mitochondrial Cs. We will describe the two protein families and report on protein/protein interactions between them, PPR proteins and RIP/MORF proteins.

Conclusion: With the detection of these two additional gene families, we are approaching an understanding of the composition of the mitochondrial RNA editing complex.

SCIENTIFIC SESSION IV:
MITOCHONDRIAL MEMBRANE BIOGENESIS, DYNAMICS AND TRANSPORT

Chairmen:
David Logan (Angers University, France), Shin-Ichi Arimura (The University of Tokyo, Japan)

LECTURE 14

Identification and Characterisation of a tRNA Import Receptor into Plant Mitochondria

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It is well established that mitochondria are required to import the majority of their protein complement and lipids to function. The identity of the molecular machines involved in these processes is intensively studied, and the underlying regulatory processes are also beginning to be uncovered. While mitochondria contain a genome, the proteins required for replication, transcription and translations are also required to be imported from cytosolically synthesized, nuclear encoded proteins.

In general the rRNA and tRNA molecules required for translation of mitochondrially-encoded proteins are encoded in mitochondria. However in some organisms, in particular plants, the mitochondrial genome does not encode sufficient tRNA molecules to support translation, although as many as 50 protein are encoded in plant mitochondrial genomes. Thus is it firmly established that plant mitochondria import tRNA from the cytosol.

However while the import of tRNA into plant mitochondria has been known for over a decade, there is little understanding of the machinery involved in this process. In order to identify proteins that are involved in tRNA import into mitochondria candidate proteins on the mitochondrial outer membrane were investigated to determine their role in tRNA import. Two proteins displaying high levels of sequence identity were identified as receptors for tRNA into mitochondria. The identification and characterization of the proteins will be outlined in this presentation, with possible implications for mitochondrial biogenesis and signaling.

LECTURE 15

Post-translational regulation of mitochondrial dynamics in Arabidopsis

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Keywords: post-translational modification, mitochondrial dynamics, dynamin-related protein, phosphorylation, ubiquitination, de-ubiquitination

One research direction of our group is to elucidate mechanisms that govern the dynamic behavior of plant energy organelles, including their morphogenesis, fission and fusion, distribution, and movement. Post-translational modifications (PTMs), such as protein phosphorylation and ubiquitination, play critical roles in animal and yeast mitochondrial dynamics. How these mechanisms contribute to mitochondrial dynamics in plants is just beginning to be elucidated. Arabidopsis dynamin-related protein 3 (DRP3) proteins are large GTPases that serve as the main executors of mitochondrial fission. We have shown that phosphorylation of DRP3 inhibits its function in organelle division, possibly through reducing its GTPase activity. In addition, we identified a mitochondrial outer membrane-localized ubiquitin protease, which promotes the conversion of mitochondrial morphology from rod to spherical shape and reduces the mitochondrial association of DRP3. Finally, we have shown that a mitochondrial outer membrane-anchored ubiquitin ligase affects the distribution of mitochondria. In summary, our study has begun to uncover PTM mechanisms that regulate plant organelle morphogenesis and dynamics. Some of these mechanisms are shared by mitochondria, chloroplasts and/or peroxisomes.

LECTURE 16

AtPAP2 and STY kinases regulate the import of certain precursor proteins into chloroplasts and mitochondria

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Keywords: AtPAP2, MORF, Phosphorylation, Protein Import, STY kinase

Cytosolic STY kinases were reported to phosphorylate the transit peptides of certain precursor proteins and regulate their import into chloroplasts. Here we showed that the presequences of several multiple organellar RNA editing factor (MORF) family proteins, which play role in RNA editing in mitochondria and plastids, are phosphorylated by cytosolic STY kinases. STY-phosphorylated pMORF3, but not its non-phosphorylatable mutant (pMORF3-T17/20/35A), can complex with 14-3-3 and HSP70 and the import rate of wheat germ lysate (WGL)-synthesized pMORF3-T17/20/35A into mitochondria was much faster than that of WGL-synthesized pMORF3. *Arabidopsis thaliana* purple acid phosphatase 2 (AtPAP2), anchored on the outer membranes of both chloroplasts and mitochondria, can selectively interact with certain chloroplast precursor proteins and the presequences of MORFs. The import rates of these proteins into chloroplasts and mitochondria isolated from *pap2* T-DNA line were slower than into that isolated from the wild-type plants. We hypothesize that STY kinases and AtPAP2 regulate the import of proteins into chloroplasts and mitochondria by phosphorylating and dephosphorylating their transit peptides/presequences. Based on the phenotypes of AtPAP2 overexpression lines, we proposed a model on how STY kinases and AtPAP2 orchestrate the energy outputs from these two organelles.

LECTURE 17

TOM9: a calmodulin-regulated protein involved in TOM complex assembly in Arabidopsis thaliana

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Keywords: protein import, calcium regulation, TOM complex

Nuclear encoded mitochondrial proteins are imported into the organelle through multi-subunit protein translocases in the outer and inner membrane. At the interphase with the cytosol, the translocon on the outer membrane of mitochondria (TOM complex) initiates the import of proteins and provides the first place for potential regulation. However, very little is known about how mitochondrial protein import is regulated. We have discovered a potential role of TOM9 as a regulator of TOM complex assembly in Arabidopsis. RNAi knock-down plants with severe lack of TOM9 have shortened hypocotyls and show an early flowering phenotype. Western Blot analysis showed that their mitochondria have a significantly reduced content of TOM40 and TOM20 and a corresponding severe reduction in TOM complex, indicating that lack of TOM9 affects the assembly of the TOM complex. Furthermore, TOM9 was shown to be a calmodulin (CaM)-binding protein with a potential CaM-binding domain within its cytosol exposed N-terminal domain. CaM interferes with the interaction between TOM9 and the import receptor TOM20. Binding of CaM to TOM9 could thus be part of a plant specific signalling network involved in the regulation of mitochondrial protein import.

LECTURE 18

Identification of a mitochondrial super-complex involved in lipid trafficking in plant mitochondria

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Keywords: Lipid trafficking, plant mitochondria, chloroplast

The mitochondrion is an organelle delineated by two membranes and little is known about their biogenesis in plants. In yeast, extensive exchanges of lipids occur between mitochondria, endoplasmic reticulum and vacuolar membranes. In plants, the transfer of digalactosyldiacylglycerol (DGDG), a chloroplast-synthesized lipid, to mitochondria has been shown previously during phosphate starvation, suggesting a role of chloroplasts in mitochondrial membrane construction. In this study, we have identified a mitochondrial super-complex enriched in DGDG during phosphate deprivation in *Arabidopsis thaliana* cells culture. Using mass spectrometry, we found that this DGDG-enriched super-complex contains proteins involved in respiration, protein import, or mitochondrial division. We isolated a knock out plant for one candidate gene present in this complex and we observed a perturbation of mitochondrial lipid composition in this mutant. We also observed a decrease of the *in vitro* incorporation of DGDG into mitochondria. To decipher the role of this protein in mitochondrial lipid trafficking, we are looking at its direct partners by co-immunoprecipitation and at its ability to bind lipids *in vitro*. Our data suggest we have identified for the first time a super-complex playing a role in plant mitochondria lipid trafficking.

LECTURE 19

Functional characterization of NAD+ transporters in Arabidopsis thaliana

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Keywords: Mitochondrial Carrier Family, Arabidopsis, AtNDT1, AtNDT2

Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are essential compounds in all organisms being highly connected within the metabolic network. This fact notwithstanding little is known about the transport of both NAD^+ and $NADP^+$ in plants. We have investigated the role of two NAD^+ carrier proteins in *Arabidopsis*, namely *At*NDT1, which resides in the chloroplasts, and *At*NDT2, located in the mitochondria. *Arabidopsis thaliana* T-DNA insertion lines as well as antisense lines deficient in the expression of NDT1 or NDT2 were analyzed in details at both physiological and biochemical levels. NDT1 deficient plants displayed significant increases in glucose, sucrose, starch, malate and fumarate levels at midday and at the end of the day, in comparison to wild type. In turn, NDT2 deficient plants exhibited lower photosynthesis and stomatal conductance and decreases in starch content and turnover. Collectively, our results are consistent with the crucial role of both transporters in maintaining a balanced metabolism in plant cells.

SCIENTIFIC SESSION V: INTERORGANELLAR SIGNALLING AND INTERACTION

Chairmen:
Christine Foyer (University of Leeds, United Kingdom), Kathleen Soole (Flinders University, Australia)

LECTURE 20

Mitochondrial Signalling

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La Trobe University

Keywords: Retrograde Signalling, Sensor, Stress, Development

It is widely accepted that in addition to the extensive biochemical metabolic functions of mitochondria that they play an essential role as a cellular signalling hub. Mitochondria act as a sensor to environmental and cellular signals to trigger signal transduction pathways to modify nuclear gene expression. Much of the investigation in this area has focused on stress induced components of mitochondria, e.g. alternative oxidase (AOX), and the identification of the signalling pathways and components that are involved in induction of AOX under a variety of different conditions. However in addition to responding to adverse conditions it is hypothesize that mitochondrial signalling is required during normal growth and development.

To gain further insight into such pathways a number of mitochondrial proteins were targeted for inactivation to determine their role, if any, in mitochondrial signalling. Proteins of the mitochondrial outer membrane and intermembrane space were targeted as any signals generated in mitochondria need to be relayed to the nucleus and it is considered that proteins in these compartments may play a role in the initiation and/or rely of such signals.

In this presentation the identification and characterisation of a variety of proteins in mitochondria will be presented and how they may play a role in mitochondrial signalling.

LECTURE 21

Organellar gene expression and retrograde signalling

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Keywords: organellar gene expression, retrograde signalling, transcript maturation

Retrograde signals originating from chloroplasts and mitochondria modulate nuclear gene expression (NGE). *A. thaliana* PRORS1 encodes a prolyl-tRNA synthetase, which is targeted to both plastids and mitochondria. Work on the Arabidopsis *prors1* mutant showed that disturbances of organellar gene expression (OGE) in both chloroplasts and mitochondria cooperate to trigger retrograde signalling. The *prors* mutant was identified in a screen for photosynthesis affected mutant (*pam*) lines – just as was the *pam48* mutant. Interestingly, *prors* and *pam48* mutants show very similar NGE profiles, attracting our attention to this mutant.

PAM48 is dually targeted to plastids and mitochondria, and its knockout perturbs plastid development and results in seedling lethality. In the leaky *pam48-1* mutant, a defect in photosynthesis is associated with reduced levels of photosystem subunits although corresponding mRNA levels are unaffected. Bacterial one-hybrid screening, electrophoretic mobility shift assays and coimmunoprecipitation experiments reveal a specific interaction between PAM48 and a RNA sequence in the chloroplast *rrn* operon. In consequence, rRNA maturation is perturbed in *pam48-1* mutants, which reduces levels of *16S* and *23S* rRNAs and, in turn, translational capacity. Because *pam48-1* displays a *genomes uncoupled* (*gun*) phenotype and *gun1 pam48-1* plants exhibit a synergistic phenotype, we conclude that disturbed OGE in *pam48-1* triggers retrograde signalling.

LECTURE 22

Mitochondrial transcriptome control and genetic coordination in plant cells

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Keywords: Genetic regulation, signaling, mitochondria, ribozyme, RNA trafficking

Regulation of mitochondrial gene expression and inter-compartment genome coordination is still little understood in plants. We addressed this question through a novel genetic approach, circumventing the absence of mitochondrial transformation methodologies for multicellular eukaryotes. We developed the use of a tRNA-like structure to target RNAs expressed from nuclear transgenes into mitochondria through the natural tRNA import pathway. Inducible expression and mitochondrial targeting of *trans*-cleaving ribozymes attached to the tRNA-like structure caused transient knockdown of individual mRNAs in the organelles of nuclearly transformed *Nicotiana tabacum* cells or *Arabidopsis thaliana* plants. Differing from classical mutant analyses, this strategy allowed to characterise the early regulation and signaling response triggered by the altered expression of a mitochondrial gene. Whereas it has been considered so far that mitochondrial genetic processes are mostly controlled at the post-transcriptional level, our observations imply that the mitochondrial transcriptome is reactive to the knockdown of a major organellar mRNA. Furthermore, microarray analyses showed a general response at the whole cell level. Knockdown of the *nad9* mRNA in mitochondria induced retrograde hormone signaling and altered expression of nuclear genes encoding pentatricopeptide (PPR) proteins. Cytosolic, plastidic and mitochondrial protein synthesis, as well as transport processes, were subsequently affected, followed by a further signaling response. These results bring a new integrated view of the genetic regulation processes in plant cells.

LECTURE 23

Mitochondrial redox as the major signal to mediate the crosstalk with chloroplasts and other organelles in plant cells

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Keywords: mitochondria, redox, chloroplasts, peroxisomes, proline, menadione, glycolate

The metabolic interactions between mitochondria and chloroplasts are well accepted. However, the exact signals originating from mitochondria to modulate chloroplasts and other organelles are not clear. Wehave been studying the consequences of the modulation of mitochondrial metabolism in *Arabidopsis* by suitable inhibitors and mutants. The importance of mitochondria shows up only when mitochondrial electron transport or redox status is disturbed, but not by modulation of TCA cycle. Menadione (MD) was used to induce ROS production in mitochondria. Thelevels of H_2O_2 were monitored by DAB staining and superoxide by the reduction of NBT. The patterns of O_2 uptake/evolution as well as the activities of key anti-oxidative enzymes were examined. The levels of important metabolites,including amino acids, were determined. The oxidative stress in mitochondria created by MD led to increases in ROS, catalase/anti-oxidative enzyme activities, operation of alternative pathway and proline levels. In contrast, MD decreased the rate of photosynthesis, glycolate levels and glycolate oxidase activity. Our results suggest that modulation of mitochondrial redox by MD results in decreased photosynthesis,restricted photorespiratory glycolate metabolism and modification of amino acidmetabolism, leading to proline accumulation. Based on our results andliterature, we propose that mitochondrial redox ROS could act as a major signal to modulate the metabolism of chloroplasts and peroxisomes.

LECTURE 24

A two-phase stress response to energy organelle inhibition is controlled by WRKY and NAC transcription factors

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Keywords: mitochondria, chloroplasts, retrograde signalling, stress, transcriptional regulation

Plants respond to organelle dysfunction with extensive transcriptional changes that result in reprogramming of cellular homeostasis. Here we show that two distinct phases of expression changes follow treatment with a variety of elicitors and energy organelle inhibitors. The first phase occurs rapidly after treatment, and is related to touch and pathogen defence responses. A second phase peaks at 3 to 6h after treatment and comprises a wide range of stress responsive genes. To identify which regulators control the respective expression phases, a wide range of Arabidopsis lines with altered expression of known regulators of organelle-to-nuclear (retrograde) signalling were compared, including ABI4, WRKY and NAC transcription factors. Our results indicate that several WRKY factors are also induced in the first stress-response phase and exert negative feedback. Furthermore, we show that NAC transcription factors are almost solely responsible for induction of the second phase and are master regulators of both mitochondrial and chloroplast retrograde signalling. When NAC function was abolished in mutants with mitochondrial and/or chloroplast defects, their constitutive retrograde responses were almost completely suppressed. This loss of retrograde signalling exacerbated growth deficiencies under normal and stress conditions, underlining the importance of organelle-to-nucleus signalling *in vivo*.

SCIENTIFIC SESSION VI:
RESPIRATORY CHAIN STRUCTURE, ASSEMBLY AND REGULATION

Chairmen:
Allan Rasmusson (Lund University, Sweden), Wieslawa Jarmuszkiewicz (Adam Mickiewicz University in Poznań, Poland)

LECTURE 25

The carbonic anhydrase domain of mitochondrial complex I

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Keywords: Respiratory chain, NADH dehydrogenase complex, complex I, carbonic anhydrase

The mitochondrial NADH dehydrogenase complex (complex I) consist of several functional domains which independently arose during evolution. In higher plants, it contains an additional domain which includes proteins resembling gamma-type carbonic anhydrases. The extra domain is composed of three proteins but the genome of Arabidopsis encodes five distinct gamma-type carbonic anhydrases (CA1, CA2, CA3, CAL1, CAL2), all of which form part of complex I. Evidence will be presented that the extra domain includes one copy of either CAL1 or CAL2 plus two copies of the CA1/CA2 proteins. Thus, the carbonic anhydrase domain can have six distinct subunit configurations. Single and double mutants with respect to the CA/CAL proteins were employed to genetically dissect the domain. New insights into complex I biology in plants will be presented and discussed.

LECTURE 26

CMS-G of Beta vulgaris ssp maritima contains an unusual cytochrome c oxidase

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Keywords: cytoplasmic male sterility, respiratory chain composition, cytochrome c oxidase

In sea beet (*Beta vulgaris ssp maritima*), ten types of mitochondrial genomes (mitotypes) have been identified. Three of these mitotypes induce male sterility. The mitotype G, responsible for CMS-G, has recently been sequenced and, surprisingly, no new chimeric ORFs likely to cause sterility were detected. Instead, many point mutations in protein coding genes were found. In particular, the initial methionine of Cox1 is mutated and the STOP codons of Cox2 and Nad9 are changed.

We have characterized the mitochondria of CMS-G plants. Using Blue Native-PAGE, we observed the presence of a heavy form of the cytochrome *c* oxidase (complex IV). Other OXPHOS complexes appear unchanged. When investigating the subunit composition of complex IV, we identified a larger form of Cox1. The *cox1* transcript in CMS-G encodes a form of Cox1 containing an N-terminal extension of 138 amino acids. This configuration originates from a CMS-G-specific insertion in the mitochondrial genome that modifies the intergenic region between *nad9* and *cox1*.

The capacity of the CMS-G-specific heavy complex IV is reduced compared with normal complex IV but the dark respiration of leaves is increased in CMS-G. This suggests that the heavy complex IV acts as uncoupler as other CMS-inducing factors. Preliminary work on fertility restoration indicates that male fertile plants carrying the CMS-G cytoplasm possess exclusively the heavy cytochrome *c* oxidase form.

LECTURE 27

From gene expression to respiratory complex assembly: the roles of maturases in mitochondria biogenesis in plants

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Keywords: Maturase, Group II intron, Splicing, Mitochondria, Plants

Mitochondria (mt) functions are required for cellular energy production and metabolism. The respiratory activity is subjected to regulation by physiological, developmental and environmental signals. The expression of the mtDNA in plants is complex, particularly at the posttranscriptional level. The primary mtRNAs undergo extensive processing events, which include editing and the splicing of numerous group II-type introns that reside in many essential genes. The removal of these introns from the genes they interrupt is essential for mt functions. Proteins that regulate the expression of the mtDNA have the ability to link mt functions with environmental or developmental signals. The main factors that influence group II intron splicing in bacteria include maturases (MATs), an ancient group of proteins encoded within the introns themselves.

Plants harbor several proteins that are closely related to bacterial MATs. These including MatR in the mtDNA and several nuclear encoded mitochobndria-localized proteins (nMATs). Reverse-genetic and biochemical studies established the roles of the MATs in the splicing of many mtRNAs. *nmat* mutants show altered growth and developmental phenotypes, which are tightly associated with defects in the biogenesis of the respiratory machinery. Interestingly, the five MATs in Arabidopsis (i.e. nMAT1 to 4 and MatR) are all involved in the expression of Nad1, a highly conserved subunit that is likely incorporated at the earliest stage in complex I assembly. These results indicate that the maturation of *nad1* pre-mRNAs serves as a key step in regulating complex I assembly in angiosperms mitochondria.

LECTURE 28

Multiple AOX isoforms in Arabidopsis: What makes the difference?

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Keywords: Alternative Oxidase, Arabidopsis thaliana, enzyme activity, Escherichia coli, plant mitochondria, protein expression

The Alternative Oxidase (AOX) is a non-energy conserving terminal oxidase in plant mitochondria using electrons from the ubiquinone pool to reduce oxygen to water. Arabidopsis possesses five AOX isoforms – AOX1A-D and AOX2 – each containing two conserved cysteine residues (CysI and CysII) in the N-terminal domain. CysI inactivates AOX by forming a disulfide bond with the corresponding CysI on the adjacent subunit of the AOX homodimer. Reducing conditions lead to an active protein which can be further activated by alpha-keto acids through the formation of a thiohemiacetal with CysI.

To analyze the post-translational regulation of different AOX isoforms, Cys mutants of AOX1A, C and D were generated and expressed in *E. coli*. Many different mutants with single, double or triple substitutions of CysI, CysII and/or CysIII (which is only present in AOX1A and B) were investigated for their activity and the influence of various alpha-keto acids. Additional mutations were introduced to mimic the covalent modification of Cys with the alpha-keto acid leading to an additional negative charge. From the permanently increased activity of such mutants we conclude that a negative charge has impact on the conformation and on the position of the di-iron ligands important for the catalytic reaction. The fact that the wild-type AOX1A activity can be increased by amino acid exchange gives further insight into the regulation of AOX, since AOX1C and D activity was not significantly enhanced by mutations. This might explain the earlier finding that *aox1a* knock-out plants which compensate the deficit by increasing AOX1D are not completely recovered.

LECTURE 29

A putative auxiliary role of cytochrome c oxidase assembly factor AtCOX11 in ROS scavenging

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Keywords: COX complex, ROS, copper

COX11 (cytochrome *c* oxidase 11) is a conserved family of copper chaperones found in many eukaryotic and prokaryotic species. These chaperones are involved in copper delivery to complex IV (COX) of the respiratory chain. We recently identified an Arabidopsis COX11 homolog and confirmed that it serves the same function. As COX11 homologs in yeast and in rice have been proposed to exert an additional function in scavenging reactive oxygen species (ROS) we investigated such a role for AtCOX11. The putative *AtCOX11* promoter region reveals the presence of at least six putative *cis*-acting ROS-responsive elements. qPCR analyses suggested that these regulatory elements are indeed functional because *AtCOX11* levels doubled in seedlings when exposed to oxidative stress compared with the untreated control. Unexpectedly, however, ROS levels were the same in wild-type and *AtCOX11* overexpressing plants and even lower in the knock-down lines, contradicting a function of AtCOX11 as ROS scavenger. Considering the insensitivity of the ROS detection assays we took a different approach: we expressed several soluble versions of AtCOX11 in the cytoplasm of Saccharomyces cerevisiae and monitored the growth of the transformed yeast strains under oxidative stress. Interestingly only the yeast strain expressing a variant of AtCOX11 which can form a disulfide bridge grew significantly better under stress conditions. Based on these observations, we propose that AtCOX11 might have a secondary function as a ROS scavenger, possibly by forming intracellular disulfide bridge.

SCIENTIFIC SESSION VII: MITOCHONDRIA AND STRESS RESPONSE

Chairmen:
Miquel Ribas-Carbó (Universitat De Les Illes Balears, Spain), Anna M. Rychter (University of Warsaw, Poland)

LECTURE 30

Tips and tricks of antibody production and validation process - how to obtain good results?

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Antibodies are a popular tool used in plant cell biology research. They can be either custom made or purchased from a commercial supplier. In either case their production is a complex process, consisting of three very important components which has to be carefully considered. These are: Antigen-Animal-Testing. Which source of antigen is most optimal for your project: peptide, recombinant protein or a native protein isolated from tissue? Which animal species to choose? Are certain species making better antibodies compare to others? Do I have any controls to validate produced antibody? What controls should be used? What to do if my antibody is not giving any signal in a western blot? Over 15 years spent in antibody production and validation process will aid to answer such questions during this presentation.

LECTURE 31

Integration of organellar and epigenomic behavior during reprogramming for abiotic stress response in plants

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Plants display a remarkable range and capacity in their response to environmental stress. In some cases, stress responses may be transgenerational. The MSH1 Effect is an organellar-epigenetic phenomenon that appears to be unique to plants and conditions developmental reprogramming. Our group has investigated the epigenetic and plastid features of the system. The MSH1 gene product localizes to a specialized plastid type, and disruption of this association appears to condition non-genetic heritable changes in the plant growth pattern. However, we have not previously reported on the mitochondrial effects on growth during MSH1 disruption. Some plants display a curly or wrinkled leaf phenotype together with changes in metabolism and gene expression that will be described. Once selected, the phenotype is completely penetrant, maternal and retained following replacement of MSH1, implying that the changes are likely due to mitochondrial genome changes. What is particularly striking is the apparent cooperation evidenced in organellar and epigenetic behavior to elicit a full range of developmental and stress responses. These behaviors are recapitulated in all plant species that we have tested to date, suggesting that they likely comprise components of adaptation.

LECTURE 32

Mitochondrial respiration in the light during drought

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Keywords: alternative oxidase, chloroplast ATP synthase, drought, mitochondria, Nicotiana tabacum, photosynthesis, photosystem stoichiometry adjustment, mitochondrial respiration in the light

Chloroplasts have means to manage excess reducing power but these mechanisms may become restricted by rates of ATP turnover. Alternative oxidase (AOX) is a mitochondrial terminal oxidase that uncouples the consumption of reducing power from ATP synthesis. Physiological and biochemical analyses were used to compare respiration and photosynthesis of *Nicotiana tabacum* wildtype (WT) plants with that of transgenic lines overexpressing AOX, under both well-watered and drought stress conditions. With increasing drought severity, AOX overexpression acted to increase respiration in the light (R_L) relative to WT. CO_2 and light response curves indicated that overexpression also improved photosynthetic performance relative to WT, as drought severity increased. This was not due to an effect of AOX amount on leaf water status or the development of the diffusive limitations that occur due to drought. Rather, AOX overexpression dampened photosystem stoichiometry adjustments and losses of key photosynthetic components that occurred in WT. The results indicate that AOX amount influences R_L, particularly during severe drought, when cytochrome pathway respiration may become increasingly restricted. This impacts chloroplast redox state, influencing how the photosynthetic apparatus responds to increasing drought severity. In particular, the development of biochemical limitations to photosynthesis are dampened in plants with increased non-energy conserving R_L. The results suggest that increasing the capacity of non-energy conserving electron sinks may be a useful biotechnological approach to preserve photosynthetic potential during drought.

