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Prediction of Novel genes for Heat and Cold adaptation

Presentation Overview

- Introduction
- NASA Astrobiology Centers
- Astrobiology and University of Hawaii
- Origin of Life
- Gene Adaptation overview
- Heat Adaptations
- Cold Adaptations
- Lab Work and Terraforming

Introduction: About Us

- Students of Belgrade University Dept of Biology and Astrophysics.
- Simona earned PhD in Biomedicine from University of Hawaii in 2002
- Miodrag earned Masters in Astronomy from University of Western Sydney in 2001
- Used to live and work in Hawaii for 9 years
- Moved to Minnesota last year. Simona works as an assistant professor at UMN, Miodrag involved in biostatistical analyses

NASA & Astrobiology

- Astrobiology is relatively new scientific area, connecting work and knowledge of several major sciences
- In year 2000, NASA started funding of several astrobiology centers worldwide
- Proposals for funding involved multidisciplinary studies and wide spectrum of areas
- University of Hawaii, primarily because of its specific location and possibility of research of deep ocean and vulcanic environments got one of the center
- In 2003, we wrote a proposal, and got funding for our research on genetic adaptations to extremely hot environments

NASA & Astrobiology

NASA Astrobiology Institute: NAI:

http://nai.arc.nasa.gov/

Mission statement:

The mission of astrobiology is to study the origin, evolution, distribution, and future of life on Earth and in the Universe.

Dr Carl Pilcher, director 2006-

Chemist, discovered water on Saturn rings, worked as an astronomer at the University of Hawaii, discovered “weather” on Neptune and worked on the discovery of methane ice on Pluto.

Then went to work for NASA.

NAI centers

http://nai.arc.nasa.gov/team/index.cfm

November 2003 - October 2008 (Currently Active)

Carnegie Institution of Washington *
University of Colorado, Boulder *
NASA Ames Research Center *
NASA Goddard Space Flight Center
Indiana University
Marine Biological Laboratory *
Pennsylvania State University *
SETI Institute
University of Arizona
University of California, Berkeley
University of California, Los Angeles *
University of Hawaii, Manoa
***** = *Teams on their second 5-year Cooperative Agreement.*

NASA Hawaii Astrobiology

Hawaii Astrobiology NASA Center

Head of Center: Dr Karen Meech, also involved in Deep Impact project

Object of interests:

- **The Origin, History, and Distribution of Water and its Relation to Life in the Universe**
- Deep Ocean
- Volcano National Park
- Lava Ocean interaction
- Mauna Kea complex

NASA Hawaii Astrobiology

Departments involved in multidisciplinary studies:

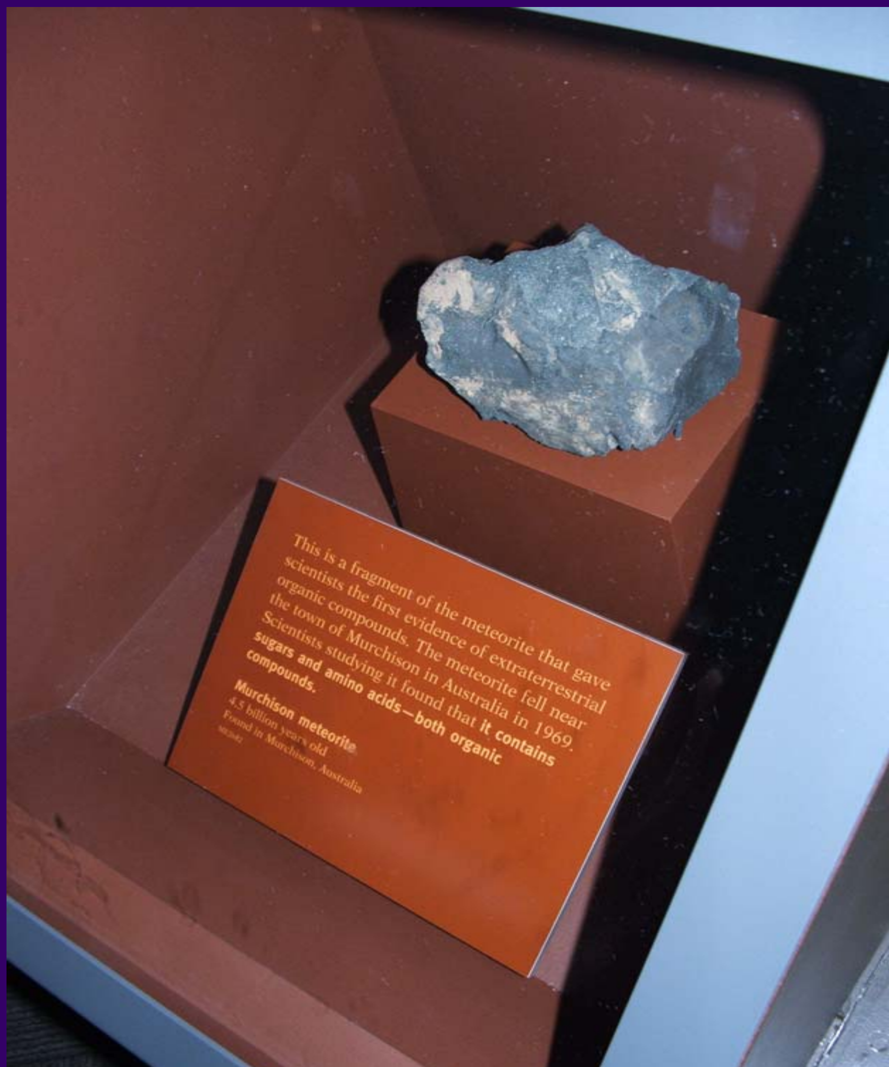
- Astronomy
- Geology and Vulcanology
- Marine science
- Biology and Microbiology
- Biochemistry
- Genetics
- Computer science