LECTURE 33

Mitochondrial Calcium Dynamics - Checks and Balances of Energy Physiology

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Keywords: Mitochondria, in vivo imaging, fluorescent protein sensors, calcium, energy physiology

Mitochondria conserve the energy released from metabolic redox reactions and supply the cell with ATP. When the rate of respiratory metabolism does not match ATP demand active regulation of mitochondrial function is essential. For plants particularly sophisticated regulation strategies can be expected, to ensure maintenance of homeostasis in the presence of frequent environmental changes. Yet, the mechanisms by which such control is achieved *in vivo* are poorly understood.

Calcium acts as a key regulator of mitochondrial energy metabolism in mammals by modulating the activity TCA cycle dehydrogenases. Calcium flux into the matrix is controlled by the recently identified mitochondrial uniporter complex. Plants contain homologues of components of the uniporter, but their function has been unclear. To understand how mitochondrial calcium dynamics are regulated and what their impact is on energy metabolism, we have combined reverse genetics with *in vivo* sensing of calcium. Fluorescent protein sensors and quantitative confocal imaging allow monitoring of mitochondrial energy physiology in living Arabidopsis tissues. We have found that several homologues of components of the mitochondrial calcium uniporter complex localize to mitochondria in Arabidopsis. Mutant lines have shown severely altered mitochondrial calcium levels and abnormal organellar calcium transients, providing a novel genetic handle on the dissection of the role of calcium regulation in plant mitochondria. We will discuss the specific impact of de-regulated mitochondrial calcium on the physiological network and the function of plant mitochondria.

LECTURE 34

Unravelling the molecular mechansims of cold and salinity stress/tolerance in wheat mitochondria

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Keywords: Cold, Salinity, Respiration, Oxidative Phosphorylation, Wheat.

One of the most prevalent environmental challenges encountered by crop plants is the exposure to a broad range of temperatures. Fluctuations in temperature affect respiration and can have dramatic implications on biosynthesis, cellular maintenance and biomass allocation. Similarly, salinity exposure is a major impairment to agricultural production due to the toxic effects of salts upon plant growth. Whilst the costs of these environmental stresses on crop production are well known, the molecular responses within plant cells are not. Using a wide array of approaches including SRM mass spectrometry, metabolomics, biochemistry and physiology of lab and field grown wheat we are beginning to characterise the specific control points of mitochondrial metabolism in responce to cold and salinity exposure. For example proline has long been known to be induced during chilling and act as a compatible solute, here we will present evidence that proline dehydrogenase has a major role in the cold responce of wheat mitochondria by maintaining respiration during chilling and for the removal of proline during recovery. Similarly it has been known that exposure to salt can reduce the activity of a number of enzymes, here we will show that in wheat plants under environmental salt concentrations that components of the TCA cycle are specifically inhibited. This inhibition leads to a restriction of TCA cycle function and the induction of the GABA shunt to maintain mitochondrial function.

LECTURE 35

The role of mitochondrial proteins in stress responses

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Keywords: Arabodopsis, stress response

High soil salinity or drought result in accumulation of reactive oxygen species (ROS), leading to oxidative stress. Although chloroplasts are main source of ROS, mitochondria are also important in the maintenance of the cellular redox homeostasis, where Complex I and III of mitochondrial electron transport chain are major sites for ROS production. To reveal the importance of genes encoding the mitochondrial proteins in stress responses, we are characterizing Arabidopsis thaliana mutants, in which the mutations are localized in genes encoding the subunits of Complex I and III of the mitochondrial electron transport. When compared to wild type, several mutants showed morphological and physiological changes under stress conditions. Phenotypic alterations and differences in tolerance to drought and salinity were revealed through in vitro germination and growth tests, as well as by complex phenotyping in collaboration with European Plant Phenotyping Network. Several mutants were identified, which showed altered tolerance to osmotic or salt stress. Two mutant alleles were characterized in detail in which the mutations disrupted the NDUSF8a gene. Both *ndusf8a* mutants were hypersensitive to oxidative stress, although less hydrogen peroxide formed in them and lipid peroxidation level were lower in high osmotic stress. Changes in chlorophyll fluorescence under stress treatments suggested that these Complex I mutations influence photosynthesis as well. Our data shows that the NDUSF8a gene has important affect on plants stress responses.

This research was supported by OTKA Grant NN-110962 and the EPPN program.

LECTURE 36

Investigating succinate dehydrogenase as a central regulator of stress response in plants

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Keywords: Arabidopsis, Mitochondria, Complex II, Succinate Dehydrogenase, plant defence, respiration

Mitochondrial complex II (Succinate Dehydrogenase: SDH) plays a role in the electron transport chain as well as in the tricarboxylic acid cycle by oxidizing succinate to fumarate and transferring electrons to ubiquinone which is reduced to ubiquinol. The classical complex II contains four subunits, SDH1-4, and two assembly factors (SDHAF1 and SDHAF2) have been identified in humans. We previously reported a point mutation in SDH1-1 of *Arabidopsis thaliana* (*dsr1*), with disrupted cell signaling in response to salicylic acid, lower production of reactive oxygen species (ROS) and higher susceptibility to plant pathogens. Plant defense mechanism is of global interest due to the annual loss in crop production caused by plant diseases. Therefore understanding of how plants respond to stress is of great interest. We also reported a second SDH assembly mutant line (*sdhaf2*) that affects FAD binding into SDH1, with lower SDH activity and reduced root elongation. Although both mutant lines are affected in the same subunit (SDH1-1) of SDH, the resulting phenotypes are quite different. We will present evidence that observed differences may be caused by kinetic differences in SDH enzyme activity that have impacts on biotic and abiotic stress tolerance associated with SDH.

SCIENTIFIC SESSION VIII:
PROTEIN SYNTHESIS, MODIFICATION AND QUALITY CONTROL

Chairmen:
Linda Bonen (University of Ottawa, Canada), Hanna Janska (University of Wroclaw; Poland)

LECTURE 37

Lysine acetylation and its regulatory roles in Arabidopsis mitochondria

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Keywords: lysine acetylation, posttranslational modifications, acetyl-CoA, metabolism

Posttranslational modifications are essential regulators of protein functions as they can modify enzyme activities or protein-molecule interactions by changing the charge state or chemical properties of their target amino acid. The acetyl moiety of the central energy metabolite acetyl-CoA can be transferred to the ⊠amino group of lysine, a process known as lysine acetylation which is implicated in the regulation of key metabolic enzymes in various organisms. Since plant mitochondria are of great concern for plant growth and development and as they house key enzymes of oxidative phosphorylation and photorespiration, it is essential to investigate the occurrence and regulation of this posttranslational modification. In this talk, I will give an overview on our current knowledge about the role of lysine acetylation in Arabidopsis mitochondria.

LECTURE 38

Mitoribosome-mediated regulation of protein synthesis in plant mitochondria

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Keywords: protein synthesis, mitoribosome, Arabidopsis

The ribosome filter hypothesis postulates that ribosomes are not simple non-selective translation machines but may function as regulatory elements in protein synthesis. Growing studies indicate that ribosomes *in vivo* form heterogeneous population. It is postulated that such populations contribute to the functional selectivity of translation in response to environmental or developmental signals.

RNAi-mediated silencing of the nuclear *RPS10* gene encoding S10 protein of mitochondrial ribosome leads to the generation of artificial, heterogeneous population of mitoribosomes. We found that *rps10* mitoribosomes show an imbalanced level of small and large ribosomal subunits, with an excess of the large ones. Moreover, a portion of the small subunits are incomplete, lacking at least the S10 protein. This polymorphic mitoribosome population diversified translation of both upregulated mitochondrial OXPHOS and ribosomal transcripts in *rps10*. As a consequence, the ribosomal proteins were oversynthesized, while the OXPHOS subunits were translated with a reduced yield. Based on microarray data, we suspect that mechanism responsible for selective protein synthesis is rather not connected with initiation of translation.

Our finding unambiguously indicate that translation of certain groups of mitochondrial transcripts can be differentially affected by alterations in mitoribosomes composition and translation is able to override the result of transcription in plant mitochondria.

LECTURE 39

The mitochondrial SHOT1 protein is required for OXPHOS biogenesis

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Keywords: OXPHOS biogenesis, mitochondrial protein synthesis, mTERF

Members of the mTERF (mitochondrial transcription termination factor) family are superhelical nucleic acid binding proteins that were initially characterised in animal mitochondria, where they have roles in transcription and translation. In land plants, mTERFs are found in mitochondria as well as in plastids. To date, only two of the many plant mTERFs have been characterised with respect to their molecular function, revealing roles for mTERFs in transcript splicing. Mutation of another plant mTERF, SHOT1, located in mitochondria, was found affect redox balance and to enhance thermotolerance, but had no effect on mitochondrial transcript splicing.

To elucidate SHOT1 function and determine the cause of altered *shot1* mutant physiology, we examined the accumulation of OXPHOS complexes in *shot1* using blue-native PAGE. Compared with wild type, *shot1* displayed reduced amounts of complexes I and IV and lower in-gel activity for these complexes. These results were matched by immunoblots showing reduced steady-state levels of several OXPHOS subunits. RNA gel blots detected wild type-like mitochondrial cox transcript patterns in *shot1*, indicating that complex IV reduction was potentially due to a defect in mitochondrial protein synthesis or stability. In organello protein synthesis assays suggest a general stimulation of translation in *shot1* mitochondria, as observed in other mitochondrial mutants. However, profiles of labelled proteins differed between *shot1* and wild type, indicating altered translation in *shot1* mutants. Work is in progress to substantiate a role for SHOT1 in mitochondrial protein synthesis.

LECTURE 40

Shredding the signal: Systems for targeting peptides degradation in Mitochondria and Chloroplasts

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Keywords: peptidases, peptide degradation, targeting peptides, biogenesis of mitochondria, quality control

In plant cells, both mitochondria and chloroplasts contain a multitude of proteolytic systems participating either in targeted proteolysis related to preprotein maturation upon their import into these organelles or in a general degradation of damaged proteins. A consequence of these reactions is the production of presequences, transit peptides and other short peptides that due to their toxic effects must be either exported or degraded. Up to now only the Presequence Protease, PreP, was shown to degrade targeting peptides in the endosymbiotic organelles.

We have recently identified a new component of the organellar proteolytic system in Arabidopsis thaliana, the Organellar Oligopeptidase, OOP. OOP is dually targeted to the mitochondrial matrix and the chloroplastic stroma where it degrades short mitochondrial and chloroplastic targeting peptides or their fragments ranging between 8 and 23 a.a in length (partially overlapping with PreP, which cleaves peptides 10-65 a.a long). Functional overlap between PreP and OOP suggests that these two systems might cooperate and complement each other constituting a targeting peptide degradation pathway. In agreement with this *prep oop* crosses in *Arabidopsis thaliana* display more severe phenotype than *prep* and *oop* knockouts alone. Further analysis of the *prep oop* knockouts might give clues about the role of the organellar peptide degradation in the plant development.

LECTURE 41

Identification of Cleavage sites and Substrate Proteins for Two Mitochondrial Intermediate Peptidases in Arabidopsis thaliana

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Keywords: Protein import, Precursor processing, ICP55, OCT1, ChaFRADIC

Most mitochondrial proteins contain an N-terminal targeting signal which is removed by specific proteases following import. In plant mitochondria, only mitochondrial processing peptidase (MPP) has been characterised to date. Therefore, we sought out to determine the substrates and cleavage sites of the Arabidopsis thaliana (Arabidopsis) homologs to the yeast Icp55 and Oct1 proteins, utilising the newly developed method ChaFRADIC for N-terminal protein sequencing. We identified 88 and 7 putative substrates for Arabidopsis ICP55 and OCT1, respectively. It was determined that the Arabidopsis ICP55 contains an almost identical cleavage site to that of Icp55 from yeast. However, it can also remove a far greater range of amino acids. The OCT1 substrates from Arabidopsis displayed no consensus cleavage motif, and do not contain the classical -10R motif identified in other eukaryotes. Arabidopsis OCT1 can also cleave presequences independently, without the prior cleavage of MPP. It was concluded that while both OCT1 and ICP55 were probably acquired early on in the evolution of mitochondria, their substrate profiles and cleavage sites have either remained very similar or diverged completely.

SCIENTIFIC SESSION IX: MITOCHONDRIA AND PLANT DEVELOPMENT

Chairmen:
Per Gardeström (Umeå University, Sweden), Bozena Szal (University of Warsaw, Poland)

LECTURE 42

Mitochondrial genotype determines fate of pollen development in cytoplasmic male sterile rice.

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Keywords: pollen development, CMS

A nuclear substitution sometimes causes defects of male organ development, because of an incompatibility between substituted nuclear and mitochondrial genomes of recipient cell. Such a phenomenon is called cytoplasmic male sterility (CMS) and caused by the expression of mitochondrial unique *orf* termed CMS associated gene. The function of the CMS associated gene is prevented by the presence of a particular nuclear gene, called *Rf* (*Restorer of fertility*).

We have been studying BT-, CW-, and WA-CMS in rice. In the BT-, CW and WA-CMS line, the mitochondrial *orf79*, *orf307* and *WAf352* respectively, are known as CMS associated gene. We have cloned *Rf1* for BT-CMS, *Rf17* for CW-CMS and *Rf4* for WA-CMS. These researches revealed that *Rf1* and *Rf4* encode pentatricopeptide repeat protein and *Rf17* encodes an acyl-carrier protein.

Recently we obtained new type CMS rice, RT98- and RT102-CMS developed from wild rice. To reveal the CMS associated gene of RT98- and RT102-CMS, we determined the whole sequence of both type of mitochondria. We found *orf113* and *orf352* respectively, as CMS associated gene. To reveal the fertility restorer gene for RT98- and RT102-CMS, we are now performing map-based cloning of these genes.

Phenotype comparison of mature pollen among these five CMS lines revealed that the mitochondrial genotype possibly determines the timings of pollen abortion.

LECTURE 43

Cytochrome c connects carbon metabolism with plant growth and developmental transitions.

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Keywords: cytc mutants, Arabidopsis, flowering time, development, metabolism, GA

We studied mutants in the two genes encoding cytochrome c (CYTc) in Arabidopsis. Knock down-double cytc mutants have shorter roots, decreased biomass and show an increase in plastochron length. Developmental transitions to flowering and senescence are also delayed. Treatment of mutants with gibberellins (GA) suggests that some of these characteristics may be due to GA deficiency. Mutant rosettes also exhibit alterations in carbohydrate metabolism, with higher levels of starch and soluble sugars compared to those of wild-type plants during the diurnal cycle. Remodeling of plant metabolism and development is also supported by the observed changes in gene expression. Several genes related to sugar metabolism, photosynthesis, flowering and the circadian clock show altered expression in cytc mutant plants. We postulate that CYTc interconnects energy metabolism with plant growth and developmental responses and that its deficiency affects the balance between carbon storage and utilization.

LECTURE 44

Reactivation of mitochondrial bioenergetics and dynamics during germination of Arabidopsis thaliana seed

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Keywords: seed, germination, mitochondrial dynamics

Mitochondria are dynamic organelles in shoots and roots. Mitochondria move on F-actin using myosin motors, but movement can also result from remodelling of the actin cytoskeleton. This dynamism of mitochondria underpins their function and maintenance. For example, mitochondrial quality control underpins cell health and requires inter-mitochondrial fusion due to the uneven distribution of mtDNA within physically discrete mitochondria. Mitochondria are inactive in dry seed but must reactivate rapidly upon imbibition to provide ATP for germination. This reactivation requires not only a reactivation of mitochondrial metabolism but also of mitochondrial dynamics. Using a bioimaging approach we investigated reactivation using Arabidopsis as a model. Bioenergetic reactivation, visualised as the presence of a membrane potential, is almost immediate upon rehydration. However, reactivation of mitochondrial motility only occurs after transfer to optimal germination conditions. This late reactivation of mitochondrial motility appears specific: the actin cytoskeleton is present and dynamic early during imbibition as are other organelles that move in actin (e.g. ER). Reactivation of mitochondrial bioenergetics and dynamics is followed by dramatic reorganisation of the chondriome due to transient massive fusion, to form a perinuclear reticulum, followed by division. These data will be presented alongside results testing our hypothesis that the delay in activating mitochondrial motility is associated with the activation of mitochondrial quality control mechanisms to repair mtDNA damage incurred during maturation drying and imbibition.

LECTURE 45

Identification of the unique alternative oxidase (ClAOX) in watermelon (Citrullus lanatus)

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Keywords: alternative oxidase, abiotic stress, watermelon

Alternative oxidase (AOX) is encoded by a small gene family divided into 2 subfamilies (*AOX1* and *AOX2*) in plant. Here, we identified one *AOX* (*ClAOX*) based on genome blast and degenerated RT-PCR amplification in watermelon, which belongs to *AOX2* subfamily. *ClAOX* can be induced by different abiotic stresses, like cold, salt, osmotic and ABA in watermelon. To clarify the abiotic stress responsive role of *ClAOX*, we checked the phenotypes under cold, salt, osmotic and ABA in WT, *aox1a* knock-out and *ClAOX* complement to *aox1a* (35S::ClAOX$^{(K/N)}$-aox1a) of *Arabidopsis*. *ClAOX*^{K}-*aox1a* plants show much more tolerance capacity to cold, osmotic, ABA and salt stresses than *aox1a* mutant and *ClAOX*-*aox1a*^{N}plants. These abiotic stress treatments suggested the complementary role of *ClAOX*^{K} and non-complementary role of *ClAOX*^{N} to *AOX1*. Tissue specific expression analysis use qRT-PCR found that *ClAOX* has different expression levels in watermelon leaf, root, stem, flowers, fruit and *ClAOX*-prompter-GUS analysis showed the expression of *ClAOX* is also associated with developmental changing suggesting the *AOX2* characters. These results indicate that the unique *ClAOX* may possess dual functions of *AOX1* and *AOX2* in watermelon. Moreover, 5 members of *AOX* were isolated in *Cucurbita moshata*, of which all 5 *AOXs* were classed into *AOX2* subfamily. Likewise, only one *AOX* that was also belonged to *AOX2* subfamily was identified in *Cucumis sativus* and *Cucumis melo* genome. The distinctive *AOX* members found in several genus of *Cucurbitaceae* family may suggest the evolutionary diversification and taxonomic distribution for AOX family in planta.

SCIENTIFIC SESSION X: MITOCHONDRIAL BIOCHEMISTRY AND METABOLISM

Chairmen:
Jan A. Miernyk (University of Missouri, USA), David Day (Flinders University, Australia)

LECTURE 46

A smelly business: Persulfide metabolism and trafficking in mitochondria

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Keywords: Cofactor, iron-sulfur, ABC transporter, cysteine desulfurase, glutathione

A smelly business: Persulfide metabolism and trafficking in mitochondria

Mitochondria and plastids contain a cysteine desulfurase that provides sulfur for several essential cofactors, including iron-sulfur (FeS) clusters. The enzyme cleaves cysteine into alanine and sulfane sulfur (S^0, valency is zero), also called persulfide. Interestingly, the mitochondrial desulfurase is also required for FeS clusters in the cytosol and nucleus. It was proposed by Lill (2009, Nature 460, 831-8) that a sulfur conjugate, X-S, is exported to the cytosol by an ATP-binding cassette Transporter of the Mitochondria (ATM). Using a combination of in-vitro and in-vivo approaches, we have found that ATM3 (ABCB25) from Arabidopsis and Atm1 from yeast can transporting oxidized gluthathione (GSSG) and persulfide in the form of GS-S-SG (Schaedler *et al* 2014, J Biol Chem 289: 23264-74). Although we could not detect GS-S-SG in isolated mitochondria from *atm3* mutants, we found a genetic interaction between *atm3* and mutant alleles of *ETHE1*. This single copy gene encodes a sulfur dioxygenase that uses GS-SH as substrate to produce sulfite. ETHE1 is located in the mitochondrial matrix and plays a key role in the catabolism of amino acids such as cysteine (Krüssel *et al* 2014, Plant Physiol 165: 92-104). While these studies provide a first glimpse of persulfide metabolism in plant mitochondria, many steps remain to be identified.

*in collaboration with TA Schaedler, I Kruse, JD Thornton, L Browning, HW van Veen, M Schwarzländer, AJ Meyer, T Hildebrandt and others.

LECTURE 47

Manipulation of cellular metabolism by stabilizing an enzyme-enzyme interaction

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Keywords: tricarboxylic acid cycle, metabolon, citrate synthase, aconitase

Enzymes of the tricarboxylic acid (TCA) cycle are known to form hetero-enzyme complexes in mammals, yeast and bacteria. Especially when the enzymes catalyzing sequential reactions interact and metabolite channeling takes place, a protein complex is called as “metabolon” and considered as an important component of metabolic regulation. Recently we demonstrated that plant TCA cycle enzymes also form metabolon in mitochondria (presentation by Zhang Y). In the present study we tried to stabilize an interaction between mitochondrial citrate synthase (CSY) and aconitase (ACO) and investigated the effect on cellular metabolism. CSY and ACO proteins were fused with split-Venus protein at the C-terminus (CSY-VC and ACO-VN, V/V pair) to stabilize the protein complex by re-constructed Venus protein. The CSY-GFP fusion protein was used instead of CSY-VC as negative control (G/V pair). These two pairs of proteins were expressed in Arabidopsis PSB-D cell culture under the control of 35S promoter. Both cell lines accumulated recombinant enzymes at the similar levels. The levels of most amino acids were decreased in the V/V line although those of the TCA cycle intermediates were unchanged in comparison to G/V line. This indicates the impact of stabilization of metabolon to cellular metabolism. The in vitro evidence of complex stabilization and enzyme kinetics measurement would also be presented.

LECTURE 48

Plants Possess a Mitochondrial Metabolic Repair Cycle Involving Malate Dehydrogenase and L-2hydroxyglutarate Dehydrogenase

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Keywords: L-2hydroxyglutarate; L-2hydroxyglutarate dehydrogenase; malate dehydrogenase; metabolite repair

Introduction

Enzymatic side-reactions can give rise to wasteful/toxic products that are removed by metabolite repair pathways. Recently, we showed that L-2hydroxyglutarate (L-2HG) is found at low levels in Arabidopsis leaves. Here, we investigated if plants possess a mitochondrial damage-control system involving L-2HG similar to mammals.

Material and methods

Arabidopsis recombinant mitochondrial malate dehydrogenase 1 (mMDH1), mMDH2 and L-2hydroxyglutarate dehydrogenase (L-2HGDH) were produced and the enzymatic properties were analysed. T-DNA insertion lines of L-2HGDH and the electron transfer protein (ETF) were characterized at the molecular and physiological level.

Results

The kinetic properties of the mMDH isoforms indicated that they produce L-2HG from 2-ketoglutarate (2-KG) in side reactions. We identified Arabidopsis L-2HGDH as a mitochondrial FAD-containing oxidase that converts L-2HG back to 2-KG. We showed that the electrons produced in the L-2HGDH reaction are transferred to the mitochondrial electron transport chain through the ETF. Accumulation of L-2HG in plants does not adversely affect their development under a range of tested conditions.

Conclusion

Plant mitochondria possess the biochemical components of an L-2HG metabolic repair system identical to that found in mammals. While deficiencies in the metabolism of L-2HG result in fatal disorders in mammals, plants are not severely affected. However, orthologs of L-2HGDH are found in all examined genomes of *viridiplantae*, indicating that the repair reaction we identified makes an essential contribution to plant fitness in as yet unidentified conditions in the wild.

LECTURE 49

Integration of amino acid catabolic pathways at the sulfur dioxygenase ETHE1

Tatjana Hildebrandt, Hans-Peter Braun
Plant Proteomics, Leibniz University Hannover, Germany

Keywords: carbohydrate starvation, amino acid degradation, sulfur catabolism

In situations of decreased photosynthesis such as drought or extended darkness carbohydrate supply becomes limiting and plants degrade cellular proteins as an alternative energy source. The role of branched-chain amino acids and lysine as substrates for ATP production has already been well established. These amino acids are oxidized in the mitochondria and transfer electrons into the respiratory chain via the ETF/ETFQO complex. We present an additional mitochondrial pathway that is physiologically relevant under carbohydrate starvation conditions. The sulfur dioxygenase ETHE1 together with a sulfurtransferase catalyzes the oxidation of persulfides derived from cysteine catabolism to thiosulfate while the carbon skeleton is converted to pyruvate. An Arabidopsis knockdown line with 99 % decreased sulfur dioxygenase activity shows a severe starvation phenotype under short day growth conditions and is more sensitive to extended darkness than the wildtype. Metabolite profiles in combination with biochemical assays reveal that ETHE1 in addition to its role in sulfur catabolism is required for efficient oxidation of branched-chain amino acids and lysine. In fact, toxic intermediates of leucine rather than cysteine catabolism accumulate in the mutant. Proteome analysis indicates that ETHE1 deficiency leads to major changes in amino acid metabolism and plants adapt by increasing alternative pathways of energy generation such as the degradation of lipids and cell wall constituents.

LECTURE 50

Glutaredoxin S15 is involved in Fe-S biogenesis in mitochondria influencing lipoic acid-dependent enzymes, plant growth and arsenic tolerance in Arabidopsis

Elke Stroeher, A. Harvey Millar
ARC CoE Plant Energy Biology, University of Western Australia

Keywords: Glutaredoxin, Fe-S cluster, arsenic, lipoic acid

Glutaredoxins (Grxs) are small proteins that function as oxidoreductases with roles in deglutathionylation of proteins, reduction of antioxidants and assembly of Fe-S cluster-containing enzymes. Which of the 33 Grxs in Arabidopsis perform roles in Fe-S assembly in mitochondria is unknown. We have examined in detail the function of the monothiol GrxS15 in plants. Our results show its exclusive mitochondrial localisation and we conclude it is the major or only Grx in this subcellular location. It had a very low deglutathionylation and dehydroascorbate reductase activity, but it bound a Fe-S cluster. Its involvement in Fe-S dependent processes in mitochondria was found to be especially important for lipoic acid-dependent enzymes. Partially removing GrxS15 from mitochondria slowed whole plant growth and respiration. The enhanced effect of the toxin arsenic on the growth of GrxS15 knockdown plants compared to wildtype, highlight the role of mitochondrial glutaredoxin Fe-S binding in whole plant growth and toxin tolerance.

SCIENTIFIC SESSION XI:
OMICS APPROACHES IN MITOCHONDRIAL BIOLOGY

Chairmen:
David Macherel (Angers University, France), Michał Rurek (Adam Mickiewicz University in Poznań, Poland)

LECTURE 51

Protein turnover by progressive 15N labelling for analysis of plant mitochondrial biogenesis and maintenance

Harvey Millar, Clark Nelson, Lei Li, Shaobai Huang
University of Western Australia

Keywords: proteomics, mass spectrometry, protein turnover, proteases, ETC assembly

Intro: Analysing protein synthesis and degradation rates with progressive stable isotope labelling provides an interesting new window on the control of protein abundance. With this approach we can determine the 'relative age' of the proteins that we see and define the energetic effort employed by the cell to build or maintain particular activities.

Materials and Methods: We are using progressive ^{15}N labelling of plant cells from nitrate and ammonium salts and modelling incorporation fits, to calculate the rate at which mitochondrial proteins which are often static in abundance in the proteome, turning over as tissues develop and mature. We have developed pipelines to undertake these studies in plant cells, plant leaves and in whole plants through the use of hydroponics.

Results: Through combining such labelling with separation of protein complex and subcomplexes by native electrophoresis, we can observe the *in vivo* turnover rate of assembly intermediates of protein complexes in plant mitochondria. We can use turnover rate coupled to absolute abundance and amino acid length to measure the energetic cost of mitochondria maintaining different aspects of their protein machinery. We are also using this approach to study the role of mitochondrial proteases in mitochondrial maintenance and show that removal of a single protease can dramatically affect the in vivo turnover rate of a variety of mitochondrial proteins.

Conclusions: Omics rocks. These new tools allow peptide mass spectrometry to propose answers to a number of key questions in plant mitochondrial biology.

LECTURE 52

Mitochondria connections in plant meiosis

Stefanie Dukowic-Schulze, Anthony Harris, Changbin Chen
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Keywords: meiosis, RNA-seq, transcriptome

Meiosis and meiotic recombination are crucial for propagation and seed setting of most plants. Our research aims at elucidating the meiotic transcriptome and its regulatory mechanisms, and also at deciphering which factors govern the recombination landscape. To do so, we couple next-generation sequencing technology and a special microcapillary collection technique for isolating male meiocytes (the cells undergoing meiosis) from Arabidopsis and maize.

RNA-seq results indicated highly up-regulated mitochondrial genes in meiosis, which was at first attributed to mitochondrial genome insertions in the nuclear genome but could be shown to derive from mitochondria themselves. Although detectable in other datasets, this observation is usually neglected or cautiously set aside. This, together with other indicators pointing to the importance of mitochondria in meiosis, led us to study the connection of meiosis and mitochondria further.

The investigations we have been working on include:

1) Characterization of the pathways in which the up-regulated mitochondria genes are involved in.

2) Cytological examination of mitochondria during meiosis.

3) Meiotic defects in chromosome behavior in a CMS line.

Coming from the plant meiosis research area, we happily take this conference as an opportunity to connect with the plant mitochondria community and to gain more knowledge and insight into future research directions and collaborations.

LECTURE 53

Gene expression in ever changing mitochondrial genomes

Helena Storchova[1], James D Stone[1,2], Pavla Kolouškova[1]

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Keywords: Transcriptome, Mitochondria, Gender, Silene vulgaris

Gene expression in ever changing mitochondrial genomes

Silene vulgaris has been used as a model for the study of gynodioecy for decades. Several CMS candidates were found in 5 completely sequenced mitochondrial (mt) genomes of this species (Sloan et al 2012). The genomes were highly rearranged, differed in intergenic regions and gene content. An extraordinary variation in DNA sequence affected promoters, which resulted in a high variation of atp1 transcripts (Muller and Storchova 2013; Elansary et al 2010). This variation raises the question about how are the diverse motifs recognized and interpreted by nuclear factors. S. vulgaris is an ideal model for the study of cyto-nuclear interactions in plant mitochondria.

We adopted RNA-seq method to study transcribed regions and editing events across an entire mt genome (Stone and Storchova 2014). We chose Kov haplotype of S. vulgaris with no predicted CMS candidate gene to compare female and hermaphrodite transcriptomes. We detected several regions differentially expressed between females and hermaphrodites and no differences in editing between the genders. Only a few “islands” of significant transcription, not corresponding to the predicted ORFs, were discovered outside the annotated genes. The absence of stop codons was a common feature of mt transcripts. The analysis of cytoplasmic portions of the same transcriptomes detected a signature of a programmed cell death in females.

LECTURE 54

Mitochondria and Plastids in Moss: From Quantitative Proteomics to Dynamics

Stefanie Mueller, Daniel Lang, Sebastian Hoernstein, Ralf Reski
Plant Biotechnology, Albert-Ludwigs-Universität Freiburg, Germany

Keywords: metabolic labelling, Physcomitrella patens, organellar interfaces

Each eukaryotic subcellular compartment constitutes a specific context for biochemical reactions in terms of protein composition, substrate concentration and connectivity to other organelles. Moreover, during evolution organellar proteomes are modified by gene gain and loss, by gene subfunctionalisation and neofunctionalisation, and by changes in protein targeting.

We used metabolic labelling in the model moss *Physcomitrella patens* to quantify the plastid and mitochondrial proteomes, to map metabolic pathways, and to study the effect of post-endosymbiotic evolution on organellar pathway partitioning.

Using single protein tagging, several proteins with conspicuous proteomics data showed a dual localisation, or were present at organelle interfaces, including contact sites between mitochondria and the ER. As interorganellar contact sites might impact mitochondria biogenesis, metabolism and dynamics, these proteins are interesting candidates for novel pathways at organelle interfaces. The specific subcellular localisation and role of these proteins in organelle dynamics is being investigated using functional analysis of protein domains, targeted knock-in of fluorescent reporters into the moss genome, as well as targeted knock-out to reveal gene function by reverse genetics.

LECTURE 55

A Deep-Learning Method for Predicting Mitochondria-Localized Proteins in Plants

Ning Zhang[1], R. Shyama Prasad Rao[2,3], Fernanda Salvato[2,4], Jesper F. Havelund[5], Ian Max Møller[5], Jay J. Thelen[2], Dong Xu[6]

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Keywords: deep learning, subcellular localization, mitochondira

Targeting and translocation of proteins to the appropriate subcellular compartments is crucial for cell organization and function. Newly synthesized proteins are transported to mitochondria with the assistance of targeting sequences, which are complex, containing either an N-terminal presequence or a multitude of internal signals to target this organelle. Compared with experimental approaches, computational predictions provide an efficient and cost-effective way to infer subcellular localization for any given protein. However, it is still challenging to predict plant mitochondrial localized proteins accurately due to various limitations, and the performance of current tools is unsatisfactory. We present a novel computational approach for large-scale prediction of plant mitochondrial proteins. We collected protein subcellular localization data in plants from databases and literature, and extracted different types of features from the training data, including amino acid composition, protein sequence profile, and gene co-expression information. We then trained deep neural networks for predicting plant mitochondrial proteins. Testing on a non-redundant dataset of potato mitochondrial and Swiss-Prot proteins, our method achieves considerable improvements over existing tools in predicting mitochondria-localized proteins in plants.

SCIENTIFIC SESSION XII: BREAKTHROUGH TECHNOLOGIES

Chairmen:
Philippe Giegé (IBMP-CNRS, France)

LECTURE 56

Can plant research provide insight into Alzheimer's disease?

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Keywords: Targeting peptides, protease, peptidolysis, Alzheimer´s disease

Can plant research provide insight into Alzheimer´s disease?

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The majority of mitochondrial and chloroplastic proteins are synthesized in the cytosol as precursor proteins containing N-terminal targeting peptides, TPs, which are cleaved off inside the organelle after completed import. TPs have potential toxic effects as they dissipate membrane potential and therefore need to be degraded. Recent work elucidated a pathway involved in the degradation of organellar TPs, with two proteolytic components: the Presequence Protease, PreP, and the Organellar Oligopeptidase, OOP. Thorough characterization of the two proteases showed that PreP and OOP are specialized in degrading peptides of different lengths, with the substrate restriction being dictated by the structure of their proteolytic cavities. Elimination of both oligopeptidases severely affects growth and development of Arabidopsis. Both PreP and OOP have human homologues, hPreP and neurolysin, respectively. hPreP was shown to degrade not only TPs but also amyloid-beta peptide, Ab, associated with Alzheimer's disease. Neurolysin, was initially identified as a peptidase involved in degrading the neuropeptide neurotensin, however, we found that it not only complements hPreP in the degradation of TPs, but also in degradation of Ab through a specific cleavage profile. Furthermore, our studies suggest that mitochondria-associated endoplasmic reticulum membranes, MAM, constitute a potential site of Aβ production, from which Aβ is transported into mitochondria.

LECTURE 57

Potential uses of synthetic RNA binding proteins

Kate Shearston[1], Ben Gully[2], Peter Kindgren[3], Aaron Yap[3], Catherine Colas des Francs-Small[3], Joanna Melonek[3], Charles Bond[2], Ian Small[3]

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Keywords: PPR proteins, synthetic RNA binding proteins

Hundreds of RNA binding proteins participate in the complex post-transcriptional processing that prepare plant mitochondrial RNA for translation. They act in determining transcript termini, splicing, editing, endonucleolytic cleavage and promoting or blocking translation. Most show a high degree of sequence specificity in their binding preferences and generally the physiological role of the protein is entirely determined by which target RNA it recognises and where on that target it binds. Large families of organellar RNA binding proteins (for example, PPR and mTERF families) share several interesting features from a synthetic biology viewpoint. They are modular, composed of arrays of repeated motifs each of which probably contacts a single nucleotide. We have shown that the largest of these families (the pentatricopeptide repeat or PPR family) recognizes single-stranded RNA sequence-specifically via a simple two-amino-acid code, with each combination preferring either A, C, G or U. This has made binding sites of the natural proteins predictable from amino acid sequence alone, and the protein sequence can be altered to change RNA binding specificity in vitro and in vivo. Synthetic RNA binding proteins with predictable properties can now be constructed to target almost any RNA. Potential applications of these synthetic RNA processing tools to engineer mitochondrial gene expression will be discussed.

LECTURE 58

Redesigning PPR proteins to block the expression of selected organelle genes

Catherine Colas des Francs-Small, Ian Small
ARC CoE Plant Energy Biology, University of Western Australia

Keywords: Organelle gene expression, PPR proteins, complex I

PPR proteins belong to a large family of RNA-binding proteins found in all eukaryotes, but particularly prevalent in plants. They play key roles in plant development and crop breeding, and can be associated with mitochondrial disorders in humans. PPR proteins are essential for the expression of genes required for the construction and function of the major protein complexes involved in photosynthesis and respiration. They are thus vital during germination and early seedling development and some are absolutely required for autotrophic growth.

The recent discovery of a 'code' describing how PPR proteins recognize their target RNAs (Barkan et al., 2012) is a major breakthrough as it allows us to predict binding sites and construct custom-designed proteins to bind desired targets. To test this, we are working with PPR proteins related to fertility restorer factors that are known to block expression of specific mitochondrial transcripts. We have modified some of these PPR proteins to bind the coding sequence of selected mitochondrial and plastid transcripts. These new PPR proteins are being tested for their effect on organelle gene expression. Preliminary results show that this approach works as we have generated plants devoid of assembled respiratory complex I by targeting the mitochondrial nad6 transcript. This system has potential for many biotechnological applications in plant breeding and medicine.

POSTER ABSTRACTS

LIST OF POSTER SESSIONS

SESSION: MITOCHONDRIAL GENOME DYNAMICS

POSTER 1

Loss of a RadA/Sms-like protein results in instability of the mitochondrial genome by recombination

Julien Schmitz, Cédric Nadiras, Monique Le Ret, André Dietrich, José Gualberto
IBMP-CNRS

Keywords: Recombination, repeated sequences, stoichiometric shifting, genome evolution

Introduction

The plasticity of plant organellar genomes is due to recombination processes that modulate their structure. Several proteins have already been identified as implicated in homologous recombination (HR) dependent pathways, but the interplay of these factors and their roles in mtDNA maintenance and segregation is not well known.

Results

We have identified a homolog of bacterial RadA/Sms, conserved in all plants, that we named RADA. In bacteria RadA influences recombination and recombinational repair processes, most likely involving the stabilization or processing of branched DNA molecules, or the recruitment of DNA polymerases. It is described as a possible RecA-paralog, but its biochemical function is unknown.

GFP gene fusion showed that Arabidopsis RADA is dually targeted to mitochondria and plastids. T-DNA insertion *radA* plants were all affected by phenotypes of dwarfism, slow growth, variegation and very little fertility. We showed that they are apparently the result of increased ectopic recombination involving medium size repeats, which correlates with the non-stoichiometric replication of mtDNA subgenomes generated by recombination. However, no effects of the *radA* mutation were observed on the chloroplast genome.

A recombinant RADA protein was expressed, and its affinity to different DNA structures was studied.

Conclusion

Our results show that RADA is an additional factor that modulates HR processes controlling the maintenance and segregation of the plant mtDNA.

POSTER 2

Tracing of forest species with DNA markers as a support in the combat with illegal logging in Poland

J.A. Nowakowska[1], A. Tereba[1], T. Oszako[1], T. Pasternak[2], M. Borys[1], J. Bieniek[1], M. Klisz[1], A. Konecka[1]

1. Forest Research Institute; 2. General Directorate of State Forest

Keywords: DNA genotyping, forensic science, deciduoues trees

Introduction

DNA analysis are widely use in forensic and medicine research. Doubtful origin of samples can be unambiguously determined on the basis of DNA profiles. Recently the molecular analysis have become a tool in the combat against illegal logging and can support District Courts and the Police.

Materials and Methods

The most frequent cases concern on: oak, European beech, black alder, silver birch, European hornbeam, common ash.

Basis of molecular identification methods is to determine the genotypes of analyzed samples and compare them i.e. the evidence (wood fragments originating from stolen timber) and the comparative material (stumps remained in the forest stand). Briefly, the procedures used in the comparative analysis are based on the extraction of DNA, amplification at least four nuclear loci and one cytoplasmic loci and genotyping in automatic sequencer. Finally, obtained genetic profiles are compared taking into account the probability of identity settled for the chosen markers.

Results and Conclusion

Presented results of molecular analyzes have successfully allowed the identification of genetic profiles in all investigated forest tree species with high 98-99% of probability. The method based on DNA "fingerprints" can be used for diagnoses of individual sample, but power of discrimination of used markers must be taken into account. These data successfully contributed to forensic investigations carried out in many cases of illegal logging in Poland. Molecular methods for identification of wood samples provide a new evidence in controversial issues concerning by the Forest Guard Service in Poland.

POSTER 3

Species and substrate specificity of DNA import into plant mitochondria

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Keywords: DNA import, ADP/ATP carrier, voltage-dependent anion channel

Import of nucleic acids into mitochondria is a firmly established process in eukaryotic cells but little is known on the involved membrane translocation mechanism(s). Using DNA as a substrate for in vitro uptake assays, we show that there are definite differences in the translocation process into isolated mitochondria for DNA of various lengths and structures. Addition of the terminal inverted repeats from the Brassica napus 11.6 kb linear plasmid to large (> 9 kb) DNA fragments leads to greater import efficiency. The uptake of large DNA shows similarities with that of DNA with medium size (0.7-3 kb) and involves the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator (ANT). DNA fragments of small (0.1-0.3 kb) and medium (0.7-3 kb) size are imported into mitochondria apparently by partly overlapping but independent mechanisms. The interrelationships of DNA with a small or medium size are non-competitive in the import. Transport of small size DNA is little affected by ANT and VDAC inhibitors, but is sensitive to inhibitors of the ADNT1 transporter or of the phosphate carrier, as well as to the presence of tRNA. We propose a model of transport into plant mitochondria based on the idea of alternative mechanisms for the import of DNA with different lengths and structures. Funded by RFBR 15-04-05046.

POSTER 4

Identification of differentially expressed genes in cucumber MSC lines possessing complex mitochondrial DNA rearrangements

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Keywords: Cucumis sativus, mitochondrial mutants, mtDNA rearrangements, RNA-seq, differentially expressed genes

Cucumber is a plant species characterized by a large, paternally transmitted mitochondrial genome that accumulates short repetitive DNA. Passage of highly homozygous cucumber line B through cell cultures induces complex rearrangements in mitochondrial DNA. Certain types of mtDNA rearrangements are associated with a mosaic (MSC) phenotype that is characterized by slower growth, chlorotic mosaic on the leaves and fruit, and lower fertility. Several cucumber MSC lines possessing different mtDNA rearrangements and mitochondrial genes expression profiles have been developed. We used an Illumina platform and RNA-Seq approach to investigate transcriptomic profiles of three MSC lines (3, 12, and 16) in comparison to wild type line B. Total RNA was isolated from whole shoots of 2-week old, phytotron-grown plants. cDNA libraries were synthesized and sequenced using HiSeq2000. Analysis of the reads using TopHat2 and Trinity based bioinformatics pipeline allowed to identify a set of differentially expressed genes (DEGs) shared by all three MSC lines (minimum 4-fold expression change, $p<0.01$). Based on Gene Ontology and functional annotation DEGs were classified in several groups related to the biological processes including: response to stimulus (HSP23, SAP12, NUDIX1), metabolic processes (NDA1-2, FTSH-like), cellular processes (Ntn-hydrolases, GST), and regulation of transcription (NAC, CRF6, Bhlh92). Altered expression profiles of DEGs was confirmed by Real-Time qPCR. This study provides insights into mitochondria to nucleus interplay and shows that cucumber is an interesting model to study this interactions.

POSTER 5

Mitochondrial DNA variability in Polish Pinus sylvestris (L.) forest stands

Justyna A. Nowakowska[1], Ewa Aniśko[2], Małgorzata Borys[1], Jolanta Bieniek[1], Władysław Kantorowicz[2], Marcin Klisz[2], Jan Kowalczyk[2], Jan Matras[2], Paweł Przybylski[2], Iwona Szyp-Borowska[2], Małgorzata Sułkowska[2], Krystyna Szczygieł[2], Anna Zawadzka[3]

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Keywords: STS markers, mitochondrial gene, genetic variation

Introduction: Scots pine (Pinus sylvestris L.) is one of the most important coniferous species in Poland and its origin from postglacial refugia is practically unknown. The aim of this study was to identify the polymorphism of mtDNA fragments with the mitochondrial Sequence-Tagged-Site (STS) markers among 90 native Scots pine populations in Poland.

Material & Methods: Two specific pairs of STS primers amplifying the B/C intron of NADH dehydrogenase mitochondrial nad1 and nad7 genes were used to perform the genetic diversity analysis. The amplified fragments were analyzed via chip-electrophoresis and GenePop software. Genetic similarity analyses were conducted using PopGene v. 3.2, MEGA v. 4.0, and MCMC simulations using BAPS 2.0 software.

Results: Our studies of mtDNA genetic diversity among studied P. sylvestris stands in Poland indicated that 2 variants of haplotypes, i.e. “a”- 220 bp and “b”- 254 bp in locus nad1 were present, and two variants of haplotypes, “c” and “d”, composed by 3 different alleles, i.e. 320, 368 and 425 bp for “c”; and 317, 421 and 439 bp for “d” were distinguished in locus nad7.

Conclusions: The overall mitotype variability among all studied Scots pine provenances in Poland was low (H_T = 0.246, F_{ST} = 0.010). The distribution of mitochondrial gene frequencies and low fixation-index (F_{ST}) value obtained for Scots pine stands in Poland comparing to those obtained in Spain and Scotland, may result from: i) different gene pools migration routes deriving from South-European and Central Russia glacial refugia, ii) high gene flow within the stands, and iii) transfer of seed-material in the past.

POSTER 6

RECG Maintains Mitochondrial and Plastid Genome Stability by Suppressing Extensive Recombination between Short Dispersed Repeats

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Keywords: recombination, genome rearrangements, moss, plastid

Maintenance of mitochondrial and plastid genome stability is crucial for respiration and photosynthesis, respectively. Recently, we have reported that RECA1 maintains mitochondrial genome stability by suppressing gross rearrangements induced by aberrant recombination between short dispersed repeats in the moss *Physcomitrella patens*. In this study, we studied a newly identified *P. patens* homolog of bacterial RecG helicase, RECG, some of which is localized in both plastid and mitochondrial nucleoids. *RECG* partially complements *recG* deficiency in *Escherichia coli* cells. A knockout (KO) mutation of *RECG* caused characteristic phenotypes including growth delay and developmental and mitochondrial defects, which are similar to those of the *RECA1* KO mutant. Analyses of *RECG* KO plants showed that mitochondrial genome was destabilized due to a recombination between 8–79 bp repeats and the pattern of the recombination partly differed from that observed in the *RECA1* KO mutants. Some of the induced recombination caused efficient genomic rearrangements in *RECG* KO mitochondria. Such loci were sometimes associated with a decrease in the levels of normal mitochondrial DNA and significant decrease in the number of transcripts derived from the loci. In addition, the *RECG* KO mutation caused remarkable plastid abnormalities and induced recombination between short repeats (12–63 bp) in the plastid DNA. These results suggest that RECG plays a role in the maintenance of both plastid and mitochondrial genome stability by suppressing aberrant recombination between dispersed short repeats; this role is crucial for plastid and mitochondrial functions.

POSTER 7

Are mitochondrial non-coding regions good markers for phylogenetic analyses in rye species?

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Keywords: Secale, phylogenesis, non-coding regions

Understanding genetic variation among species of rye is important for the process of breeding new cultivars. Many of the traits associated with resistance to adverse soil and climatic conditions, diseases and pests are found in wild rye forms. Determination of the phylogenetic relationships among taxa of wild and cultivated species will facilitate transfer of the genes encoding these traits to the cultivated species.

The present study examined sequence variability in internal transcribed spacer (ITS) of mitochondrial DNA among rye species. These regions are useful molecular markers in phylogenetic studies of closely related species, at the interspecific level, too. Two pairs of universal primers were used in this study for the amplification of ITS: *rps12*/*nad3* and *rrn*5/*rrn*18. The intergenic region *rps12*/*nad3* amplifies the two genes *nad3* and *rps12*, and the intergenic region between them. The intergenic sequences of ribosomal region *rrn*5/*rrn*18 amplifies the intergenic region only.

The sequences obtained from 42 *Secale* accessions, which included both cultivated and non-cultivated rye and which represented four species and seven subspecies of the genus *Secale*, showed a low level of polymorphism. The results showed that the investigated regions of mitochondrial genome are conservative in he tested taxa and can't be used as a molecular markers in phylogenetic studies of *Secale* species.

This study was supported by the State Committee for Scientific Research grant No. N N310 435498 "Degree of Relatedness Within the Genus *Secale* Using non-coding Chloroplast and Mitochondrial Sequences, and Nuclear rDNA IGS Regions".

POSTER 8

Tracing of forest tree species with DNA markers as a support in the combat with illegal logging in Poland

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Keywords: DNA genotyping, forensic sciences, deciduous trees

Introduction

DNA analysis are widely use in forensic and medicine research. Doubtful origin of samples can be unambiguously determined on the basis of DNA profiles. Recently the molecular analysis have become a tool in the combat against illegal logging and can support District Courts and the Police.

Materials and Methods

The most frequent cases concern on: oak, European beech, black alder, silver birch, European hornbeam, common ash.

Basis of molecular identification methods is to determine the genotypes of analyzed samples and compare them i.e. the evidence (wood fragments originating from stolen timber) and the comparative material (stumps remained in the forest stand). Briefly, the procedures used in the comparative analysis are based on the extraction of DNA, amplification at least four nuclear loci and one cytoplasmic loci and genotyping in automatic sequencer. Finally, obtained genetic profiles are compared taking into account the probability of identity settled for the chosen markers.

Results and Conclusion

Presented results of molecular analyzes have successfully allowed the identification of genetic profiles in all investigated forest tree species with high 98-99% of probability. The method based on DNA "fingerprints" can be used for diagnoses of individual sample, but power of discrimination of used markers must be taken into account. These data successfully contributed to forensic investigations carried out in many cases of illegal logging in Poland. Molecular methods for identification of wood samples provide a new evidence in controversial issues concerning by the Forest Guard Service in Poland.