Scientifical perspective

Understanding Life

Understanding life in extreme environments on Earth can tell us a great deal about the potential for life in similar environments on other planets. Understanding the limits of life as we know can also help determine what makes a planet habitable.

Scientifical perspective



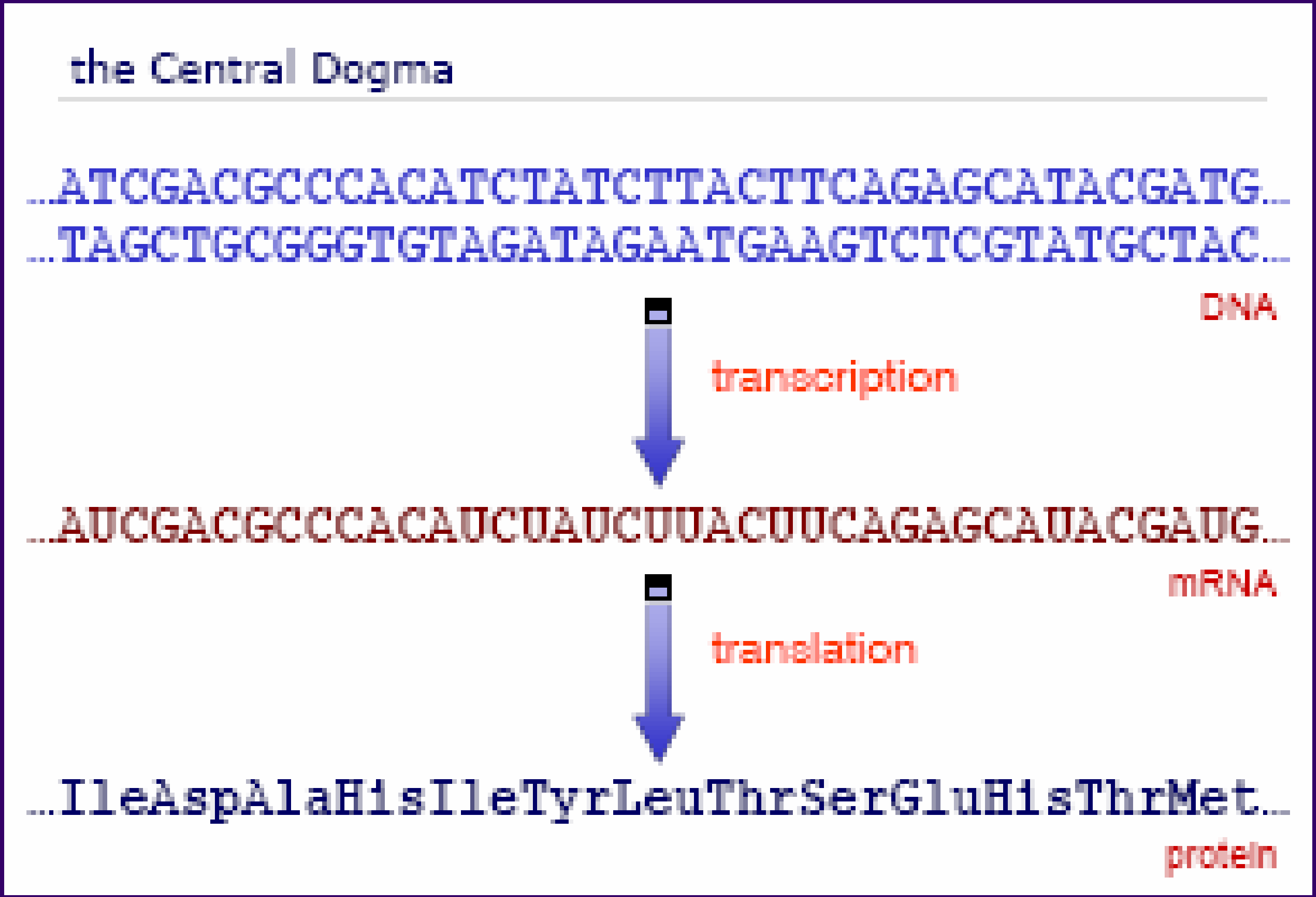