POSTER 9

A new configuration of mitochondrial genome found in CMS onion

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Keywords: onion, NGS, master circle, CMS

Onion (Allium cepa) is one of the popular vegetables grown worldwide, belonging to the family Alliaceae. In onion, several types of cytoplasm have been found and some are known to cause cytoplasmic male-sterility (CMS). CMS has been traditionally used in onion breeding programs to produce commercial hybrid varieties. Previous studies showed that orf725 is a candidate mitochondrial (mt) gene for onion's CMS. However, a complete nucleotide sequence of any mt genomes has not been determined, and other orfs have not been characterized. Here, we report the data for mt genome of CMS onion (A. cepa cv. Momiji-3). Mitochondria were isolated from onion bulbs and mtDNA was purified by CsCl/EtBr centrifugation. MtDNA was subjected to 454 pyrosequencing. A large number of reads (87,611) were assembled into 22 contigs, and ten of them were selected to have mtDNA sequences (distinguished by read depth). Linkage study among the ten contigs yielded two separate circles, MC1 (173,132bp in size) and MC2 (143,157bp). The sizes of two circles are similar to each other, and 48 protein-coding genes are located in either of two circles. Considering the size and gene contents, it is quite unlikely that both circles are categorized as extrachromosomal plasmids that are occasionally found in angiosperm mitochondria (e. g. those in sugar beet). Although two circles share sequences in common (the largest one is 451bp), there is no evidence that homologous recombination occurs with this sequence. Therefore Momiji-3 mitochondrial genome may consist of two master circles. Our finding shows a new configuration of mitochondrial genome in higher plants.

POSTER 10

Multipartite chloroplast genome in the transplastomic tobacco plant as a model to study homologous recombination in organelle genomes

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Keywords: multipartite structure, homologous recombination, chloroplast genome

One of the characteristics of plant mitochondrial (mt) genome is multipartite organization with a predicted master circle (MC) and sub-circles (SCs). SCs are generated by homologous recombination (HR) between large repeats in a MC. However, factor(s) that determines the recombinogenic activity of each repeat is poorly understood. Chloroplast (ct) genome also has a large number of repeats, however, HR is suppressed, except that occurred in a large inverted repeat. Therefore ct genome exists as a single circle. Since deregulation of HR results in genomic instability, the molecular mechanism underlying HR should be clarified to understand organelle genome evolution.

In our laboratory, many transplastomic tobacco plants have been constructed. Most of the transplastomics had a similar appearance to wild type, however, two transplastomic lines containing apx gene exhibited leaf variegation. Ct DNAs were isolated from variegated plants, and their genome structures were analyzed by next-generation sequencing. The results showed the variegated plants have multipartite ct genome. The genome is divided into two circles; a large- (140kb in size) and a mini-circle (22kb in size). A mini-circle is generated by HR between rps16 terminator fused to a transgene and that in a native rps16 gene. Real-time PCR showed that copy number of a mini-circle is one order of magnitude higher than that of a large-circle, suggesting that the mini-circle contains replication origin. Our study demonstrates that ct genome, like mt genome, can exist in multipartite organization. This will be a good model to study HR in organelle genomes.

POSTER 11

Increased recombination at intermediate repeats in a mitochondrial Uracil-DNA glycosylase mutant of Arabidopsis thaliana

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Keywords: DNA Repair, recombination

Plant mitochondrial genes have very low mutation rates in genes, yet expand, rearrange and mutate in non-gene regions. One possible explanation for this peculiar difference is that most DNA repair is done via double-strand break repair pathways. This leads to very accurate repair through gene conversion and homologous recombination, but also inaccurate repair through nonhomologous end-joining, break-induced replication, and ectopic recombination between intermediate-length repeated sequences. Selection removes the products of inaccurate repair when they occur in genes, but non-gene regions show evidence of these processes. Base excision repair also occurs in mitochondria, as evidenced by the function of a mitochondrially-targeted uracil-N-glycosylase (UNG) protein. A prediction of this model is that an increase in DNA damage or mismatches should also lead to an increase in double-strand break repair processes. We have used homozygous ung- mutants to allow uracil to accumulate in the DNA, and asked whether double-strand break repair increases. Quantitative PCR shows an increase in recombination at the intermediate repeats relative to wild-type Arabidopsis thaliana. This provides evidence for an increase in double-strand break repair in the ung knockout lines. We propose a model in which double-strand breaks can be initiated at mismatches and repaired by the double-strand break repair machinery using a homologous template.

POSTER 12

Neutral Mutation in Plant Mitochondrial Genes and Pseudogenes

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Keywords: neutral mutation. pseudogenes

Plant mitochondrial genes appear to have very low mutation rates, while non-gene regions expand, diverge and rearrange quickly. One possible explanation for this disparity is that mutation rates in genes are measured using the assumption that synonymous substitutions are neutral. If this assumption is not true, then selection makes the measured mutation rate appear much lower than the true neutral mutation rate. Rps14 is cotranscribed with cob and rpl5 in most plant mitochondrial genomes. In some genomes, rps14 has been duplicated to the nucleus and is a pseudogene in the mitochondria. This offers a unique opportunity to test whether synonymous substitutions represent the neutral mutation rate. Neutral substitution rates in rps14 pseudogenes and synonymous substitution rates in rps14 genes have been measured, and are not significantly different, providing evidence that synonymous substitutions in plant mitochondria are neutral. The vast differences between the mutational patterns of mitochondrial genes and non-genes appears to be due to indels and rearrangements.

POSTER 13

Comparative mitochondrial genome analysis provides insights into the mitochondrial genome evolution in Brassica

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Keywords: Brassica, mitochondrial genome evolution, mitochondrial genome rearrangement, repeated sequence

The *Brassica* family possesses many important economic species for oil, vegetable and other use, which was regularly selected to understand the mitochondrial genome structure and evolution because of their evolutionary position among angiosperm families. In this study, we newly assembled a *B. nigra* mitochondrial genome sequence with 232,407 bp in size, which is the last mitochondrial genome in ‚U'-triangle of *Brassica*. Genome rearrangement caused by repeated sequences resulted in numerous variations on gene, tRNA and unknown function ORFs contents, genome size in *capitata*-type *B. oleracea*. Principal component analysis and phylogenetic tree deduced the maternal ancestor of three allotetraploid species in ‚U'-triangle of *Brassica*. Allotetraploid *B. juncea* was synthesized from *B. rapa* as maternal ancestor. Allotetraploid *B. carinata* was derived from *B. nigra* as maternal ancestor. Diversification of mitochondrial genome donor in allotetraploid *B. napus* was observed, in which *nap*-type *B. napus* was derived from *B. oleracea* as maternal ancestor; however, *polima*-type *B. napus* was inherited from *B. rapa* as maternal ancestor. In addition, mitochondrial genome of *nap*-type *B. napus* was more closed to *botrytis*-type than *capitata*-type *B. oleracea*. Our results clarified the role of diploid species in the maternal origin of allotetraploid species in ‚U'-triangle of *Brassica*, which points out the direction of potential genetic pools expansion by wide hybridization among them.

SESSION: RNA TRANSCRIPTION, MATURATION AND REGULATION

POSTER 14

Interaction network of protein-only RNase P in plant mitochondria.

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Keywords: RNase P, Pentatricoppetide repeat proteins, immuno-precipitation, polysomes

RNase P activity is essential to obtain functional tRNAs. It allows to cleave additional nucleotide sequences 5' of tRNA precursors. PRORP1 is a member of a novel class of protein-only RNase P enzymes containing PPR domains. It has been shown that PRORP1 is the only enzyme responsible for RNase P activity in vivo in Arabidopsis mitochondria and chloroplasts. In order to understand the functon of PRORP1 in the wider context of mitochondrial gene expression, we have determined the protein interaction network of PRORP1 in vivo. For this, we have used complementary approaches based on Blue Native PAGE and on the immunoprecipitation of protein complexes and identification by mass spectrometry. Results suggest that PRORP1 is associated to polysomes and that it might be part of RNA maturation complexes containing PPR proteins and other proteins predicted to be involved in mitochondrial gene expression.

POSTER 15

Multiple splicing pathways of trans-introns in wheat mitochondria

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Keywords: splicing, introns, evolution, RNA, wheat

DNA rearrangements have scattered segments of the nad1, nad2, and nad5 genes around the wheat mitochondrial genome, yet functional mRNAs can still be produced through trans-splicing. The independently-transcribed precursor RNAs assemble into splicing-competent structures and this is facilitated by higher-order folding of these group II-type introns as well as by splicing factors. The latter are expected to be especially important for the introns which have lost most of the conventional ribozyme features over evolutionary time. Group II splicing typically releases lariat-form introns or Y-branched for trans-introns. However in some cases, introns are also excised as linear halves via a hydrolytic pathway (eg. nad1 intron 4 and nad5 intron 2) or solely by a hydrolytic pathway (eg. nad1 intron 1). Surprisingly, such half-introns are present at high steady-state levels rather than being rapidly degraded. We also observe a third productive pathway for nad5 intron 2, yielding full-length excised introns in which the termini are joined in vivo. Moreover, when wheat embryos are germinated in the cold rather than at room temperature, differences are seen in the complexity of splicing pathways, suggestive of environmental effects on intron RNA structure. The elevated level of precursor RNAs in cold-treated embryos also facilitates tracking other RNA processing events such as mRNA end-maturation. Our observations provide insights into intron evolution and the complexity of RNA processing events in plant mitochondria.

POSTER 16

Various approaches to identify novel RNA editing factors associated with PPR proteins in plant organelles

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Keywords: plant mitochondria, RNA editing, PPR proteins, Arabidopsis thaliana

RNA editing is a posttranscriptional process that alters specific cytidines (C) to uridines (U) in plant organelle transcripts. In *Arabidopsis thaliana* two protein families have been identified as necessary factors in the RNA editing process. PLS class pentatricopeptide repeat (PPR) proteins recognize specific target nucleotides within the transcript and thereby provide a contact point for other cofactors like multiple organellar RNA editing factors (MORF) whose function is not yet known as well as other cofactors not yet identified. Besides their participation in the specific RNA recognition, the DYW-motif (a C-terminal extension of some PLS class PPR proteins) is a candidate for catalysing the C to U deamination. To further understand the role of PPR proteins in the RNA editing process in plant mitochondria various experimental approaches are necessary.

POSTER 17

Refinement and Verification of the RNA Recognition Code for PPR RNA Editing Factors

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Keywords: RNA editing, PPR code

The transcripts of plant organellar encoded genes undergo numerous posttranscriptional processing steps including 5'- and 3'-terminal processing, splicing and RNA editing during their maturation. Members of the PPR protein family (pentatricopeptide repeat proteins) consist of tandem arrangements of 31-40 amino acids long modules and are involved in all of these steps. The various tasks of PPR proteins require a potential for specific binding to the respective target RNAs at distinct nucleotide sequences. However, the specificity of the protein-RNA recognition machinery has been unclear until recently. Matching analysis found that amino acid positions 6 and 1' (1st of the next repeat) in the respective PPR module correlate with the respective nucleotide identity for two types of PPR modules, P (canonical: 35 aa) and S (short: 31 aa) (Barkan et al. 2012). These correlations allowed prediction of the target RNA sequence for a given PPR protein. In these analyses another type of PPR module, L (long: 35-40 aa), was not considered. We have now included the L module in the PPR nucleotide alignment and could thus improve the specific correlation between a given PPR type RNA editing factor and its target RNA sequence (Takenaka et al. 2013). Based on these findings, we developed a bioinformatic program tool not only to predict the potential target sites for a given PPR protein, but also to identify candidate PPR recognition factors for specific RNA editing sites.

POSTER 18

Characterization of protein-only RNase P in complex with tRNA in plants.

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Keywords: RNase P, Pentatricoppetide repeat proteins, substrate specificity, small angle X-ray scattering

RNase P is the essential activity that removes 5'-leader sequences from transfer RNA precursors. "PRORP" (PROteinaceous RNase P) defines a novel category of protein only RNase P. Before the characterization of PRORP, RNase P enzymes were thought to occur universally as ribonucleoproteins (RNP). The characterization of PRORP revealed a two-domain enzyme, with an N-terminal domain containing multiple PPR motifs assumed to achieve target specificity and a C-terminal domain holding catalytic activity.

We use a combination of biochemical and biophysical approaches to characterize the PRORP / tRNA complex. For instance, the complex was analysed by small angle X-ray scattering and Kd values of the PRORP / tRNA interaction were determined by analytical ultracentrifugation. We produced a collection of mutants where PRORP PPR domains were mutated. Their analysis reveals to what extent PRORP target recognition process conforms to the PPR code described for the interaction of PPR proteins with linear RNA. Altogether, our analysis reveals an interesting case of convergent evolution. It suggests that PRORP has evolved an RNA recognition process similar to that of RNP RNase P.

POSTER 19

Analysis of the RNA editing protein interaction network

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Keywords: RNA metabolism, RNA editing, MORF proteins

Almost 30 years ago C to U RNA editing was discovered in plant organelles. Recent genetic approaches revealed the involvement of two types of editing factors, E-class PPR (pentatricopeptide repeat) proteins and MORF (multiple orgenellar RNA editing factor) proteins. It becomes apparent that the loss of a MORF abolishes or decreases editing at multiple sites, implicating the importance of this protein family in RNA editing in both plant organelles. All MORF proteins contain a conserved central domain of 100 amino acids (the MORF box), which is supposed to mediate interaction between several proteins in the editosome. In order to elucidate the function of the MORF proteins in the editosome, the interaction between different parts of the MORF box and different RNA editing factors, including mitochondrial- and plastid- PPR type RNA editing factors as well as other MORF proteins, were analysed. So far, a preference in interactions between the N-terminus of the MORF box and parts of PPR proteins is observed. Furthermore, the results suggest that MORF homo- or heteromers connect between their respective N- and N-, N- and C- , and C- and C-terminal MORF box-sections.

POSTER 20

Identification of CMS-associated gene and gene mapping of the Rf gene for RT98-CMS

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Keywords: Cytoplasmic male sterility

Cytoplasmic male sterility (CMS) is a maternally inherited trait resulting in the failure to produce functional pollen and is often observed when an alien cytoplasm is transferred into a cultivated species. An RT98A CMS line and an RT98C fertility restorer line were obtained by successive backcrossing between *Oryza rufipogon* W1109 and *O. sativa* cultivar Taichung 65 (T65).

To uncover the CMS-associated mitochondrial genes, we determined the complete sequence of the RT98-CMS mitochondrial genome using next-generation pyrosequencing. The *orf113* gene, which is absent in a report mitochondrial genome of *O. sativa* Nipponbare, showed different transcript size between RT98A and RT98C on Northern blot analysis and was shown to be co-transcribed with *atp4* and *cox3*. The 5' end of the RNA of RT98C was approximately 50 bp shorter than that of RT98A, and located inside of the *orf113* coding sequence.

To identify the fertility restorer (*Rf*) gene for RT98-CMS, named *Rf98*, F_2 mapping population was generated from the cross between an RT98C with T65. We used 1248 plants from the RT98C×T65 F_2 population and 6432 from the RT98C×T65 BC_1F_2 for the mapping. As a result, the *Rf98* locus was narrowed down to a 170-kb region between the two makers. Within the region, 17 putative genes have been predicted by the Flowering Plant Gene Picker. We assume one of these genes is the *Rf* gene for the RT98-CMS.

POSTER 21

Two putative nucleases are involved in 5' processing of distinct mitochondrial transcripts in Arabidopsis thaliana

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Keywords: Arabidopsis thaliana, michondria, 5' processing, putative endonucleases

In *Arabidopsis thaliana* (*A.thaliana*) the generation of mature mitochondrial mRNAs includes processing of both 5' and 3' ends. Up to now, the processes underlying the 5' processing are poorly understood. Recent studies identified several P-class PPR proteins (RNA PROCESSING FACTORs 1-7) involved in this process. These factors have no enzymatic activity and thus so far unknown endonucleases were suggest to be involved in the generation of 5' ends of mitochondrial transcripts.

We now analyzed two putative MITOCHONDRIAL NUCLEASES (MNU1, MNU2) which are involved in the processing of at least four mitochondrial mRNAs (*atp*8, *ccmF*$_{N2}$, *nad*3-*rps*12 and *nad*9) in *A.thaliana*. In *MNU1*/*MNU2* double mutants large *ccmF*$_{N2}$ and *nad*3-*rps*12 precursor transcripts suggesting a participation of the two proteins in mRNA processing. Furthermore the lack of one of the two main *nad*9 transcripts in *MNU1*/*MNU2* double mutants suggests an important role of MNU1 and MNU2 in the generation of the *nad*9 mRNA with a 5' end at -243. In contrast to that a strong reduction of the main *atp*8 mRNA in *mnu1-2*/*mnu2-1* indicates that the proteins are important for the stability of this transcript.

Since the lack of MNU1 and MNU2 results in various phenotypes it seems that these two proteins have multiple functions but their exact role in RNA processing remains unclear.

POSTER 22

RPF3 and RPF6 are involved in ccmC mRNA maturation in mitochondria of Arabidopsis thaliana

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Keywords: Arabidopsis thaliana, michondria, 5' processing, pentatricopeptide repeat proteins

In *Arabidopsis thaliana* (*A.thaliana*) the maturation of mitochondrial transcripts includes the post-transcriptional formation of 5' ends. Proteins involved in this step are the RNA PROCESSING FACTORS (RPFs), belonging to the P-class pentatricopeptide repeat (PPR) proteins.

Two of these proteins, RPF3 and RPF6, play a role in the maturation of *ccmC* transcripts. Dependent on a 66 bp sequence segment around 500 bp upstream of the *ccmC* gene, defining two *ccmC* genotypes, distinct transcripts are generated in different *A.thaliana* accessions. In Col, the 5' ends of *ccmC* transcripts map to positions -484/482. These transcripts are formed in the presence of RPF3. In contrast to that, RPF6 is responsible for the generation of *ccmC* mRNAs with 5' ends around -391/390. Such transcripts are undetectable in Col, but are present in C24. The investigation of around 50 *A.thaliana* accessions revealed a strong correlation between the *ccmC* genotype (Col or C24) and the *ccmC* transcript lengths (like in Col or C24), but identified also accessions deficient in *ccmC* mRNA maturation.

By using the recently described RNA recognition code of PPR proteins we suggest, that the 66 bp sequence segment upstream of the processing sites, different between Col and C24, contains the binding sites of RPF3 and RPF6, respectively. Because of the high similarity between RPF3 and RPF6 the specific binding seems to be defined only by a few PPR-nucleotide interactions.

POSTER 23

A PLS-type PPR protein is involved in RNA splicing of nad5 pre-mRNA in the moss Physcomitrella patens

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Keywords: PPR protein, RNA splicing, nad5

Pentatricopeptide repeat (PPR) proteins are localized in either mitochondria or plastids, where they play a crucial role in various aspects of RNA metabolism at the posttranscriptional level in plant organellar gene expression. However, many of their functions remain to be characterized. PPR proteins are divided into four types, P, PLS, E/E+, and DYW. In *Arabidopsis* and rice, several P-type PPR proteins are known to be involved in RNA splicing or RNA cleavage and many E/E+ or DYW-type PPR proteins are shown to be required for RNA editing in mitochondria. In contrast to studies performed in flowering plants, knowledge regarding the PPR proteins required for organelle biogenesis is limited in the basal land plants. The moss *Physcomitrella patens* has at least 105 PPR proteins (Sugita et al. RNA Biol. 9, 1439-1445, 2013). To investigate their functions, we have generated and characterized 40 *PPR* gene knockout and/or knockdown mutants. Here we identified a PLS-type PPR protein, PpPPR_31, as a splicing factor. Knockout of the *PpPPR_31* gene reduced the extent of RNA splicing of the third intron of *nad5* pre-mRNA. This intron contains an ORF encoding a maturase-related protein. A possible role of PpPPR_31 in RNA splicing will be discussed.

POSTER 24

Dynamic RNA editosomes in plant organelles

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Keywords: RNA editing, PPR proteins, MORF proteins, protein-protein interactions

In flowering plant mitochondrial mRNAs, RNA editing alters 400-500, and in plastid transcripts 30-40 nucleotides from C to U. Pentatricopeptide repeat (PPR) proteins have been assigned to be involved in processing single or several RNA editing sites in plant mitochondria or chloroplasts. The structures of individual PPR domains in these RNA editing factors suggest that these proteins mediate the sequence specific recognition of editing sites. Each PPR module binds to one nucleotide whose identity is determined by the few contacting amino acids. Several other proteins influence RNA editing, directly involved in RNA editing in both plant organelles are the members of the MORF protein family, the multiple organellar RNA editing factors. Each of these MORF proteins is essential for or influences editing at many sites, for several of which individual PPR proteins are also required. Requirement of two or three MORF proteins at identical editing sites suggests the involvement of multiple MORF proteins in an RNA editosome. To determine whether MORF and PPR proteins physically interact, we tested various combinations of PPR editing factors and MORF proteins by Y2H, BiFC and CoIP analyses. In these assays we observe selected connections in vivo, in yeast cells and in vitro. These homo- and heteromer interactions suggest that in plant organelles several distinct protein moieties assemble on the target mRNA into a protein complex to form the functional RNA editosome specific for each editing event.

POSTER 25

Strategy for the purification of RNA editing complexes from isolated mitochondria

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Keywords: RNA editing, in vitro editing reaction

The C to U RNA editing in flowering plant mitochondria specifically addresses hundreds of C-nucleotides and converts these to U presumably by deamination. So far, the enzyme involved has not been identified. Site-specificity appears to be mediated by individual MEF proteins of the PPR family through sequence specific interaction with the target RNA molecule. The identification of the MORF proteins as essential co-factors shows that beyond selected specific MEF proteins, additional proteins may be part of the editosome which performs the RNA editing. Several biochemical approaches have been initiated to identify the in vitro interaction between MEF proteins, MORF proteins and their respective RNA targets and to investigate the overall sizes of the respective editosomes. Furthermore, the editosome complexes are being analysed to identify further components in *Brassica oleracea*. We will discuss our results from these analyses with respect to the composition of the RNA editing protein complexes in plant mitochondria.

SESSION: MITOCHONDRIAL MEMBRANE BIOGENESIS, DYNAMICS AND TRANSPORT

POSTER 26

A comparative study of molecular mechanisms underlying mitochondrial fission in Arabidopsis thaliana and a model liverwort, Marchantia polymoropha.

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Keywords: Mitochondrial fission, Mitochondrial biogenesis

Mitochondria increase in number by the fission of existing mitochondria. Mitochondrial fission is also necessary for providing mitochondria to daughter cells after cell division. In Arabidopsis, four kinds of genes have been reported to be involved in mitochondrial fission. The nucleotide sequences of two of them, *DRP3* (*Dynamin-Related Protein 3*) and *FIS1* (*Fission 1 /BIGYIN*), are well conserved in eukaryotes. The other two, *ELM1* (*Elongate Mitochondria 1*) and *PMD* (Peroxisomal and Mitochondrial Division) are found specifically in plants. A model liverwort, *Marchantia polymorpha,* has single copies of homologues for *DRP3, FIS1* and *ELM1* in the genome*,* but does not appear to have a *PMD* homologue. GFP-fusion proteins with DRP3, FIS1 and ELM1 of Marchantia were also localized to mitochondria in *M. polymorpha*. Marchantia *ELM1* and *DRP3* knockouts (*elm1*^{KO} and *drp3*^{KO}) grew more slowly and had elongated mitochondria, suggesting that mitochondrial fission was blocked. On the other hand, growth and mitochondrial morphology of a Marchantia *FIS1* knock-out plant (*fis1*^{KO}) did not seem to be different from those in the wild type. These results suggest that homologues of *ELM1* and *DRP3* but not *FIS1* are essential for mitochondrial fission in *M. polymorpha.* The diversification, conservation and evolution of molecular mechanisms underlying mitochondrial fission in terrestrial plants and in other eukaryotes will be discussed.

POSTER 27

Two paralogous proteins differing in subcellular localization reveal essential features of the targeting sequence for mitochondrial import

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Keywords: mitochondrial targeting sequence, subcellular localisation, paralogous genes

In a previous study focused on the localization of late embryogenesis abundant (LEA) proteins in Arabidopsis, two paralogous genes were shown to encode proteins with different subcellular localisations. LEA38 (At4g02380) was found to be targeted to mitochondria, while LEA2 (At1g02820) displayed a cytosolic localisation. Most imported mitochondrial proteins carry a N-terminal mitochondrial targeting sequence (MTS) that is usually cleaved upon import. Because of the low consensus and high variability of MTSs, subcellular targeting predictors cannot determine with high confidence their occurrence nor length, indeed, both LEA proteins were predicted to localize to mitochondria or/and plastids.

Since these two small (10 kDa) proteins have very similar primary sequences, we have experimentally investigated the determinants of their differential targeting. Relevant differences in the N-terminus were used to generate mutated synthetic genes which were expressed as translational fusions with GFP in Arabidopsis leaf protoplasts.

Subcellular localisation analysis and structural modelling revealed that specific organisations of charged and hydrophobic residues need to be preserved in the N-terminus for mitochondrial targeting.

Our study contributes to a deeper understanding of import, cleavage mechanisms, and duplicated gene evolution.

POSTER 28

Plants do it differently: The role of Tom9 in plant mitochondria

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Keywords: Tom9, TOM complex

In yeast and other fungi, the translocon on the outer membrane (TOM complex) is composed of several subunits: Tom40 is the pore forming component and Tom20, Tom22 and Tom70 are the receptors. In plants, homologs for Tom40, Tom20 and Tom22 (called Tom9 in plants) have been detected. Tom9 is significant smaller than Tom22 due to a reduction in the cytosolic domain. Moreover, the function of Tom9 is still unknown and only an interaction with Tom20 has been detected. Here we have identified a potential role of Tom9 as a regulator of the TOM complex assembly in Arabidopsis. RNAi knock-down (kd) plants of *tom9* have a severely reduced content of TOM complex. This is in line with the observation that overall levels of both Tom40 and Tom20 are reduced. No severe phenotypic abnormalities were observed in *tom9* kd-mutants under normal growth conditions; however, they display shortened hypocotyls and an early flowering compared to wild-type plants. Despite only minute amounts of TOM complex in the *tom9* kd-mutant, the rate of protein import *in vitro* showed no or only minor differences between wild-type and mutant mitochondria. Furthermore, we identified Tom9 as a binding protein of the calcium sensor, calmodulin (CaM), with the CaM-binding site located within the cytosol exposed N-terminal domain of Tom9 and we observed that CaM interferes with the interaction between Tom9 and the import receptor Tom20. These findings indicate that Tom9 is essential for the assembly of the TOM complex and that this process might be integrated into the calcium signalling network of the cell.

POSTER 29

A novel outer mitochondrial membrane beta-barrel protein

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Keywords: mitochondria, beta-barrel protein, OM47, outer membrane, orgenelle communication

The mitochondrial outer membrane has been well known to involve in recognition and translocation of mitochondrial preproteins. Also, as the interface between mitochondria and the cytosol, the outer mitochondrial membrane is proposed to play essential role in signaling, via the transport of molecules into and out of mitochondria, and by the presence of proteins that may act as regulators and transmit signals to regulate NGEMPs or mitochondrial encoded genes. A recent study on Arabidopsis mitochondrial outer membrane identified a novel plant specific beta-barrel protein OM47 encoded by At3g27930. It is known that many beta-barrel proteins evolved distinct functions among different organisms, and as an outer membrane protein, it may involve in influencing mitochondrial function and regulating the expression of NGEMPs. Therefore, functional characterisation of this protein is of particular importance. The role of this protein in plants was characterised under a variety of conditions, showing that it impacted on a variety of cellular processes. These results will be presented and discussed with respect to communications between mitochondria, chloroplasts and the nucleus.

POSTER 30

Protease AtFtsH4 as a regulator of mitochondrial membrane phospholipid composition in A. thaliana

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Keywords: phospholipid metabolism, mitochondrial membrane, mitochondrial proteases

The number of data indicates that the mitochondrial membrane phospholipid composition and therefore the regulation of membrane integrity in yeast and mammals are under proteolytic control. In yeast it was reported that the stability of proteins involved in the regulation of accumulation of cardiolipin (CL) and phosphatidyloethanolamine (PE) is determined by Atp23 and Yme1 proteases. However in plants there is a lack of such data. Using the thin layer chromatography (TLC) approach and phosphate assay we have shown that in mitochondria of Arabidopsis mutants lacking AtFtsH4 protease, the homologue of yeast Yme1 protease, the phospholipid content is reduced. Moreover, *ftsh4* plants displayed heterogeneous population of mitochondria, consisting of large, spherical mitochondria among normal ones. The observed simultaneous decrease of phospholipid content and increase of mitochondria with abnormal morphology were particularly significant when plants were grown at moderately elevated temperature. Our study uncovered that AtFtsH4 protease, like its yeast and mammalian counterparts, influences mitochondrial inner membrane phospholipid composition, possibly by regulating the turnover of proteins engaged in phospholipid metabolism.

This work was supported by Grant 2013/11/N/NZ3/00061 from the National Science Centre, Poland.

POSTER 31

Does ATP synthase form the PTP in Pea stem mitochondria?

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Keywords: ATP synthase, BN-PAGE, Cyclophylin D, Cyclosporin A, Permeability Transition Pore

The mitochondrial Permeability Transition (PT) is mediated by the opening of a mitochondrial channel named Permeability Transition Pore (PTP). Recently, it has been demonstrated, in mammals, yeast and Drosophila, that the PTP is a channel formed by dimers of F-ATP synthase. Since plants possess all the components needed for PTP formation, this work was undertaken to verify if plant F-ATP synthase possesses features similar to those already seen in other species, as this would provide a significant contribution to understand evolution of the channel function of F-ATP synthases.

Pea stem mitochondria showed a substrate-dependent electrical potential formation. In the presence of the Ca^{2+} ionophore ETH129, mitochondria were able to accumulate Ca^{2+} up to 0.2 mM, which was followed by an abrupt collapse of the membrane potential that was delayed by Cyclosporin A (CsA) and Pi. The Ca^{2+} retention capacity was also evaluated, which showed a trend similar to that of membrane potential. These results suggest that plant mitochondria, when permeabilized to Ca^{2+}, can undergo a PT. We detected F-ATP synthase activity after blue-native (BN)-PAGE and the active bands, corresponding to the dimers, were eluted from the gel. The active protein bands were then analyzed for the presence of F-ATP synthase components, and for the presence of Cyclophylin D (CyPD), which associates to mammalian mitochondria. The dimers were also inserted into an artificial bilayer, and we will report our preliminary results on their channel activity in electrophysiology experiments. Supported by MIUR grant PRIN 2010CSJX4F.

SESSION: INTERORGANELLAR SIGNALLING AND INTERACTION

POSTER 32

Selective mitoribosome-dependent translation influences on chloroplast genome expression

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Keywords: Arabidopsis thaliana, mitoribosome, chloroplast transcription, pTAC complex

Silencing of *RPS10* gene encoding a small-subunit protein of the mitochondrial ribosome in *Arabidopsis thaliana* led to the generation of heterogeneous population of mitoribosomes. The altered mitoribosomes have differential effects on mitochondrial proteins and in consequence the OXPHOS proteins were translated less efficiently, whereas mitoribosomal proteins were translated with an enhanced efficiency.

Alterations in mitoribosome-dependent translation in *rps10* plants affect also on chloroplast genome expression. We found a decrease in the steady-state level of all chloroplast transcripts. Based on *run-on* transcription assay we concluded that the reduction in steady state mRNA level of chloroplast genes results mainly from the decline in their transcriptional activity. This decline reflected the impairment in rate of chloroplast protein synthesis, implying that the decrease of chloroplast proteins in *rps10* was simply due the decreased level of chloroplast transcripts.

Our study provide evidence for transcriptional regulation of chloroplast gene expression in response to selective mitoribosome-dependent translation observed in *rps10* plants. Given the fact that in *rps10* we observed significant downregulation in expression of components of plastid transcriptionally active chromosome complex (pTAC), which mediates in chloroplast transcription, we postulated that changes in chloroplast transcription in *rps10* plants were associated with decreasing activity of chloroplast transcription apparatus.

This work was supported by Grant 2013/11/D/NZ1/00288 from the National Science Centre, Poland.

POSTER 33

Molecular mechanism of cytoplasmic male sterility in the new CMS source PET2 in sunflower and its fertility restoration

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Keywords: Cyotplasmic male sterility, fertility restoration, sunflower, PET2

In sunflower, only a single CMS source has so far been used in commercial hybrid breeding, which represents an increasing risk for pathogen attacks. However, a new CMS cytoplasm called PET2 might be an interesting alternative. The PET2 cytoplasm was characterized by the expression of a new 12.4 kDa protein in the *in organello* translation products of isolated mitochondria. Southern hybridizations showed differences for *cob*, *atp6* and *atp9*. Cloning and sequencing of the fragments revealed two new open reading frames, *orf288* and *orf231*, specific for the PET2 cytoplasm. A hypothesis for the creation of these two new open reading frames has been developed. Overexpression of both open reading frames in *E. coli* lead to reduced growth of the bacteria indicating that the products might be cytotoxic. The *orf288* codes for a predicted protein of 10.6 kDa containing a transmembrane domain, whereas *orf231* codes for a predicted 8.4-kDa-protein. Antibodies against peptides specific for the *orf*s were successfully made. RT-PCR analyses were performed in leaves, disk florets and anthers and showed that the two open reading frames are cotranscribed. In addition, a reduction of the cotranscript of *orf288* and *orf231* in the anthers of fertility restored F_1-hybrids could be demonstrated. Blue native gel electrophoresis was performed to identify associations with mitochondrial complexes. Fertility restoration requires one dominant restorer gene *Rf_PET2*. AFLP-markers linked to this restorer gene have been identified.

POSTER 34

The contribution of mitochondria in acclimation to ammonium-nutrition of Arabidopsis thaliana

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Keywords: alternative pathway, ammonium syndrome, oxidative stress, redox homeostasis

Cultivation using ammonium (NH_4^+) as the sole nitrogen source for many plants leads to severe growth disorders known as 'ammonium syndrome'. Ammonium-based nutrition affects plant metabolism in a manner that leads to an intracellular redox imbalance; meanwhile a surplus of reductants was expected in the cytosol and chloroplasts. However, plant mitochondria can be efficient reductant sinks oxidising superfluous reductants in their electron transport chain. On the other hand, increased respiratory activity that occurs during NH_4^+ nutrition can result in higher mitochondrial ROS formation. A protective mechanism is the induction of alternative pathways (including the alternative oxidase, AOX, external and internal type II dehydrogenases) to lower ROS formation.

The purpose of this study was to determine the role of mitochondria in NH_4^+-grown *Arabidopsis thaliana*. In order to identify the primary effect of NH_4^+ toxicity to plants, the response to long-term NH_4^+ fertilization was compared to short-term NH_4^+ treatments. Results indicate that prolonged NH_4^+-nourishment leads to excess mitochondrial ROS production, despite up-regulation of *AOX2*. In contrast to this the induction of all AOX isozymes during short-term NH_4^+ supply prevents elevated ROS production in plant tissues. It may be concluded that plants rapidly acclimate to NH_4^+ availability. Only prolonged alterations in redox balance during long-term NH_4^+ feeding lead to oxidative stress. The communication of chloroplasts and mitochondria during NH_4^+ nutrition is important to prevent major perturbations in ROS/redox homeostasis.

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POSTER 35

A novel forward genetic screen to identify respiratory complex I mutants in Chlamydomonas

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Keywords: mitochondria chloroplast interactions screening method complex I

Introduction

The structural subunits of the complex I are well characterized but little is known about how they assemble. The current screening method in *C. reinhardtii* is based on the slow growth of complex I mutants in heterotrophic conditions. This is probably the reason why most complex I mutants isolated so far are null mutants because intermediate phenotypes are not easy to distinguish.

Methods

This work aims to identify complex I mutants using a new screening method. We started to use a *pgrl1* deficient strain for transformation. The *pgrl1* strain lacks cyclic electron transport in the chloroplast disrupting the ATP/NADPH balance, which causes photosynthesis to rely on respiration. Indeed, a double mutant affected in both complex I and *pgrl1* displays a reduced photosynthetic yield compared to individual mutants. We thus performed insertional mutagenesis on *pgrl1* and screened for complex I mutants based on their photosystem II yield using chlorophyll fluorescence measurement directly on plate.

Results

About 3000 *pgrl1* transformants were screened and 2% showed a photosystem II yield between WT and *CI-/pgrl1* double mutants. Amongst them, 4 showed a decrease in complex I activity ranging from 20% to 70% of WT. These mutants were subjected to genetic and molecular analysis to identify the lesion responsible for the phenotype. 2 out of 4 lesions were identified. 1 mutation has been showed to be tagged and 2 remains to be verified.

Conclusion

Using a *pgrl1* deficient strain for transformation allowed us to identify respiratory mutants based on their fluorescence parameters.

SESSION: RESPIRATORY CHAIN STRUCTURE, ASSEMBLY AND REGULATION

POSTER 36

Cardiolipin deficiency has pleiotropic effects on plant mitochondria

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Keywords: mitochondria, lipid composition, cardiolipin, respiratory chain, cytochrome c, protein complexes, supercomplex

Introduction:

Mitochondrial membranes possess a unique lipid composition due to the presence of cardiolipin (CL) which is not found anywhere else within the eukaryotic cell. This dimeric phospholipid influences mitochondrial biology in several ways, including mitochondrial structure, fission, and function. The presence of CL is not limited to membranes but also forms part of the protein complexes and protein supercomplexes involved in oxidative phosphorylation system. In non-plant systems CL deficiency is accompanied by severe implications of mitochondria functions.

Materials and Methods:

By comparing a complemented Arabidopsis knock out line of the last enzyme of CL biosynthesis, CL synthase (CLS) with WT plants using blue-native gels, immunoblots, functional assays and mass spectrometry the dependence of the plant OXPHOS system on CL is investigated.

Results:

Functional assays indicate a severe obstruction of electron flow along the respiratory chain. By large, this stems from a reduction in mitochondrial cytochrome c content and the nearly complete absence of the most abundant plant respiratory supercomplex. Restricted electron flow along the mitochondrial electron transfer chain also has implications for the reactions taking place in the mitochondrial matrix as shown by proteomic analyses.

Conclusions:

By reducing respiratory capacity and protein complex abundances cardiolipin affects plant mitochondrial biology in several ways and on different levels. This is caused by primary and secondary effects.

POSTER 37

Unravelling the in vivo regulation and role of the alternative oxidase (AOX) in leaves

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Keywords: Alternative oxidase, oxygen isotope fractionation, photosynthesis, metabolite profiling, thioredoxin

The mitochondrial alternative oxidase (AOX) pathway provides flexibility in cellular energy and carbon metabolism under stress conditions, particularly in leaves, where it strongly interacts with photosynthesis. Although several studies have reported evidence for such a general role in plants, it is still not clear when and how AOX activity is regulated *in vivo*. Here we present experiments in which *in vivo* electron partitioning to AOX together with metabolite profiles and photosynthesis measurements were determined in different species and genetically modified plants. *In vivo* evidence for the mitochondrial thioredoxin system in mediating redox regulation of AOX and its role in photosynthetic metabolism and amino acid synthesis under high light conditions is presented and discussed.

POSTER 38

Double mutants with respect to subunits of the “carbonic anhydrase domain” of mitochondrial complex I have developmental and respiratory defects

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Keywords: complex I, development, gamma carbonic anhydrase, respiratory defects

NADH dehydrogenase complex (complex I) is part of the oxidative phosphorylation system (OXPHOS) in mitochondria and has an important function in energy metabolism. Unique to photoautotrophic eukaryotes such as plants and green algae is the extra gamma carbonic anhydrase (gamma CA) domain attached to the matrix side of the membrane arm of complex I. Altogether, three gamma CA (gamma CA1, gamma CA2, gamma CA3) and two “gamma carbonic anhydrase like (gamma CAL)” (gamma CAL1, gamma CAL2) proteins are known, but only three of them are simultaneously present in individual complex I gamma CA domains. The function of these proteins is still unclear. In earlier studies, a reduction of complex I activity was reported for gamma *ca2* mutants, but this did not come along with alterations in phenotype. Crosses between *various ca/cal* mutants have been carried out. The resulting double mutants have prominent developmental and respiratory defects.

POSTER 39

Cytosolic pH is a major modulator of mitochondrial NADPH oxidation in plants

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Keywords: Calcium, Cytosol, Mitochondrial electron transport, NADPH, NADPH dehydrogenase

NADPH is a key reductant carrier that maintains redox and antioxidant status, and that links biosynthetic, catabolic and signalling pathways. In the electron transport chain of plant mitochondria, alternative pathways for NAD(P)H oxidation are catalysed by type II NAD(P)H dehydrogenase. NDB1 is a type II NADPH dehydrogenase, which has a high capacity to oxidise cytosolic NADPH and can modulate whole cell NADPH status. NDB1 is known to be Ca^{2+} and pH dependent, but all studies have been done in the presence of high, unphysiological concentrations of Ca^{2+}. In this study, $K_{0.5}(Ca^{2+})$ of the NDB1 from potato mitochondria and membranes of *E. coli* expressing Arabidopsis NDB1 was determined under various pHs over the physiological pH range using oxygen and decylubiquinone as electron acceptors. The result shows that $K_{0.5}(Ca^{2+})$ for NDB1 is significantly modified by pH, which indicates that Ca^{2+}-regulation is subordinate to pH regulation. In contrast, for potato external NADH dehydrogenases, there is no significant pH-regulation of $K_{0.5}(Ca^{2+})$ observed. Thus, a decrease in cytosolic pH below normal steady state levels is essential for external NADPH oxidation, and in addition high Ca^{2+} concentrations are needed for activation. Besides, the results also show that NADPH oxidation responded to changes in Ca^{2+} concentrations more rapidly than NADH oxidation did. This knowledge is important for further understandings of NADP(H) homeostasis and redox dependence, and predicting the impact on NADPH/$NADP^+$-ratios in transgenic plants modified for the NDB1 NADPH dehydrogenase.

POSTER 40

The indirect regulation of the plant and fungal Alternative Oxidases via GMP

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Keywords: AOX, GMP, Regulation,

The alternative oxidase (AOX) is an integral monotopic membrane protein that catalyses the reduction of oxygen to water via the oxidation of ubiquinol in a non-protonmotive fashion. The AOX is found within all higher plants, various fungi, protozoa and even some ciliates. AOXs show large differences in terms of regulation, for example AOXs from non-thermogenic (*A. thaliana, AtAOX)* or thermogenic plants (*S. guttatum,* SgAOX), either are sensitive or insensitive respectively to the addition of pyruvate. On the other hand fungal AOXs (e.g. *N. crassa* and *P. stiptis)* display higher AOX activities when stimulated by purine nucleotides such as GMP. The protozoan *A. castellanii,* also displays increased AOX activity in the presence of GMP but not pyruvate in contrast to the trypanosomal AOX (TAO), which is not stimulated by either compound. Understanding the mechanisms behind AOX regulation and activity is of importance for the future design of highly efficient quinol oxidases suitable for gene therapy.

Our findings indicate that 1.5 mM GMP results in a 2-fold increase in quinol oxidase activity of membrane-bound recombinant AtAOX and SgAOX expressed in *E. coli* comparable to that observed with fungal and protozoan mitochondria. A 4.5-fold increase in activity was also observed in CfAOX (AOX from *Chalara fraxinea*). Importantly, purification of SgAOX and CfAOX resulted in the loss of GMP stimulation. Such data suggests that the observed GMP stimulation is not a result of a direct effect on the AOX *per se* but rather on another membrane bound protein. Results will be presented as to the identification of this GMP sensitive protein.

POSTER 41

Atypical structure and subunit composition of respiratory complexes in Euglena gracilis

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Keywords: Euglenozoa, Trypanosomatidae, Oxidative phosphorylation, Dimeric mitochondrial complex V, Protein electrophoresis.

Euglena gracilis, a non-parasitic secondary green alga related to trypanosomes, has a complex mitochondrial oxidative phosphorylation system constituted by atypical respiratory enzymes (complexes I - V). Recently, the analysis of the subunit composition of respiratory complexes by 2D BN/SDS PAGE has shown that at least 41 of the non-canonical subunits reported in trypanosomes are also present in this alga along with 48 classical subunits described in other eukaryotes including green plants. In the present study the complexes I, III, IV and V were further purified from isolated mitochondria using liquid chromatography after solubilization with n-dodecyl-maltoside. Using a 3D BN/SDS/SDS PAGE analysis, we resolved their subunit composition and confirmed the atypical subunit composition of Euglena respiratory complexes. The apparent molecular mass of purified complexes I and V (1.5 and 2.2MDa, respectively) is far above the classical ones. Single-particle analysis from transmission electron microscopy revealed some unusual features for complex V, including smaller angles between monomers and additional membrane extensions. Complex I also shows an unusual long matricial arm.

POSTER 42

BN-PAGE analysis of the respiratory chain complexes in mitochondria of sulphur-deficient Arabidopsis thaliana

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Keywords: BN-PAGE, Complex I, type II NADH dehydrogenases, sulphur deficiency, supercomplexes

Plant mitochondrial metabolism depends on sulphur (S) availability due to the presence of thiol moieties in proteins and Fe-S clusters in the respiratory Complexes I, II and III in the electron transport chain (mtETC). Although we have previously found that the plasticity of mitochondria allow plant to adapt to S deficiency, there is still a gap between the observed changes in mitochondrial ultrastructure and the restriction of mtETC capacity. One of the unanswered issues is whether S deficiency influences the structure of mtETC in S-deficient plants. The aim of our research was to analyze the mtETC complexes in mitochondria isolated from the leaves and roots of S-deficient *A. thaliana*. One-dimensional Blue-Native polyacrylamide gel electrophoresis (1D BN-PAGE) and densitometric measurements showed that the level and in-gel capacity of Complex I were lower in S-deficient mitochondria as compared to control. Two-dimensional BN/SDS-PAGE indicated that the band abundance for all of the subunits of Complex I was lower in S-deficient plants as compared to control although there were no differences in the qualitative composition of Complex I and supercomplex I+III_2. Complex I impairment under S deficiency could be compensated to some extent by the type II external NADH dehydrogenase ($NDII_{ex}$). This was proved by higher in-gel capacity of NDII after 2D BN/BN-PAGE. The present study demonstrates that in *A. thaliana* S deficiency does not strongly modify the structure of mtETC and confirms previously shown alterations in the capacities of mtETC components.

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POSTER 43

Structural Analysis of the Plant Mitochondrial OXPHOS System Using Chemical Cross-Linking

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Keywords: Arabidopsis, OXPHOS system, structure, chemical cross-linking, DSP, 3D-PAGE

Introduction: Multi-protein complexes form the basis for the regulation of metabolic processes. In *Arabidopsis thaliana* the protein complexes building the oxidative phosphorylation system (OXPHOS) include several plant specific subunits of so far unknown function. To understand their functions, knowledge about the localization of individual subunits within the OXPHOS complexes is needed.

Material&Methods: A novel approach to resolve the internal architecture of the OXPHOS complexes of mitochondria isolated from an *Arabidopsis thaliana* cell culture is presented. It is based on a combination of chemical cross-linking and a three dimensional polyacrylamide gel-electrophoresis (3D-PAGE). Subunits in close proximity inside the complexes are reversibly cross-linked by the chemical cross-linking agent Dithiobissuccinimidyl propionate (DSP). Thereafter, the cross-linked subunits are visualized by a 3D-PAGE system and identified by mass spectrometry.

Results and Conclusions:

The efficiency of the method was demonstrated comparing the results to previously obtained structural data. The structures of complexes III and V are well characterized in other eukaryotic organisms. Regarding these complexes 13 different cross-linking products were obtained and all of them match to known structural data. Following this approach, the localization of several subunits of the plant OXPHOS system could be shown in detail for the first time. Most interestingly, the localization of some plant specific subunits like the F_Ad subunit of complex V was resolved. This knowledge will help to better understand the diverse functions of these complexes in plants.

SESSION: MITOCHONDRIA AND STRESS RESPONSE

POSTER 44

Analysis of Physiological Roles of AOX under Phosphorus Deficient Condition

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Keywords: alternative oxidase, phosphorus deficiency, organic acids exudation

Introduction

Alternative oxidase (AOX) is a non-energy conserving pathway in the plant respiratory chain. AOX is induced by stresses such as phosphorus (P) deficiency and oxidizes excess reductants. Under P deficiency, roots exudate organic acids to dissolve and absorb insoluble P in soil. In process of synthesis of organic acids, reductants are synthesized and AOX is considered to contribute to exudate organic acids by reductant oxidization. However, this hypothesis has not been examined well. Then, we tried to clarify roles of AOX under P deficient condition.

Material and Methods

Arabidopsis thaliana, Col-0 and *aox1a* (transgenic line lacking AOX1a) were cultivated under hydroponic culture conditions, and transferred to phosphate (Pi) deficient culture during vegetative growth stage.

Results

Under P deficiency, shoot FW decreased and root FW increased. Shoot-root ratio and Pi amount decreased similarly between Col-0 and *aox1a*. The mRNA levels of alternative NAD(P)H dehydrogenase 2 (NDA2), NAD(P)H dehydrogenase B2 (NDB2) and AOX1a increased in Col-0. Although the mRNA levels of NDA2 and NDB2 increased similarly in *aox1a*, the AOX genes other than AOX1a were not induced and AOX capacity of *aox1a* was maintained low. The exudation rate of citrate and malate were lower in *aox1a* than Col-0. However, total respiratory rates in shoots increased and those in roots decreased similarly in both Col-0 and *aox1a*.

Conclusions

In this study, organic acids exudation rate was lower in *aox1a* than Col-0 under P deficient condition. This result corresponds to the above hypothesis.

POSTER 45

Global variability in leaf respiration and its temperature response

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Keywords: acclimation, leaf respiration, temperature, climate

Leaf respiration (R) represents a massive flux of carbon to the atmosphere. Currently, neither physiological models nor terrestrial biosphere models are able to disentangle sources of variation in leaf R among different plant species and contrasting environments. Similarly such models do not adequately describe the short-term temperature (T) response of R. Even minor differences in the underlying basal rate of leaf R and/or shape of the T-response curve can significantly impact estimates of carbon released and stored in ecosystems. Given this, we have recently assembled nad analysed two new global databases (arctic-to-tropics) of leaf R and its T-dependence. The results highlight variation in leaf R among species and across global gradients in *T* and aridity, with leaf *R* at a standard *T* (e.g. 25°C) being greatest in plants growing in the cold, dry Arctic and lowest in the warm, moist tropics. Arctic plants also exhibit higher rates of leaf R at a given photosynthetic capacity or leaf N concentration than their tropical counterparts. The results also point to convergence in the short-term temperature response of respiration across biomes. The utility of these results from predicting variation in rates of leaf R in terrestrial ecosystems across the globe will be discussed.

POSTER 46

Plant mitohormesis: UCP1-induced hypoxia links altered carbon fixation and energy expenditure to increased stress tolerance

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Keywords: uncouplig; hypoxia; mitohormesis

Introduction:

The mitochondrial uncoupling protein one (UCP1) plays an important role on controlling ROS production under stressful conditions by uncoupling the electrochemical gradient from ATP synthesis. We accessed the mechanisms that links altered mitochondrial metabolism and increased carbon assimilation to the increased performance of plants overexpressing UCP1 under abiotic stresses.

Methods:

We used the transcriptome of transgenic plants to hypothesize that these plants may be dealing with a hypoxic stress.To proof this hypothesis we submitted wild-type and UCP1 overexpressors to a 5% O_2 atmosphere and performed gene expression analysis together with a carbohydrate and metabolite content profile.

Results:

We found that key genes involved in hypoxic adaptation are up-regulated in UCP1 overexpressors, which together with the altered mitochondrial morphology indicates that these plants are suffering from a hypoxic stress. Interestingly, under control conditions the UCP1 overexpressors accumulate markers of hypoxic adaptation.

Conclusions:

This suggests that the increased uncoupling activity disrupts cell energy homeostasis because of the unbalance between ATP production and oxygen consumption. In this scenario, specific metabolites in the UCP1 overexpressors signalize for the increased carbon assimilation and fermentative glycolysis becomes critical to meet the cell energy demands. The uncoupled mitochondria are resistant to cytotoxic insults and oppose the activation of the intrinsic apoptotic pathways when plants are subjected to stressful conditions.

POSTER 47

Amino acids show different effects on OXPHOS system activity under carbon starvation in Arabidopsis cell suspensions

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Keywords: Carbon starvation, stress response, alternative respiration, OXPHOS system

Plant respiration is mostly dependent on carbohydrate to synthesize ATP. However, during stress situation plant cells also use amino acids as alternative substrates to donate electrons via the ETF/ETFQO complex to the mitochondrial electron transport chain (mETC). Given to this, here we investigated changes on oxidative phosphorylation (OXPHOS) system in *Arabidopsis thaliana* cell culture under carbon (C) starvation supplied with a range of amino acids. Induction of isovaleryl-CoA dehydrogenase (IVDH) activity was observed under carbon starvation which was associated with increased amounts of IVDH protein detected by immunoblotting. Furthermore, activities of the protein complexes of the mETC were reduced under carbon starvation. We also observed that OXPHOS system activity behavior is differently affected by different amino acids. Collectively, our results support the contention that ETF/ETFQO is an essential pathway to donate electrons to the mETC and that amino acids are alternative substrates to maintain respiration under C starvation.

POSTER 48

Alternative oxidase activity is not a genotype-specific component of salt stress tolerance in Medicago truncatula

Del Saz NF, Florez-Sarasa I, Clemente- Moreno MJ, Mhadhbi H, Flexas J, Fernie AR, Ribas-Carbo M.

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Keywords: Alternative oxidase, salt stress, Medicago truncatula

Under salt stress the alternative oxidase (AOX) may prevent over-reduction of the mitochondrial ubiquinone pool, reducing the formation of reactive oxygen species (ROS). Although the degree of tolerance/sensitivity to salinity has already been studied in relation to gene expression of antioxidant enzymes in different varieties of *Medicago truncatula*, it is yet to be known whether AOX activity is a genotype–specific component of salt stress. The effect of the salt stress on the *in vivo* electron partitioning to AOX was analysed together with metabolite profiles and photosynthesis in leaves and roots of three varieties of M. *truncatula* with different salinity tolerance grown under 300 mM NaCl during 1, 3 and 5 days. The results show that AOX activity is not a genotype –specific component of salt stress, as it differs between tissues and how is regulated by different amino acids and organic acids.

This work was financed by the Spanish Ministry of Science and Innovation (MICINN) - project BFU2011-23294

POSTER 49

Unraveling a role of Arabidopsis HCC2 and its yeast homolog SCO2 in redox homeostasis and UV-B stress response

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Keywords: Mitochondria, UV-B, oxidative stress, respiratory chain, COX

The inner mitochondrial membrane protein Sco1p of the yeast Saccharomyces cerevisiae has been shown to transfer copper to the Cu_A center of cytochrome c oxidase (COX). Deletion of the SCO1 gene results in respiratory deficiency. The highly similar paralog Sco2p shares 54% identity, but its deletion does not affect mitochondrial respiration. A similar scenario is observed in Arabidopsis: the SCO1 homolog HCC1 is essential due to its function in COX assembly. By contrast, knockout (KO) mutants lacking the paralog HCC2 show no apparent phenotypic alterations under normal growth conditions.

Both HCC2 and Sco2p possess a thioredoxin-like fold, possibly hinting at a role in oxidative stress defense. Interestingly, HCC2 expression is upregulated upon UV-B stress and an hcc2 KO line is more sensitive against long-term UV-B exposure than the wild type (WT). Notably, UV-B stress induces oxidative stress in plants. In order to investigate if HCC2 defends specifically against UV-B or oxidative stress in general, we phenotypically characterized the WT and hcc2 KO mutants under oxidative reagents. Preliminary results showed no significant difference between the WT and the mutants.

Our parallel investigation of a potential redox activity of Sco2p in yeast showed that the sco2 null mutant grew equally well in the presence of oxidative stressors as the WT. A double mutant lacking SCO2 and the superoxide dismutase 1 (SOD1), however, was no longer able to grow under oxidative stress conditions, while the KO of SOD1 alone still was. Currently we examine whether a similar synergistic effect of HCC2 and SOD is also observed in Arabidopsis.

POSTER 50

AOX isoforms from Arabidopsis and their post-translational regulation

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Keywords: Alternative Oxidase, Arabidopsis thaliana, enzyme activity, Escherichia coli, plant mitochondria, protein expression

Plant mitochondria contain a non-energy conserving terminal oxidase that couples the oxidation of ubiquinol to the four-electron reduction of oxygen to water without leading to ATP formation. The activity of the alternative oxidase (AOX) can be modulated *in vitro* in several ways. Oxidizing conditions result in the formation of a homodimer whereas the reduction of the intermolecular disulphide bridge increases its activity. The activity of the reduced AOX can be further enhanced by several alpha-keto acids which have been shown to interact with sulfhydryl groups, potentially by forming a thiohemiacetal. Arabidopsis AOX isoforms (AOX1A-D and AOX2) contain two conserved cysteine residues (CysI and CysII) from which CysI is subject of the sulfhydryl/disulfide change and alpha-keto acid activation.

To investigate the processes participating in post-translational regulation and the differences between the Arabidopsis isoforms, constructs containing wild-type or Cys-mutated AOXs were cloned for each isoform. We generated an *E. coli* strain which specifically lacks two of three endoxidases (cytochrome *bo* (*cyo*) and cytochrome *bd-I* (*cyd*)) from the respiratory chain of *E. coli*. The third endoxidase (cytochrome *bd-II* (*app*)) is still present in this *E. coli* strain (*delta cyd/delta cyo*) to maintain a high activity of Complex I. O_2 uptake was measured with a Clark-type electrode, using vesicles from *E. coli* cells expressing the recombinant AOX protein. As a basis for specific AOX activity, respiration rates were normalized for each sample using densitometry on immunoblots.

POSTER 51

Stress-induced mitochondrial protein degradation and peptide release

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Keywords: Protein degradation, peptide release, oxidative stress,

Mitochondrial protein turnover under oxidative stress conditions was studied in vitro by incubating mitochondria isolated from potato tubers (POM) (Solanum tuberosum L.) in a medium containing a substrate cocktail and ATP (control), in the same medium plus FCCP (uncoupled control), or in the medium plus methyl viologen and KCN to block electron transport and maximize the production of reactive oxygen species (ROS) (oxidative stress). After 15 min incubation, the mitochondria were pelleted, digested with trypsin, and iTRAQ-labelled. The pooled samples were analyzed by liquid chromatography-mass spectrometry. We found that 73 tryptic peptides decreased in amount relative to other peptides in the same protein after the oxidative stress treatment, but not after FCCP treatment. This indicates that the peptides had been modified, probably by oxidation. Amongst the modified proteins the respiratory complexes, especially complex I (7 subunits), the tricarboxylic acid cycle enzymes (6 out of 9) and ROS-detoxifying enzymes (6) were overrepresented. Many peptides were released from these POM and many more so in the presence of the pore-forming peptide alamethicin. Twice as many peptides appeared from matrix and IMM proteins during the oxidative stress treatment indicating that ROS reached larger parts of these compartments. We conclude that a detectable protein turnover can be detected observed in isolated mitochondria respiring in vitro over 15 min and that this protein turnover is changed during severe oxidative stress.

POSTER 52

Does Complex V of the electron transport chain act as a respiratory bottleneck during chilling stress in plants?

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Keywords: Complex V (F1Fo-ATP Synthase), ADP/O, ATP synthesis, respiration, chilling stress, temperature perception, Arabidopsis thaliana

Considerable effort has been made to identify physiological and biochemical changes that occur during chilling stress of plant species. However, despite such advancements, mechanistic insights into temperature perception, signal transduction and organellar responses to the cold are still rare. We have performed detailed studies of the respiration of control, cold-shocked and cold-adapted *Arabidopsis* leaves and isolated mitochondria. Here we present evidence based on respiratory rates, ADP/O ratios and ATP synthesis rates that Complex V (F_1F_0-ATP Synthase) of the electron transport chain (ETC) can act as a bottleneck in the respiratory process during chilling stress. This could provide a direct means of temperature perception by mitochondria and may in turn explain the activation of non-phosphorylating ETC bypass mechanisms to maintain or even increase respiratory rates in the cold.

POSTER 53

Biological Weighting Functions quantifying the effect of Ultra violet Radiation (UV_B) on the growth, Photo Synthesis & Photo Respiration of Ulva grown under Low light & High light Environment

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Keywords: BWF,PS2,Induction kinetics

Introduction:

Solar ultraviolet radiation (UV) (290-400 nm) is a significant stressor in aquatic environments. It has adverse effects on photosynthesis (Lorenzen 1979, Cullen et al.1992) and growth (Ekelund 1990), and damages DNA (Karentz et al. 1991) in the major primary producers in most aquatic ecosystems. Broad band UVB (290-320 nm) effectively inhibits macroalgal photosynthesis more than UVA (320-400 nm). SERC has made major contributions to the worldwide monitoring of UVB radiation through the development of sophisticated UVB-sensing instruments. Particular attention is given the more biologically damaging, short-wavelength ultraviolet-B (UVB), which is increasing as air pollutants deplete stratospheric ozone, which normally blocks UVB. Mechanistically, absorption of high energy UVB photons damages proteins associated with photosystem II (Renger et al.1989, Babu et al.1999, Vincent and Neale 2000) and degrades the carbon fixing enzyme Rubisco (Bishcof et al. 2000).

Materials and methods

Algae Source, Site and Collection. Ulva is collected from high (0 m) and low (2 m) light environments, a subestuary (RhodeRiver) of the Chesapeake Bay. stranded on the shoreline. It is often seen attached to piers or floating docks. Generally, it appears not to tolerate high temperature so it may be a difficult time to collect in the middle of the summer. Only healthy and clean fronds are used in order to avoid wound respiration (Bidwell and McLachlan 1985).

Justification**:**

These data will provide an excellent base for modeling work (biological responses to UV) and also support to identify the site of UV damage in photosystems.

POSTER 54

The role of AOX in ammonium-grown Arabidopsis thaliana

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Keywords: alternative oxidase, ammonium nutrition, oxidative stress

Plant growth on ammonium (NH_4^+) as the sole nitrogen source leads to an intracellular redox imbalance, mainly resulting in the overproduction of ROS in the mitochondrial electron transport chain (mtETC). As a consequence excess mitochondrial ROS can lead to oxidative stress and growth retardation of NH_4^+-grown plants. On the other hand mitochondria, due to their plasticity, can be efficient reductant sinks during NH_4^+ nutrition. The alternative pathways including alternative oxidase (AOX), and internal and external type II dehydrogenases (NDin and NDex) may oxidise superfluous reductants and lower ROS generation.

The purpose of this study was to analyse ROS metabolism in mutants with altered expression of AOX during long-term NH_4^+ fertilization. Transformed antisense silencing of *AOX1a* and overexpressor lines were utilized in these studies. The investigative approach focused on determining oxidative stress in NH_4^+-nourished transgenic *Arabidopsis*. ROS production, detoxification, and oxidative damage were analysed in plant tissues. *AOX1a* antisense silencing resulted in hypersensitivity to NH_4^+ nutrition, while the overexpressor line was not affected. It can be concluded that AOX is an important protein for maintaining redox homeostasis during NH_4^+ growth and prevents ROS poise of the cell.

POSTER 55

In silico analyses revealed new insights into the possible roles of TCA cycle enzymes

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Keywords: plant respiration, TCA cycle, stress

Although plant respiration is mainly dependent on carbohydrate oxidation it has been demonstrated that the oxidation of alternative substrates becomes considerably important during various stress conditions which affects carbohydrates supply. Additionally plant mitochondrial metabolism is highly reorganized under a range of stress conditions. In order to understand transcriptional changes of genes encoding TCA cycle proteins we evaluated public transcriptional database concerning a range of stress. In silico analyses suggest new insights into the possible roles of TCA cycle enzymes in different tissues. Finally, we performed co-expression analysis using mitochondrial TCA cycle genes revealing close connections amongst these genes most likely related to the higher efficiency of oxidative phosphorylation in mitochondria. Our results identify further candidates genes which might be used for metabolic engineering purposes given the importance of the TCA cycle during development and/or stress situations. Our co-expression analyses provided an explanation for the modular operation of the TCA cycle according with physiological conditions.

POSTER 56

A mitochondrial twin-cysteine protein impacts cellular wide anti-oxidant defences and response to abiotic stress

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Keywords: mitochondria, intermembrane space, twin-cysteine, anti-oxidant defence, abiotic stress

Mitochondria as important hubs can sense or integrate a variety of other internal and external signals in the cell. However, it is still unclear how these signals generated in mitochondria are transmitted to the nucleus. In order to identify mitochondrial proteins involved in mitochondrial signaling, an analysis was undertaken to identify mitochondrial proteins located in the outer membrane or intermembrane space (IMS) that respond to perturbation of mitochondrial function. One such potential protein located in the intermembrane space and cytosol, a twin cysteine protein with an apparent mol mass of 12 kDa (At12Cys) has been identified as a potential protein playing a role in mitochondrial signaling. We found that this protein was induced at a transcript level in a variety of genetic mutants that affect mitochondrial function, but not with chemical inhibitors. It is also shown that the induction of At12Cys at a protein level was evident in some but not all cases where transcript abundance was altered. Characterisation of the role of this protein revealed that it affected anti-oxidant defences throughout the cell and altered whole plant responses to abiotic stress. The underlying mechanisms by which this is achieved will be outlined.

POSTER 57

Implications of altering ATP synthase in mitochondria regulates stomatal function during abiotic stress in Arabidopsis thaliana

Jordan D Radomiljac[1], Oliver Berkowitz[1], Botao Zhang[1], Reena Narsai[1], Nathan Hall[1], Yan Wang[1], Margaretha van der Merwe[2], Jim Whelan[1]

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Keywords: mitochondria, LETM, ATP synthase, water deficit, stress response, circadian rhythm,

Mitochondria acutely respond to plant abiotic stresses. The endosymbiotic origin of mitochondria and subsequent expansion of multicellularity suggests that these energy-providing organelles developed specific acquired functions, coordinated into their host metabolism via complex regulatory mechanisms. Recently, the integration mechanisms developed and the evolutionary consequence(s) of these transductions are starting to emerge. Here we characterised two nucleus-encoded, mitochondrial-localised LEUCINE ZIPPER-EF-HAND-CONTAINING TRANSMEMBRANE (LETM) proteins 1 and 2, required as assembly factors for ATP synthase with direct impact on ATP accumulation. Reduced LETM2 expression coupled with complete deletion of LETM1 mutants (*letm1*(-/-)/LETM2(+/-)) displayed altered response to abiotic stress. Metabolic profiling revealed enhanced starch and ascorbate levels and a decrease in reactive oxygen species generation. Testing abiotic stress tolerance in *letm1*(-/-)/LETM2(+/-) double mutants showed an enhanced tolerance to water deficit and a faster recovery response upon rehydration. Transcriptomic and metabolomic profiling reveals a distinct circadian response, that results in a more tolerant response to stress. Taken together, this study provides novel insights into the role of mitochondrial function and how it impacts both circadian rhythm and stress responses.

POSTER 58

Cauliflower mitochondrial biogenesis in temperature stress and recovery

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Keywords: abiotic stress, functional analysis, proteomics, transcriptomics, translation, ultrastructure

At least 22% of stress-responsive organellar proteins comprises the ones targeted to mitochondria. Complex studies for the elucidation of the biological relevance of plant mitochondrial responses to temperature stress and recovery are particularly limited.

We investigated the biogenesis of the cauliflower curd mitochondria under cold, heat and after the subsequent stress recovery.

Using various approaches, in heat and after heat recovery we noticed variations in abundance of CI, CIII subunits and import proteins, the presence of unassembled subunits of ATP synthase accompanied by impairment in mitochondrial translation and partial disintegration of matrix complexes. Interestingly, the transcription profiles of mitochondrial genes were uncorrelated in cold and heat. The ultrastructure of mitochondria was significantly altered only in stress recovery. Contrary to general stability of respiratory chain complexes in heat, functional studies showed that their activity and the ATP synthesis yield were affected. Heat stress resulted in lowered OXPHOS efficiency. It also increased AOX activity, protein, while heat recovery reversed AOX level and activity. Cold stress, however, led to the opposite effects, which were reversed after cold recovery. Overall, cauliflower AOX was only induced by heat stress. Contrary to AOX activity, the activity of rotenone-insensitive internal NADH dehydrogenase was lowered in heat.

We conclude that cauliflower mitochondria are actively engaged in the response to various temperature treatments. However, their biogenesis at multiple steps is not equally affected by investigated conditions.

POSTER 59

The mitochondrial Proline Dehydrogenase (ProDH) of Arabidopsis thaliana

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Keywords: proline, dehydrogenase, lactate

(Introduction) Proline has multiple functions in plants. Besides being a building block for protein biosynthesis proline is an important osmolyte, it protects cellular structures during dehydration, acts as a redox buffer and a signaling molecule and can directly act as scavenger of reactive oxygen species. The proline dehydrogenase (ProDH) is the first enzyme of the L-proline break-down pathway in plants. Here, we report an analysis on the integration of ProDH into mitochondrial metabolism in *Arabidopsis thaliana*. (Material and Methods) Induction of ProDH was measured by novel photometric activity assays and by a ProDH in gel activity assay. ProDH peptides from Arabidopsis were identified by mass spectrometry for the very first time in isolated mitochondria. (Results) Effects of increased ProDH activity on other mitochondrial enzymes were systematically investigated. Activity of glutamate dehydrogenase substantially increased, indicating up regulation of the entire proline catabolic pathway, which was confirmed by co-expression analyses of the corresponding genes. Furthermore, activity of D-lactate dehydrogenase was increased. D-lactate was identified to be a competitive inhibitor of ProDH in plants. (Conclusions) We suggest that induction of D-lactate dehydrogenase activity allows rapid up regulation of ProDH activity during the short-term stress response in plants.

POSTER 60

Biological Weighting Functions quantifying the effect of Ultra violet Radiation (UV_B) on the growth, Photo Synthesis & Photo Respiration of Ulva grown under Low light & High light Environment

Kumar Kayambu, Saravanan Krishnan
Dr Murugans Bioinformaticsmedia

Keywords: BWF,PS2,Induction kinetics

Introduction:

Solar ultraviolet radiation (UV) (290-400 nm) is a significant stressor in aquatic environments. It has adverse effects on photosynthesis (Lorenzen 1979, Cullen et al.1992) and growth (Ekelund 1990), and damages DNA (Karentz et al. 1991) in the major primary producers in most aquatic ecosystems. Broad band UVB (290-320 nm) effectively inhibits macroalgal photosynthesis more than UVA (320-400 nm). SERC has made major contributions to the worldwide monitoring of UVB radiation through the development of sophisticated UVB-sensing instruments. Particular attention is given the more biologically damaging, short-wavelength ultraviolet-B (UVB), which is increasing as air pollutants deplete stratospheric ozone, which normally blocks UVB. Mechanistically, absorption of high energy UVB photons damages proteins associated with photosystem II (Renger et al.1989, Babu et al.1999, Vincent and Neale 2000) and degrades the carbon fixing enzyme Rubisco (Bishcof et al. 2000).

Materials and methodsAlgae Source, Site and Collection. Ulva is collected from high (0 m) and low (2 m) light environments, a subestuary (RhodeRiver) of the Chesapeake Bay. stranded on the shoreline. It is often seen attached to piers or floating docks. Generally, it appears not to tolerate high temperature so it may be a difficult time to collect in the middle of the summer. Only healthy and clean fronds are used in order to avoid wound respiration (Bidwell and McLachlan 1985). Justification::

These data will provide an excellent base for modeling work (biological responses to UV) and also support to identify the site of UV damage in photosystems.

POSTER 61

Salicylic acid binding of mitochondrial alpha-ketoglutarate dehydrogenase E2, affects mitochondrial oxidative phosphorylation and electron transport chain components and plays a role in basal defense against TMV in tomato

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Keywords: alpha-ketoglutarate dehydrogenase E2, alternative oxidase, mitochondrial electron transport chain, plant-pathogen interactions, SA-binding protein, salicylic acid, Solanum lycopersicum (tomato), tobacco mosaic virus (TMV)

INTRODUCTION: Salicylic acid (SA) plays a critical role in plant defense against pathogen invasion. The SA-induced viral defense in plants is distinct from the pathways mediating bacterial and fungal defense and involves a specific pathway mediated by mitochondria; however, the underlying mechanisms remain largely unknown.

MATERIAL AND METHODS: The SA-binding activity of the recombinant tomato alpha-ketoglutarate dehydrogenase (*Sl*α-kGDH) E2 subunit of the tricarboxylic acid cycle was characterized. The biological role of this binding in plant defenses against *tobacco mosaic virus* (TMV) was further investigated via *Slα-kGDH E2* silencing and transient overexpression in tomato plants.

RESULTS: *Sl*α-kGDH E2 was found to bind SA in two independent assays. SA treatment, as well as *Slα-kGDH E2* silencing, increased resistance to TMV. SA did not further enhance TMV defense in *Slα-kGDH E2*-silenced tomato plants but did reduce TMV susceptibility in *Nicotiana benthamiana* plants transiently overexpressing *Slα-kGDH E2.* Furthermore, *Slα-kGDH E2*-silencing-induced TMV resistance was fully blocked by bongkrekic acid application and *alternative oxidase 1a* silencing.

CONCLUSIONS: These results indicated that binding by *Sl*α-kGDH E2 of SA acts upstream of and affect the mitochondrial electron transport chain, which plays an important role in basal defense against TMV. This study helps elucidate the mechanisms of SA-induced viral defense.

POSTER 62

Why carbonylated proteins accumulate in mitochondria lacking FtsH4 protease?

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Keywords: Arabidopsis, mitochondria, ATP-depended proteases, quality control, oxidative stress

FtsH4 is an ATP-dependent metalloprotease localized in the inner membrane of *Arabidopsis* mitochondria, with its catalytic domain facing the intermembrane space. We observed a link between the loss of FtsH4 function, oxidative stress and altered *Arabidopsis* morphology under short-day photoperiod, 22°C, and long-day photoperiod, 30°C. Numerous carbonylated proteins from various submitochondrial compartments accumulated in *ftsh4* mutants under these conditions, although the level of ATP-dependent proteases, predicted to digest them, was increased at the transcript and protein level. When isolated *ftsh4* mitochondria were supplemented with ATP, the level of oxidized proteins was significantly declined, while a similar treatment of wild type mitochondria only slightly reduced the level of carbonylated proteins. Moreover, we observed that deficiency of ATP in *ftsh4* correlates with decreased mitochondrial membrane potential and significantly reduced amount/activity of complex V. We believe that the loss of FtsH4 activity impairs amount/activity of complex V and in consequence causes the decreased ATP level, which is insufficient for proper function of the mitochondrial ATP-depended proteases.

POSTER 63

Characterization of mitochondrial early events in plant cells undergoing programmed cell death

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Keywords: Programmed cell death, Root, Tapetum

Mitochondrial membrane potential is generated by the proton gradient between the mitochondrial matrix and the inter membrane space. Depleting the membrane potential indicates that mitochondria are dysfunctional. Mitochondria were involved in tapetal PCD and stress-induced root cell death. ROS generation and loss of mitochondrial membrane potential are critical early events in animal programmed cell death but not so clear in plant PCD. Hydrogen peroxide (H_2O_2) had been shown to cause the programmed cell death in root hairs. The tapetum, which is the innermost layer of anther, provides nutrients and components for pollen development. Tapetal cells further undergo PCD during anther development. Here we observed the cytoplasm condensation which is a character of PCD, the reducing of mitochondrial membrane potential and ROS reducing in root hairs treated with H_2O_2. We also are capable to detect the mitochondrial membrane potential in tapetal cells. These techniques will facilitate the study on the role of mitochondria in plant PCD.

POSTER 64

Analysis of Arabidopsis lines with altered expression of alternative electron transport pathway components

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Keywords: alternative respiration, electron transport, dehydrogenase, transgenic, development, stress

Plant mitochondria contain electron transport components that are involved in respiration but are not linked to energy conservation, and which together comprise what is known as the alternative pathway (AP). Such components are the alternative oxidase (AOX) and a collective group of alternative NADH and NADPH dehydrogenases (NDs). These enzymes are embedded in the inner mitochondrial membrane and each is orientated toward either the mitochondrial matrix or the cytosol. We and others have shown previously that plants in which the expression of various AP components have been manipulated, display altered growth phenotypes and metabolism, particularly in the face of environmental stress.

Here, we analyse the growth characteristics and metabolite responses of a number of Arabidopsis lines with altered expression of a cytosol-facing ND.

We present data showing the impact of these genotypes on plant growth, performance and mitochondrial function, and address the hypothesis that elevated activities of the non-energy-conserving AP pathway will reduce productivity under optimal growth conditions, whilst improving performance upon exposure to sub-optimal growth conditions.

POSTER 65

The effect of HrpWpto and HrpZpto treatment on ascorbate metabolism

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Keywords: oxidative burst, elicitor, mitochondria, ascorbate

Application of purified harpin proteins to plant cells/tissues can elicit the oxidative burst. Hypersensitive response and oxidative burst can be modified by the levels or redox status of antioxidants, such as ascorbate. The level of ascorbate is determined by its biosynthesis, recycling and by the ascorbate-consuming reactions. Thus we aimed at the investigation of the changes in ascorbate biosynthesis and recycling due to different harpin ($HrpW_{pto}$ and $HrpZ_{pto}$) treatments.

The reaction catalysed by the VTC2, VTC5 homolog pair is considered to be the rate limiting step of ascorbate biosynthesis. Thus the expression of both genes has been investigated upon $HrpW_{pto}$ and $HrpZ_{pto}$ harpin treatments. Interestingly the two genes showed different expression pattern upon the harpin treatments. There has not been significant difference in the expression of VTC2 gene. However the expression of VTC5 has been elevated by at least 5-times due to $HrpW_{pto}$ or $HrpZ_{pto}$ treatment. Beyond the expression of VTC2, VTC5 both the expression and enzyme activity of the mitochondria coupled ascorbate biosynthetic enzyme, GLDH has been followed. There has not been any difference in the gene expression of GLDH, however significantly higher GLDH enzyme activity could be observed in the mitochondria from harpin treated plants compared to the non-treated. Furthermore the activity of the enzymes of the ascorbate-glutathione cycle have also been elevated.

POSTER 66

Effect of carrot AOX1 natural sequence diversity on protein functionality

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Keywords: Alternative oxidase, AOX, carrot, abiotic stress, plant adaptation, polymorphism, wild relatives, climate changes.

The role of alternative oxidase (AOX), a key enzyme in the alternative respiration pathway, in plant tolerance to diverse abiotic and biotic stresses has been described across species, mainly focused on the AOX1-subfamily gene members. It is known that the ability of plants to adapt their phenotype to various conditions is genetically determined, and several studies show that genetic variation affects alternative respiration which is related to growth behaviour. Taken together, the hypothesis of the existence of polymorphisms within the *AOX1* gene sequence that could mark plant adaptation to specific climatic conditions is being explored by the ongoing project in *Daucus carota* L. (EXCL/AGR-PRO/0038/2012). The analysis of *DcAOX1* gene sequence (at genomic DNA level) from 450 carrot wild relatives from 45 different accessions (distributed by 3 distinct climate regions according to the Köeppen-Geiger climate classification system) allowed the identification of polymorphisms located at the exon sequences. Here we will present the results of functional studies based on the use of a yeast-expression system (*Schizosaccharomyces pombe*) to evaluate the effect of polymorphisms on protein functionality (AOX capacity).

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POSTER 67

Identification of stress tolerance alternative respiratory genes in rice

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Keywords: alternative oxidase, alternative dehydrogenase, oxidative stress

Plant mitochondria possess two distinct respiratory pathways; the cytochrome pathway and alternative pathway. The non-phosphorylating alternative pathway (AP) contains two protein groups, alternative dehydrogenases and alternative oxidases. It is suggested that these proteins play a role in stress tolerance in plants by regulating the production of Reactive Oxygen Species (ROS) within the cell, and thereby maintaining the normal growth and development of the plant under stress conditions. Three isoforms of alternative oxidases, (Aox1a, Aox1b and Aox1c) and six alternative dehydrogenases (OsNda1, OsNda2, OsNdb1, OsNdb2, OsNdb3, and OsNdc1) have been identified in rice. Transcript levels of these nine alternative respiratory proteins were assessed in rice plants exposed to oxidative stresses using Antimycin A and potassium cyanide treatments, and also in rice plants exposed mild salt stress. For salt stress, qRT-PCR data revealed that OsAox1a and OsNdb2 were the most stress responsive AP genes. A search of Gene Investigator database for responses of AP genes to salt stress indicated a great deal of variability. However, some experiments clearly showed a strong increase in Aox1a in agreement with the experimental findings presented here. Currently, transgenic rice overexpressing AtAox1a and AtNdb2 are being generated for the future experiments of AP genes on the abiotic stress responses of rice plants.

POSTER 68

Roles of the respiratory system in alleviation of photoinhibition via the photorespiratory system

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Keywords: respiratory system, photoinhibition, photorespiratory system

Leaves exposed to high light stress suffer photoinhibition that means impairs of photosystem(s) caused by excess energy. It is considered that a part of excess reducing equivalents generated in chloroplasts are transferred to mitochondria via the malate-OAA shuttle and photorespiratory system, and then dissipated by the respiratory system. It was reported that the chloroplast *nadp-mdh* (NADP-dependent malate dehydrogenase) mutant did not exhibit the photoinhibition even at high light condition and up-regulated the photorespiratory and other antioxidant systems. On the other hand, in a photorespiratory mutant, photoinhibition was induced by high light treatment. However, there are few reports that examined a relationship between respiratory activity and alleviation of photoinhibition via the photorespiratory system.

In this study, we examined effects of decrease in respiratory activity on alleviation of photoinhibition via the photorespiratory system using Arabidopsis leaves and a respiratory inhibitor.

At ambient CO_2 condition, the decrease in cytochrome pathway activity by antimycin A induced photoinhibition. However, this antimycin A-induced photoinhibition was alleviated at high CO_2 condition. In addition, *aox1a* (alternative oxidase 1a) mutants showed more severe photoinhibition than WT. The aox1a mutants also showed large decreases in qP value and slow induction of NPQ.

These results showed that once Rubisco oxygenation reaction occurred, respiration is essential to circulate the photorespiratory system. AOX deficiency would have a certain effect on the photosystem in chloroplasts.

POSTER 69

Ceramide Accumulation Potentiates Programmed Cell Death and Increases Susceptibility to Pathogen in Plants

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Keywords: ceramide, mitochondria, programmed cell death, sphingolipids

Introduction: Sphingolipids act as signaling molecules in the regulation of apoptosis, senescence, and inflammation. Ceramides are key intermediates in sphingolipid biosynthesis and catabolism. *Arabidopsis thaliana* plants that lack ceramide kinase (*acd5* mutants) display spontaneous programmed cell death late in development.

Material and methods: We identified ACD5 localization by isolating various cellular compartments and by using confocal and immunoelectron microscopy. Measurement of sphingolipids was performed by electrospray ionizationtandem mass spectrometry analysis.

Results: ACD5 and ceramide kinase activity localize to multiple membrane compartments including plasma membrane and mitochondria. Quantitative sphingolipid profiling indicated that ceramide accumulation in ceramide kinase deficient mutants paralleled the appearance of spontaneous cell death, and it was accompanied by autophagy and mitochondrial reactive oxygen species (ROS) accumulation. Ceramide kinase affects sphingolipid metabolism and sites of ROS accumulation during development and pathogen infection. The lack of ceramide kinase plants also showed an early defect in restricting fungal spore germination and growth, which occurred prior to the onset of cell death.

Conclusions: Endogenous ceramide accumulation is a key factor in plant cell death. Ceramide kinase affects ROS production and regulates defense response. Mitochondrial ROS is associated with ceramide-induced cell death. We suggest that there are multiple roles for ceramides in both cell death control and defense against pathogen infection.

SESSION: PROTEIN SYNTHESIS, MODIFICATION AND QUALITY CONTROL

POSTER 70

Functional conservation between yeast and plant mitochondrial m-AAA proteases

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Keywords: m-AAA protease, Arabidopsis thaliana, mitochondrial protein synthesis

AAA proteases (also called FtsH) are ATP-dependent metallopeptidases located in the mitochondrial inner membrane. They are bifunctional enzymes in which the ATPase module with chaperone-like properties is fused to the proteolytic domain. Previously, we identified two matrix (m) – AAA proteases - AtFtsH3 and AtFtsH10 in mitochondria of *Arabidopsis. thaliana*. Both proteases assemble with prohibitins into high-molecular–weight complexes, similarly to their yeast counterparts (Yta10 and Yta12).

In yeast, m-AAA protease mediates maturation of ribosomal subunit MrpL32 and using chaperone activity helps in processing of cytochrome c peroxidase (Ccp1) by correct positioning of this protein. Expression of *Arabidopsis* m-AAA in yeast *yta10/12* mutants recovers proteolytic activation of accumulated precursors of both MrpL32 and Ccp1. Thus, AtFtsH3 and AtFtsH10 can substitute not only proteolytic but also chaperone activity of Yta10/12 complex.

The ability of plant m-AAA to process the yeast ribosomal protein MrpL32 *prompted us* to investigate translation of mitochondrially encoded protein in *ftsh3/10* mutant. Obtained results showed impaired mitochondrial protein synthesis and in consequence decreased amount of the OXPHOS compared to the wild type. Our study imply that the plant m-AAA protease, similarly to Yta10/Yta12 complex, regulates translation within mitochondria.

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POSTER 71

Identification and characterization of novel components of Arabidopsis mitochondrial ATP-independent proteolytic machinery.

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Keywords: quality control system, ATP-independent proteases

The Arabidopsis genome contains homologues of all known yeast ATP-independent peptidases with the exception of thiol aminopeptidase. Up to now homologues of Imp complex (Imp1 and Imp2), Oma1 and Atp23 were not yet characterized. Here, using transient GFP expression assay in protoplasts and Western blot analysis we proved that these proteases are also present in Arabidopsis mitochondria. To check whether the function of identified Arabidopsis mitochondrial proteases is similar to their yeast counterparts, we performed functional complementation in yeast. Our results demonstrated that out of all investigated proteases only Oma1 is able to replace function of the yeast homologue and suggest the function of the other (Imp1, Imp2 and Atp23) proteases differs from their yeast counterparts and evolved in a specific manner. Observations *of* Arabidopsis l*ines* with *T-DNA insertions* in Oma1 and Imp1 s*howed a lack of significant morphological and developmental changes* under optimal growth conditions. However, *oma1-1* knockout plants exhibited reduced growth rate in long day photoperiod at moderate temperature of 30˚C. By combination of BN-PAGE and histochemical staining of the OXPHOS complexes we were able to show that lack of homologue of Oma1, but not Imp, caused reduced activity and protein amount of complex V. In summary, the obtained results suggest that in plants Oma1 is an important novel component in mitochondria for functionality of OXPHOS system but the mechanism of its action remains unknown.

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POSTER 72

A proteolytic pathway for amino acid recycling in mitochondria and chloroplasts

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Keywords: Peptidase; targeting peptide; amino acid; mitochondria

Despite containing their own genomes, more than 99 % of proteins localized in mitochondria or in chloroplasts are encoded in the nucleus and synthesized in cytosolic ribosomes. To ensure correct targeting, most proteins destined to the mitochondrial matrix or the chloroplastic stroma contain an amino-terminal extension designated targeting peptide.

After import the targeting peptides are cleaved off and degraded. Recently we have characterized a pathway with two proteolytic components (the Presequence Protease, PreP, and the Organellar Oligopeptidase, OOP) involved in the degradation of targeting peptides in mitochondria and chloroplasts. In the present work, we show that targeting peptides can be sequentially cleaved to generate single amino acids by a pathway including PreP, OOP and at least four aminopeptidases of distinct specificity. These findings are of importance to understand nutrient recycling in endosymbiotic organelles.

SESSION: MITOCHONDRIA AND PLANT DEVELOPMENT

POSTER 73

Identifying novel functions of mitochondria during natural leaf senescence

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Keywords: mitochondria, natural leaf senescence, transcriptomics, metabolomics

In mammals, it has been shown that mitochondria play a crucial role in the mechanisms controlling cell death induction and aging processes. Conversely, in plants the role of mitochondria and their impact on cellular metabolism during aging, in particular during natural leaf senescence (NLS) remains poorly understood. In this study, we used a multi-disciplinary approach including microscopy, transcriptomic, metabolomic and physiological analyses to gain a better understanding of the shifting role of mitochondria during NLS. First, we generated a metabolic overview of mitochondrial functions during leaf senescence, which clearly demonstrated that mitochondrial primary metabolism is stable throughout NLS, despite a progressively declining number of mitochondria, observed using microscopy. Remaining mitochondria retained an intact ultrastructure until the very late stages of leaf senescence. Intriguingly, isolated mitochondria showed a changing substrate specificity throughout leaf senescence, which led to altered respiratory capacities. Furthermore, our data strongly suggests that mitochondria play an important role in nitrogen remobilization during NLS. From the transcriptomic analysis, we identified a subset of genes of unknown functions, the products of which are predicted to be targeted to mitochondria and specifically up-regulated during NLS. We are currently investigating several candidates at the molecular, biochemical and physiological levels. Taken together, these results will help us to understand the process of natural leaf senescence better and shed light on an enigmatic player in this process: the mitochondrion.

POSTER 74

Mitochondria-derived reactive oxygen species are required for polar auxin transport in Arabidopsis organ formation

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Keywords: ros, plant development, polar auxin transport

Mitochondria are the site of reactive oxygen species (ROS) generation and act as the principal energy source in cells. Mitochondria are also known to be associated with programmed cell death, and cellular and organismal aging in eukaryotes, such as yeast, plants and animals. However, their function in developmental processes remains uncertain in higher plants. Here, we provide experimental evidence that mitochondria contribute to organ formation. The formation of organs such as leaves, flowers and roots is highly dependent on polar auxin transport. Polar-localized PIN-FORMED (PIN) proteins, auxin efflux carriers, transport auxin to the organ initiation site and promote organ growth. We performed genetic screens for novel factors involved in auxin-regulated organ formation and identified a novel mutant, *macchi-bou 1* (*mab1*), that affects the mitochondrial pyruvate dehydrogenase E1 subunit. PIN abundance and polarity were disrupted in the defective organ primordia of *mab1* mutants. Pharmacological analysis showed that PIN internalization was accelerated in the mutant. We also found ROS accumulation in a MAB1-dependent manner in organ primordia. Chemically-induced reduction of ROS altered the localization of MAB4, a negative regulator of PIN internalization, and enhanced PIN endocytosis. Our results demonstrate that *MAB1* regulates polar auxin transport through the control of ROS generation, and reveal an unexpected role for mitochondria in the control of cell polarity through ROS generation.

POSTER 75

T-DNA insertions up-stream of a cytosolic-facing NAD(P)H dehydrogenase gene (AtNdb2), alter growth and stress responses in Arabidopsis thaliana.

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Keywords: NADH dehydrogenase, ndb2, growth effects

Respiration is essential for the growth and development of plants. In addition to the conventional oxidative phosphorylation pathway, plants contain a non-phosphorylating alternative electron transport pathway in their mitochondria, which includes the alternative oxidase (AOX) and several alternative NAD(P)H dehydrogenases. In *Arabidopsis*, there are seven genes which encode these dehydrogenases; four of these proteins are localised to the outside of the inner membrane, (i.e. facing the cytosol; Atndb1-4) and three to the inside of the inner membrane (i.e. facing the matrix; Atnda1,2 and Atndc1). Together, these enzymes provide a mechanism for NAD(P)H oxidation that bypasses complex I. In this study, two T-DNA insertion lines, SALK_125427 and SALK_064041 were characterised. These lines each have a T-DNA insertion upstream of the Atndb2 gene, located adjacent to a proposed repressor site (SALK_125427) and a proposed regulatory element (SALK_064041). Both lines show altered phenotypes with respect to the timing of bolt development and the size of bolts. There was also an altered response to conditions that have been shown to cause enhanced Atndb2 expression. These T-DNA lines display lower root growth in the presence of rotenone when compared to wild type plants. These findings will be discussed in relation to the disruption of Atndb2 expression during plant development.

POSTER 76

MITOCHONDRIAL PPR-CONTAINING PROTEINS ARE ESSENTIAL TO SUSTAIN EMBRYO DEVELOPMENT IN Arabidopsis thaliana

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Keywords: Pentatricopeptide Repeat proteins, embryogenesis. artificial microRNA silencing

PPRs containing proteins are characterized by a motif of a degenerate 35 aminoacid repeat. Most PPR containing proteins are specific RNA-binding proteins which are involved in RNA processing including editing, maturation, stability, and translation. We focused on the role of PPR proteins potentially involved in embryo development. Four T-DNA insertional mutants in genes encoding for three PPR proteins (At2g02150, At1g79490 and At3g29290) were analyzed. These mutants showed around 25% of shriveled seeds in mature siliques, which do not germinate even in MS sucrose medium, suggesting embryo lethality. Transient transformation with these PPR proteins fused to GFP showed that they are localized in mitochondria. As we were unable to obtain homozygous mutant plants, silenced plants were developed by using two independent artificial microRNAs for each gene. We obtained plants in which around 70% of gene expression was silenced. Mature siliques of these silenced plants were analyzed. Most siliques were short containing mostly aborted embryos. To further investigate this phenotype, we examined female and male gametophyte development. Apparently, embryo sac proceeded normally but anther maturation did not, showing pollen grains that remain stuck inside anthers, thus pollination could not progress. We are now interested in potential target of these particular PPR proteins. These results suggest that these mitochondrial PPR proteins are involved in embryogenesis and that their expression is essential for anther maturation in A. thaliana.

POSTER 77

THE MITOCHONDRIAL ELECTRON SHUTTLE ADX-ADXR IS ESSENTIAL FOR GAMETOPHYTE DEVELOPMENT IN Arabidopsis thaliana

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Keywords: Adrenodoxin, adrenodoxin reductase, female gametophyte

In Arabidopsis, the female gametophyte is a polarized structure composed by seven cells: the egg cell, the central cell, two synergids and three antipodal cells. We here show the study of Arabidopsis mutants with insertions in *ADX* or *ADXR*, which are nuclear genes encoding for mitochondrial adredodoxin and adrenodoxin reductase, respectively. Siliques of *ADXR/adxr* plants presented around 40% of abortions and reciprocal crossings showed that transmission through the female gametophyte is severely compromised. DIC studies revealed mutant gametophytes with abnormal cell morphologies that are not able to reach complete maturation. The identity of all cells inside *adrx* embryo sacs was analyzed by studying the expression of cell specific markers for egg cell, central cell, antipodal and synergid cells. The results of anilin blue staining assays and pollination using the pollen tube marker pLAT52-RFP line as a pollen donor, suggest that attraction of pollen tubes is impaired in mutant embryo sacs. Pollen tubes showed abnormal growing patterns, including invading embryo sacs, which suggest the communication between the male and the female gametophyte is impaired. The expression pattern of *ADRX* indicates that its expression is specific for the maternal tissues of the ovules. Two hybrid assays and BiFC experiments identified mitochondrial interactors for ADX that are related to several metabolic pathways.

SESSION: MITOCHONDRIAL BIOCHEMISTRY AND METABOLISM

POSTER 78

Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria

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Keywords: Arabidopsis, redox regulation, thioredoxin TCA cycle regulation, citric acid cycle regulation, ATP-citrate lyase

Plant mitochondria have a fully operational tricarboxylic acid (TCA) cycle that plays a central role in generating ATP and providing carbon skeletons for a range of biosynthetic processes in both heterotrophic and photosynthetic tissues. The cycle enzyme-encoding genes have been well characterized in terms of transcriptional and effector-mediated regulation and have also been subjected to reverse genetic analysis. However, despite this wealth of attention, a central question remains unanswered: "What regulates flux through this pathway *in vivo*?" Previous proteomic experiments with Arabidopsis discussed below have revealed that a number of mitochondrial enzymes, including members of the TCA cycle and affiliated pathways, harbor thioredoxin- (TRX-) binding sites and are potentially redox regulated. We have followed up on this possibility and found TRX to be a redox-sensitive mediator of TCA cycle flux. We first characterized, at the enzyme and metabolite levels, mutants of the mitochondrial TRX pathway in Arabidopsis: the NADP-thioredoxin reductase double mutant (*ntra ntrb*) and the mitochondrially located *trxo1* mutant. These studies were followed by a comparative evaluation of the redistribution of isotope when ^{13}C-glucose, ^{13}C-malate or ^{13}C-pyruvate was provided as substrate to leaves of mutant or wild type plants. In a complementary approach, we evaluated the *in vitro* activities of a range of TCA cycle and associated enzymes under varying redox states. The combined dataset suggests that TRX acts as a direct regulator of carbon flow through the TCA cycle and provides a mechanism for the co-ordination of cellular function.

POSTER 79

Who makes ATP for the cytosol in photosynthetic cells?

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Keywords: mitochondria, chloroplasts, ATP

In photosynthetically active cells both chloroplasts and mitochondria have the capacity to produce ATP via photophosphorylation and oxidative phosphorylation respectively. Thus, in principle both organelles could provide ATP for the cytosol but it is not clear to what extent they actually do this and how the process is regulated. Here we report on experiments with rapid fractionation of isolated protoplasts subjected to different treatments in combination with application of specific inhibitors. In conditions where photosynthetic CO_2 fixation is high our results indicate that mitochondria supply the bulk of ATP for the cytosol. On the contrary, in conditions when CO_2 fixation is limited, ATP will build up in chloroplasts and then be exported to the cytosol, the process in which the triose phosphate translocator is involved. Thus, depending on the condition either chloroplasts or mitochondria can supply ATP for the cytosol. The results are discussed in relation to the idea that mitochondrial functions may be tuned to provide an optimal environment for the chloroplast. By balancing cellular redox states mitochondria can contribute to an optimal photosynthetic capacity.

POSTER 80

Succinate dehydrogenase assembly factor 2 is needed for assembly and activity of mitochondrial complex II and for normal root elongation in Arabidopsis

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Keywords: Succinate Dehhdrogenase, Plant Mitochondria, Electron Transport Chain

Succinate dehydrogenase (SDH) plays a central role in respiratory metabolism as a component of both the electron transport chain and the TCA cycle. We report a SDH assembly factor by analysis of T-DNA insertions in At5g51040. This gene is co-expressed with a number of genes encoding mitochondrial proteins, including SDH1-1, and has low partial sequence similarity to human SDHAF2, a protein required for flavin-adenine dinucleotide (FAD) insertion into SDH. In contrast to observations of other SDH deficient lines in Arabidopsis, the sdhaf2 line did not affect photosynthetic rate or stomatal conductance, but instead showed inhibition of primary root elongation with early lateral root emergence, presumably due to the low SDH activity caused by the reduced abundance of SDHAF2. Both roots and leaves showed succinate accumulation but different responses in the abundance of other organic acids and amino acids assayed. Isolated mitochondria showed lowered SDH1 protein abundance, lowered maximal SDH activity and less protein-bound flavin-adenine dinucleotide (FAD) at the molecular mass of SDH1 in the gel separation. The short root phenotype and SDH function of sdhaf2 was fully complemented by transformation with SDHAF2. Application of the SDH inhibitor, malonate, phenocopied the sdhaf2 root architecture in WT. Whole root respiratory assays showed no difference between WT and sdhaf2, but micro-respirometry of the tips of roots clearly showed low oxygen consumption in sdhaf2 which could explain a metabolic deficit responsible for root tip growth.

POSTER 81

Non-coupled pathways of plant mitochondrial electron transport support high rates of glycine oxidation

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Keywords: alternative oxidase, glycine decarboxylase, photorespiration, rotenone-insensitive dehydrogenases

Mitochondria of C_3 plant leaves in the light support oxidation of high fluxes of photorespiratory glycine, which is synthesized in the glycolate pathway as a consequence of the oxygenase reaction of Rubisco, while the oxidation of respiratory substrates, although partially inhibited, proceeds simultaneously with the photorespiratory glycine oxidation. High rates of glycine oxidation are sustained through the kinetic mechanisms leading to the increased capacity of the mitochondrial electron transport chain via switching to the non-coupled pathways. The expression of glycine decarboxylase (GDC) P-protein is coordinated with the expression of the alternative oxidase (AOX) as monitored by Western blotting. Glycine oxidation also engages rotenone-insensitive NADH and NADPH dehydrogenases via rising of NADH and, by transhydrogenation, of NADPH. Rising NADH due to the GDC reaction not only engages the non-coupled pathways but also results in intensification of the malate and citrate mitochondrial valves. The mechanisms of high photorespiratory flux maintenance in mitochondria are fulfilled under the conditions where concentrations of the GDC reaction products CO_2 and NADH achieve an equilibrium provided by the mitochondrially localized carbonic anhydrase and malate dehydrogenase, respectively. We discuss also a possible role of carbonic anhydrase in conversion and transport of the photorespiratory NH_3. This results in the removal of these products from the GDC multienzyme active sites and in the maintenance of their concentrations at the levels that are sufficiently low to prevent substrate inhibition of the reaction.

POSTER 82

Contribution of plant mitochondrial electron transport chain to the generation and scavenging of nitric oxide

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Keywords: nitric oxide, alternative oxidase, complex I, aconitase, anoxia

Plant mitochondria represent a major source of nitric oxide (NO) under anoxia. NO is generated via the transfer of electrons to nitrite by cytochrome c oxidase and possibly by other electron transport chain (ETC) components. We have used the transgenic tobacco plants deficient in complex I (obtained from Dr. R. de Paepe) and in the alternative oxidase (from Dr. G. Vanlerberghe) to establish the contribution of these protein complexes to NO metabolism under anoxia. The complex I-deficient plants were characterized by very low (ten times lower than the wild type) emissions of NO, which was related to a decreased flux through ETC, and by the elevated expression of class 1 phytoglobin that scavenges NO even under normoxic conditions. There was no evidence of direct involvement of complex I in NO metabolism. The alternative oxidase (AOX) tobacco mutants also showed changes in the rates of NO emissions which were lower by 30-40% in AOX downregulating plants as compared to the wild type and AOX overexpressing plants. The activity of aconitase, which is sensitive to the level of reactive oxygen and nitrogen species, was the highest in AOX downregulating plants and lowest in AOX overexpressing plants. The effect was more drastic for the mitochondrial form of aconitase as compared to the cytosolic form. We conclude that complex I and AOX, although they are likely not involved directly in NO production and scavenging, regulate NO levels via the changes in distribution of electron flow to nitrite. The importance of contribution of different ETC complexes to NO metabolism during adaptation to abiotic and biotic stresses is discussed.

POSTER 83

In search of pyruvate dehydrogenase phosphatase

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Keywords: regulation, protein phosphorylation, kinase, phosphatase, localization

We reported in 1977 that the plant mitochondrial pyruvate dehydrogenase complex (mtPDC) is regulated by reversible phosphorylation. The E1a subunit is phosphorylated on Ser292 by a dedicated, intrinsic kinase (PDK) inactivating the complex. P-E1a can be dephosphorylated by an intrinsic PP2C-protein phosphatase (PDP), re-activating the complex. The subunits of the mtPDC were cloned, expressed, and characterized in vitro in the mid-1990's, and both maize and Arabidopsis PDK's were cloned and expressed in 1998. While we have characterized native pea seedling PDP, the cloning of this important regulatory component has eluded us. In 2013, the in depth-proteomic analysis of highly purified potato tuber mitochondria yielded a peptide corresponding to a PP2C, an enzyme encoded by large multi-gene families in most plants (e.g., 76 PP2C-encoding genes in A. thaliana.). Results from sequence comparisons suggest that the putative potato mitochondrial PP2C is most closely related to A. thaliana proteins NP_565696 (PP2C26-like), NP_193391 (AtPP2C55-like), and NP_201473 (AtPP2C80-like). The next step in our odyssey involves localization of these three candidate proteins. The C-terminal GFP-chimera have been constructed and used for stable transformation of A. thaliana plants, and transient expression in onion epidermal peels.

POSTER 84

Disruption of the CYTOCHROME C OXIDASE DEFICIENT1 Gene Leads to Cytochrome c Oxidase Depletion and Reorchestrated Respiratory Metabolism in Arabidopsis

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Keywords: Complex IV mutant - Respiratory complexes - Alternative oxydase - Mitochodrial metabolism

Cytochrome c oxidase is the last respiratory complex of the electron transfer chain in mitochondria and is responsible for transferring electrons to oxygen, the final acceptor, in the classical respiratory pathway. The essentiality of this step makes it that depletion in complex IV leads to lethality, thereby impeding studies on complex IV assembly and respiration plasticity in plants. Here, we characterized Arabidopsis (Arabidopsis thaliana) embryo-lethal mutant lines impaired in the expression of the CYTOCHROME C OXIDASE DEFICIENT1 (COD1) gene, which encodes a mitochondria-localized PentatricoPeptide Repeat protein. Although unable to germinate under usual conditions, cod1 homozygous embryos could be rescued from immature seeds and developed in vitro into slow-growing bush-like plantlets devoid of a root system. cod1 mutants were defective in C-to-U editing events in cytochrome oxidase subunit2 and NADH dehydrogenase subunit4 transcripts, encoding subunits of respiratory complex IV and I, respectively, and consequently lacked cytochrome c oxidase activity. We further show that respiratory oxygen consumption by cod1 plantlets is exclusively associated with alternative oxidase activity and that alternative NADH dehydrogenases are also up-regulated in these plants. The metabolomics pattern of cod1 mutants was also deeply altered, suggesting that alternative metabolic pathways compensated for the probable resulting restriction in NADH oxidation. Being the first complex IV-deficient mutants ever described in higher plants, cod1 lines should be instrumental to future studies on respiration homeostasis.

POSTER 85

Mitochondrial Regulation through Thiol Switching in Plants

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Keywords: mitochondrial redox regulation, thioredoxin, glutaredoxin, NADPH

Plants have the remarkable ability to fix energy of the sunlight to build, maintain and reproduce themselves. On cellular level mitochondria and chloroplasts need to coordinate their respective activities. While it is well established that in the chloroplast such control can be achieved by regulatory thiol redox switches, corresponding regulation of energy metabolism in the mitochondrion is less clear. In mitochondria, a complete machinery for thiol redox regulation exist and its potential target enzymes have been identified *in vitro*. However, it remains largely unknown how exactly the mitochondrial thiol redox machinery works and what role regulatory thiol switching plays under physiological conditions. The mitochondrial thiol redox machinery includes a thioredoxin and a glutathione system; both receive their reductant from primary metabolism via NADPH. We have established a novel *in organello* assay to dissect thiol redox switching in Arabidopsis mitochondria. The setup allows to internally produce NADPH by defined substrate feeding while monitoring the thiol redox systems using redox sensitive fluorescent protein sensors and manipulating the topology of the redox machinery genetically. By establishing of physiologically meaningful conditions we are now in a position to dissect the interplay between energy and redox metabolism in plant mitochondria in a mechanistic manner and identify thiol redox switches that are actually operated, rather than just maintained, in the living plant.

POSTER 86

Methylglyoxal detoxification in plants - functional characterization of two putative Glyoxalase I proteins of Arabidopsis

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Keywords: Methylglyoxal, glyoxal, Glyoxalase I, metabolite repair

Intro: During Glycolysis, methylgloxal (MG) is formed by spontaneous loss of the phosphoryl group by beta-elimination or the enzymatic reaction of triose phosphate isomerase. MG is a highly reactive alpha-oxo aldehyde, which is detoxified to D-lactate mainly by the Glyoxalase system. The Glyoxalase system is composed of the enzymes Glyoxalase I and II, that convert alpha-oxoaldehydes into the corresponding alpha-hydroxyacids using glutathione (GSH) as a cofactor. Arabidopsis possess two putative Ni2+ dependent Glyoxalase I genes (GlyI & GlyH) that are predicted to localize to mitochondria and/or plastids depending on the use of alternative splicing sites. We started to characterize the molecular and physiological role of the different Gly I isoforms. M&M: Enzymatic properties of recombinant Gly I and GlyH were determined. Expression of splicing variants as well as their sub cellular localization was analyzed. T-DNA insertion lines were used to investigate the full MG detoxification pathway via feeding experiments. Results: Both GlyI isoforms reveal a high affinity to MG and glyoxal. Under non-stress conditions the isoforms are expressed constitutively. As the isolated single mutants show no apparent phenotype, double mutants were generated. We analysed the predicted mitochondrial localization of both enzymes. Conclusions: We identified and characterized two Ni2+ dependent GlyIs from Arabidopsis, which are involved in the MG pathway. This pathway takes place both in the cytosol and the mitochondria, where D-lactate is converted to pyruvate, avoiding the accumulation of toxic byproducts and ensuring energy conservation.

POSTER 87

Functional and proteomic comparison of V. faba root and symbiotic root nodule mitochondria

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Keywords: Symbiosis, Vicia faba, Rhizobium leguminosarum, Root, Two-dimensional gel electrophoresis, Shotgun mass spectrometry

Introduction: Mitochondria provide energy for plant growth and development. They have to adapt their functions in response to varying physiological conditions. As such also plant- rhizobia interactions in legumes are expected to influence mitochondrial biology. During the association of the legume plant *Vicia faba* with the symbiont *Rhizobium leguminosarum* specific structures (nodules) are formed. As a consequence the metabolism of the plant root changes to provide energy for the nitrogen fixing bacteria and to process nitrogen compounds. In the frame of the symbiosis the malate/fumarate metabolism, as well as other metabolic pathways of the host plant, are affected. For example, the amino acid catabolism is influenced. However, many details of the involved physiological processes, especially regarding mitochondria, are still unclear.

Material and methods: A proteomic investigation has been initiated to systematically analyze the important role of mitochondria during the symbiosis between plants and rhizobial bacteria. Our experimental approach is based on protein separation by two-dimensional (2-D) electrophoresis in combination with mass spectrometry or shotgun MS.

Results: Initial results will be presented and discussed with respect to the energetic requirements of the symbiosis.

POSTER 88

Import(ance) of Plant Mitochondrial Calcium

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Keywords: Mitochondrial calcium, calcium uniporter, respiratory control

Introduction

Mitochondria are the major cellular source of ATP in most complex life. Respiratory ATP provision needs to match cellular needs and requires tight regulation. Metabolic flexibility is particularly important in plants where respiration needs to be coordinated with photosynthesis. The mechanisms by which respiration is controlled in plants are, however, poorly understood. In mammals, matrix calcium regulates energy metabolism by modulating the activity of key TCA cycle dehydrogenases. The recently identified mitochondrial calcium uniporter mediates import of calcium into the matrix. Plants possess gene homologues of the uniporter but their function is yet unclear.

Material and Methods

We used GFP-fusions to localize uniporter homologues within plant cells. In conjunction, we worked with genetically encoded cameleon sensors in to assess calcium levels and dynamics in living plants by confocal microscopy.

Results

We have found uniporter components within *Arabidopsis* mitochondria. Knock-out mutants show altered mitochondrial calcium levels, indicating impaired calcium transport.

Conclusions

Plant mitochondria regulate calcium import through uniporter components. This suggests a yet unknown role of calcium in matching the organelle's function with cellular demands. We now seek to understand the role of calcium in the dynamic control of plant mitochondrial function. In addition, we aim to dissect the interaction between calcium dynamics and other parameters of the mitochondrial physiology network, such as pH and H_2O_2. The significance of mitochondrial calcium transport for plant performance will be discussed.

POSTER 89

New insights into the physiological role of the CA domain of Arabidopsis respiratory Complex I.

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Keywords: gamma carbonic anhydrase, complex I, photorespiration

In Arabidopsis thaliana there are five gamma carbonic anhydrase proteins, called CA1, CA2, CA3, CAL1 and CAL2. These proteins form an extra domain (CA domain) in the membrane arm of the mitochondrial complex I. Its physiological role is currently unknown. None of the T-DNA insertional single mutants show any visible phenotype under normal conditions. In order to investigate the role of the CA domain, we performed crosses of single mutants to obtain different double mutants. The mutants *ca2cal1*, *ca2cal2* and *ca2ca3* show a photorespiratory phenotype showing growth retardation in normal air. However, this phenotype is rescued by cultivating plants in a high carbon dioxide atmosphere. Moreover, under photorespiratory conditions, carbon assimilation is reduced and glycine accumulates, which suggests a photorespiratory imbalance. These results strongly suggest that the CA domain of plant complex I contributes to sustain an efficient photosynthesis at ambient (photorespiratory) conditions. On the other hand, the double mutants *ca1ca2* and *ca1ca3* present an embryo lethal phenotype due to strong respiratory problems triggering ROS accumulation. Dry seeds are shrunken and dark brown, however are able to germinate two weeks later than the WT. Indeed, double knockout seedlings die likely due to oxidative stress. This phenotype is similar to that observed in cal1cal2 silenced plants, suggesting it is neccesary at least one CA and one CAL protein to form a functional CA domain.

POSTER 90

Elucidation of the TCA metabolon and the regulation of TCA enzymes in plants

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Keywords: metabolon, protein phosphorylation, metabolism

The plant tricarboxylic acid (TCA) cycle is not only recognized as a process for energy production but also as a core of the central carbon metabolism connecting many pathways of primary metabolism. The activity of this pathway is known to be finely regulated by the combinatorial regulation of individual enzymes but the molecular basis of network level regulation is still to be investigated. Accumulating evidences support the idea of metabolic regulation by the organization of enzyme protein complexes, so called „metabolon", allowing fast and fine reconfiguration of the metabolic network.

Here I aim to identify and access the metabolic impact of the TCA cycle metabolon in the model plant Arabidopsis thaliana to gain a new insight into plant metabolic regulation. My results indicate that there is a metabolon between Fumarase (FUM), Malate Dehydrogenase (MDH), Citrate Synthase (CYS), Aconitase (ACO), Isocitrate Dehydrogenase (IDH), and the 2-Oxoglutarate Dehydrogenase Complex (ODC), Succinyl-CoA Synthetase (SCL). The Pyruvate dehydrogenase complex (PDC), Succinate Dehydrogenase (SDH) and Malic Enzyme (ME) may also participate in the metabolon. In addition, phosphorylaiton is a conserved protein post-translational modification that links primary metabolism and cellular signaling. As almost all members of the TCA cycle were found to be phosphorylated in different conditions, we find that PP6family protein may regulate the TCA enzymes by dephosphorylation.

SESSION: OMICS APPROACHES IN MITOCHONDRIAL BIOLOGY

POSTER 91

Fertility restoration: Predicting RESTORER OF FERTILITY (RF) genes from genomic sequence data

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Keywords: Fertility restoration, next generation sequencing, restorer of fertility genes, pentatricopeptide repeat proteins

To date, one of the most successful approaches to boosting seed yield of crop plants is by breeding hybrids and taking advantage of heterosis. Avoidance of self-pollination during hybrid seed production exploits mitochondrial genes that cause cytoplasmic male sterility (CMS). Nuclear *RESTORER OF FERTILITY* (*RF*) genes carried by the male parent ensure that the F1 hybrids are self-fertile to maximize seed set. The molecular basis for the nucleus-cytoplasm interactions that control male fertility is now reasonably well understood. Male sterility is induced by mitochondrial genes that cause abortion of pollen development and *RF* genes act by suppressing the expression of these CMS-specific gene products. The majority of *Rf* genes in higher plants encode pentatricopeptide repeat proteins, members of a huge family of sequence-specific RNA-binding proteins. The selection patterns on *RF* genes are extremely informative about *RF* gene function, permitting *RF* genes to be predicted amongst hundreds of similar genes. In this study we developed a bioinformatics pipeline for locating *RF* genes in the genomic data from de novo sequencing of 44 sorghum accessions. Our results have shown that different accessions within a species carry different numbers of *RF* genes. Sequence analysis revealed high sequence variability at functionally important sites within the *RF* genes. A combination of genomics and genetic mapping data is a powerful tool for prediction and identification of *RF* genes in agronomically important plant species. Application of this approach could accelerate the development of new breeding systems for high-yielding hybrid crops.

POSTER 92

Role of AtFtsH4 protease in biogenesis of mitochondria during Arabidopsis seed germination

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Keywords: mitochondrial biogenesis, AtFtsH4 protease, germination, multiple reaction monitoring

Germination is considered one of the most critical phases in the plant life cycle. We showed that a lack of mitochondrial AtFtsH4 protease delays Arabidopsis seed germination. The observed delay at the time when mitochondria undergo structural changes suggests that the loss of AtFtsH4 disturbs the process of mitochondrial biogenesis at the early stages of germination. Using GeLC-MS multiple reaction monitoring (MRM) with stable-isotope-labeled standard peptides we examined the abundance of protein targets belonging to the OXPHOS components, which are encoded by nuclear and mitochondrial DNA, throughout germination. We observed significant reduction in abundance of the components of all respiratory chain complexes in *ftsh4*, starting approximately after 12h of the germination course. Interestingly, this effect was more pronounced for mitochondrially-encoded proteins. The changes were accompanied by lowered respiration rate and cytochrome pathway activity in *ftsh4* seeds. Morphological observations of GFP-tagged mitochondria in embryos of WT and *ftsh4* lines revealed a weaker signal and abnormal shape in *ftsh4* during germination. We propose a crucial role of AtFtsH4 in assembly or stabilization of inner membrane protein complexes as well as mitochondrial morphology during fundamental processes underlying biogenesis of mitochondria in the time of seed germination.

This work was supported by Grant 2012/07/B/NZ2/01794 from the National Science Centre, Poland.

POSTER 93

The potato tuber mitochondrial proteome further expanded

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Keywords: potato tuber, mitochondria, proteomics, mass spectrometry

To better understand the role of mitochondria in maintaining and regulating metabolism in storage tissues, we have characterized the potato tuber mitochondrial (POM) proteome further. Highly purified POM were isolated from dormant potato tubers (Solanum L. cv. Folva) (Salvato et al. 2014). After digestion with trypsin, the peptides were first fractionated using hydrophilic interaction liquid chromatography (HILIC). The resulting 10 fractions were further analyzed by reversed-phase liquid chromatography-tandem mass spectrometry and peptides identified using four different search engines. In six biological replicates, we identified around 194,000 peptides belonging to >1500 proteins each present in at least two replicates. This expands the list in Salvato et al. (2014) by around 40%. The expansion can be seen in all groups of proteins, thus we found 25% more proteins belonging to the electron transport complexes and 50% more proteins belonging to the TIM and TOM complexes. Perhaps surprisingly, the new list only contains 84 pentatricopeptide repeat (PPR) proteins compared to the 71 previously identified. More detailed analyses of the data will be presented at the meeting.

Salvato, F., Havelund, J.F., Chen, M., Rao, R.S.P., Wrzesinska-Rogowska, A., Jensen, O.N., Gang, D.R., Thelen, J.J. & Møller, I.M. 2014. The potato tuber mitochondrial proteome. Plant Physiol. 164: 637–653

POSTER 94

GelMap: annotation and evaluation of gel-based proteomic data

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Keywords: Mitochondrial proteincomplex proteome, gel-based proteomics, reference map

Introduction: When the GelMap project was initiated in 2010, it was originally established for easy and quick annotations of two dimensional gels, which actually was an important advantage in comparison to other available annotation tools. In the following years, the software has developed into a multifunctional tool, providing a broad variety of options to annotate and analyze gel-based proteomic datasets.

Results: The integration of GelMap into meta protein platforms, like the GATOR, further enhances its value for systematic data mining. A major purpose of GelMap is the data exchange between researchers. Therefore, it is freely accessible via the internet (www.gelmap.de). However, if data sets should be kept confidential, projects can be protected by a password.

Here we present the most recent proteomic projects in GelMap as well as the latest updates of the GelMap software which enhance the browsing capabilities in established reference maps. Furthermore, new features are available which allow users to transfer identification data from published GelMap projects to newly prepared gel images. This allows rapid protein identification without the necessity to perform MS.

Conclusions: GelMap is a flexible tool for data annotation and evaluation. As it has been mainly used for plant research so far, linked databases and tools are from the plant field. The integration of further external sources is always possible. The structure of the software is easily expendable and allows it to be used for a multitude of future purposes.

POSTER 95

Improved RNA-seq methods for RNA editing detection and quantification.

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Keywords: editome, transcriptome, transcription, RNA editing, PPR, pentatricopeptide

RNA-seq approaches to studying RNA editing are problematic due to the high error rate of NGS data and aligner bias towards a genomic reference. We developed a workflow using proven bioinformatic tools to increase the sensitivity and accuracy of editing site detection and editing quantification. We improved RNA-seq alignment yield and accuracy in densely edited transcripts with the flexible variant- and splice-tolerant aligner GSNAP. GATK modules were successfully applied to recalibrate aligned RNA-seq reads, reducing false positive rates for site detection while increasing accuracy of editing rate quantification. Handling editing site information in standard .vcf format allowed us to leverage popular programs developed for studying SNPs and efficiently manage genome-wide editing datasets from several individuals. Applying these techniques to multiple Silene vulgaris mitochondrial transcriptomes confirmed conserved editing across CDS and revealed 17 partial RNA editing events within group II introns, as well as limited editing at 91 intergenic sites, presumably due to off-target editing. Editing site density was lowest in long open reading frames, even lower than in other intergenic regions. With care, RNA-seq can be an efficient, sensitive, and accurate method for rapidly surveying RNA editing across entire genomes of numerous individuals.

POSTER 96

Insights into expression of cytoplasmic male sterility in beets

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Keywords: mitochondrial proteome, cytoplasmic male sterility, blue-native electrophoresis, restorer genes

The aim of this study was to identify features of the mitochondrial proteome which were associated with cytoplasmic male sterility (CMS) in beets. The analyses were performed using CMS, maintainer and restored lines. The CMS condition was associated with decreased activity of complex V and enhancement of additional complexes with the ATPase activity. This was accompanied by accumulation of heptamer HSP60, preSATP6, an increase in the fraction of the free ATP9 oligomer (not bound to complex V) and decreased accumulation of glutathione reductase (GR). The ATP9 effect was reversed upon fertility restoration. The presence of preSATP6, higher accumulation of the free ATP9 oligomer and decreased accumulation of GR were also observed in CMS table (red) beet. Moreover, an attempt was made to reveal which of the restorer genes is responsible for fertility restoration in the analyzed sugar beet lines. For this purpose segregating progenies of the restored lines were subjected to genotyping using CAPS markers. According to the obtained marker data fertility restoration was determined by the Rf1 (X) gene.

POSTER 97 – AGRISERA'S POSTER

Compartment Markers for Plant Science

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Chloroplasts are ideal hosts for transgenic expression. Introduced sequences undergo homologous recombination to integrate into plastid genomes and are thereafter maternally inherited, minimizing pleiotropic effects and containment risks. Production of heterologously expressed proteins within chloroplasts also limits contamination of the cytosol and cytotoxicity toward plant cells and tissues.

Confirmation of chloroplast targeting and assessment of expression levels can be achieved by tracking introduced proteins and measuring their abundance relative to marker proteins. Fractionation of cellular compartments such as cytosol, mitochondria and chloroplasts, and further sub-fractionation into thylakoids, stroma and lumen can be followed by quantitative detection of both introduced and compartment marker proteins.

Agrisera and Environmental Proteomics have created a comprehensive set of antibodies for detection and tracking of protein compartment markers in plants and algae. Several of these are fully quantitative, allowing normalization of expression levels of heterologous proteins. We demonstrate the fractionation of chloroplasts from cytosol and other organelles with antibodies toward RbcL (chloroplast stroma), PsbA or PsbD (chloroplast thylakoid), SPS (cytosol), PEPC (cytosol, mesophyll enriched in C4 plants), AOX (mitochondrial), and VDAC (vacuole). We also show the enrichment of Rubisco and PEP carboxylase using quantitative RbcL and and PEPC markers following fractionation of mesophyll and bundle-sheath cells from maize.

POSTER 99

The characterization of RPD1, a PORR protein involved in the biogenesis of plant mitochondria

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Plant mtDNAs encode many group-II introns, which interrupt genes involved in the biogenesis and function of the respiratory chain. Genetic analyses led to the identification of various proteins required for the splicing of mitochondrial introns. These are derive from several protein families defined by atypical RNA-binding domains that function primarily within organelles (e.g. CRM, MAT, mTERF, PPR and PORR). The Plant Organellar RNA Recognition (PORR) domain is represented in a small family of proteins in angiosperms (15 in Arabidopsis and 17 in rice). These are mainly predicted to localize to mitochondria or plastids, and are thus postulated to function in organellar RNA metabolism. Indeed, one of these proteins (WTF1) was identified as a component of group II intron ribonucleoprotein particles in chloroplasts. More recently, a mitochondrial member of this family, WTF9, was shown to function in the splicing of *rpl2* i1 and *ccmFc* i1. The functions of another PORR protein in Arabidopsis, RPD1 (Root Primordium Defective 1; At4g33495), are essential during early embryonic stages. Yet, two allelic mutants harboring missense mutations in *Rpd1* are viable and show altered growth and developmental phenotypes. Here, we show that RPD1 is localized to mitochondria and facilitates the splicing of three mitochondrial introns, *nad5* i1, *nad7* i2 and *rps3* i1. While the activities of complex I are not essential in plants, the maturation defects in *rps3* may relate to the essential roles of RPD1 during embryogenesis. The global effect of *Rpd1* mutations on organelle translation is now being analyzed by ribosome profiling and mass-spectrometry analyses. We further anticipate that the co-crystal structures of PORR (i.e. RPD1, WTF9) proteins with their genetically-identified RNAs will provide with new insights into the precise roles of splicing factors in RNA metabolism.

POSTER 100

An RNA Recognition Motif Family Required for Plant Mitochondrial RNA Editing

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Introduction: Plant mitochondria modify an extraordinary number of Cs to Us by RNA editing. In Arabidopsis over 600 Cs are altered, usually changing the encoded proteins from the ones predicted by genomic sequences. Editing is carried out by a small RNA/protein complex called the editosome, whose composition is beginning to be unraveled. Recognition of the C target is mediated by pentatricopeptide repeat (PPR) motif-containing proteins that specifically recognize a cis-element near the edited nucleotide. ORRM1 (Organelle RRM protein 1), a recently identified chloroplast editing factor, belongs to a distinct clade of RNA Recognition Motif (RRM)-containing proteins, most of which are predicted to be organelle-targeted.

Materials and methods: Additional members of the ORRM clade were subjected to insertional mutagenesis or virus-induced gene silencing. Extent of editing at mitochondrial C targets in silenced and mutated tissue was compared to wild-type.

Results: We report the identification of members of the RRM family as factors essential for efficient mitochondrial RNA editing. Yeast-two-hybrid assays with these mitochondrial editing factors revealed their interaction with other known components of the RNA editosome.

Conclusion: Along with PPR and RIP proteins, some members of a family of plant RRM proteins function in plant RNA editosomes in chloroplasts or mitochondria.

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References were expressed as lecture (L) and poster (P) numbers

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Conferences for plant mitochondrial biology (1995-2015)