<u>Origin of Life</u>

Organic compounds came from space. 1969. scientist found first evidence of extraterrestrial organic compounds in Murchison meteorite in Australia.

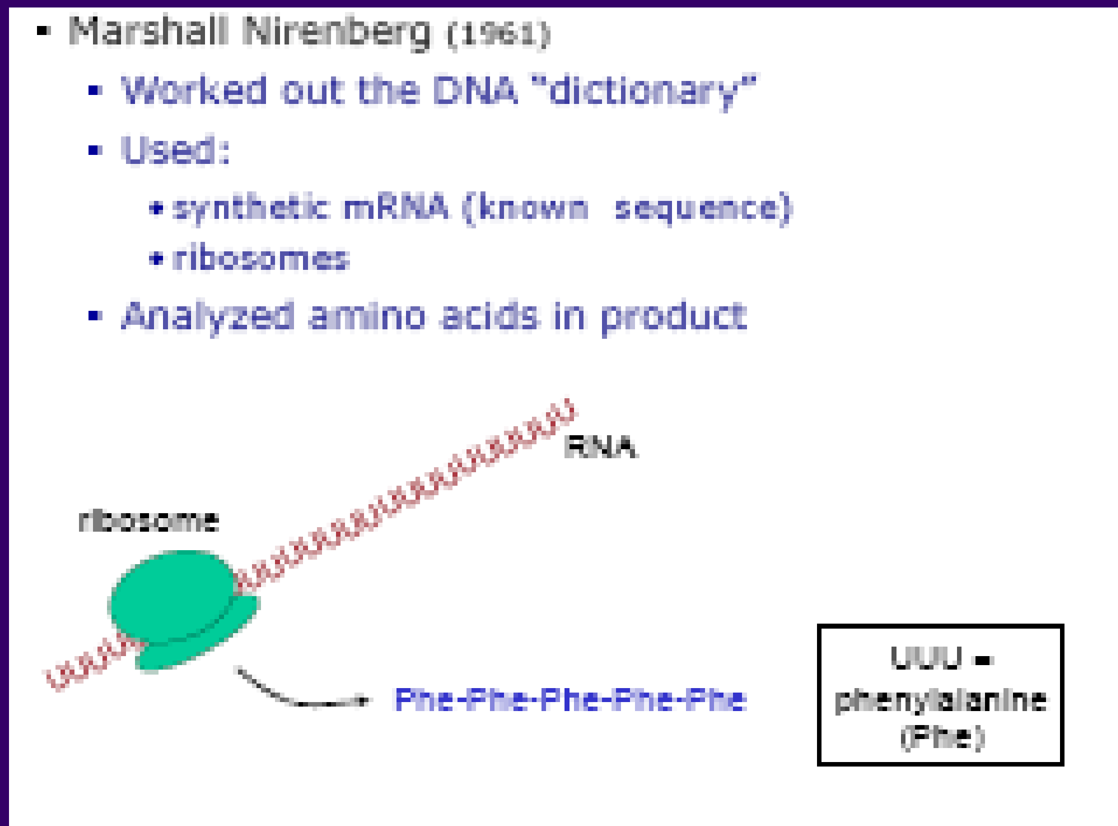
Our Proposal

We propose to investigate a gene signature responsible for adapting of microscopic life forms to the life in extreme Earth environments

Central dogma



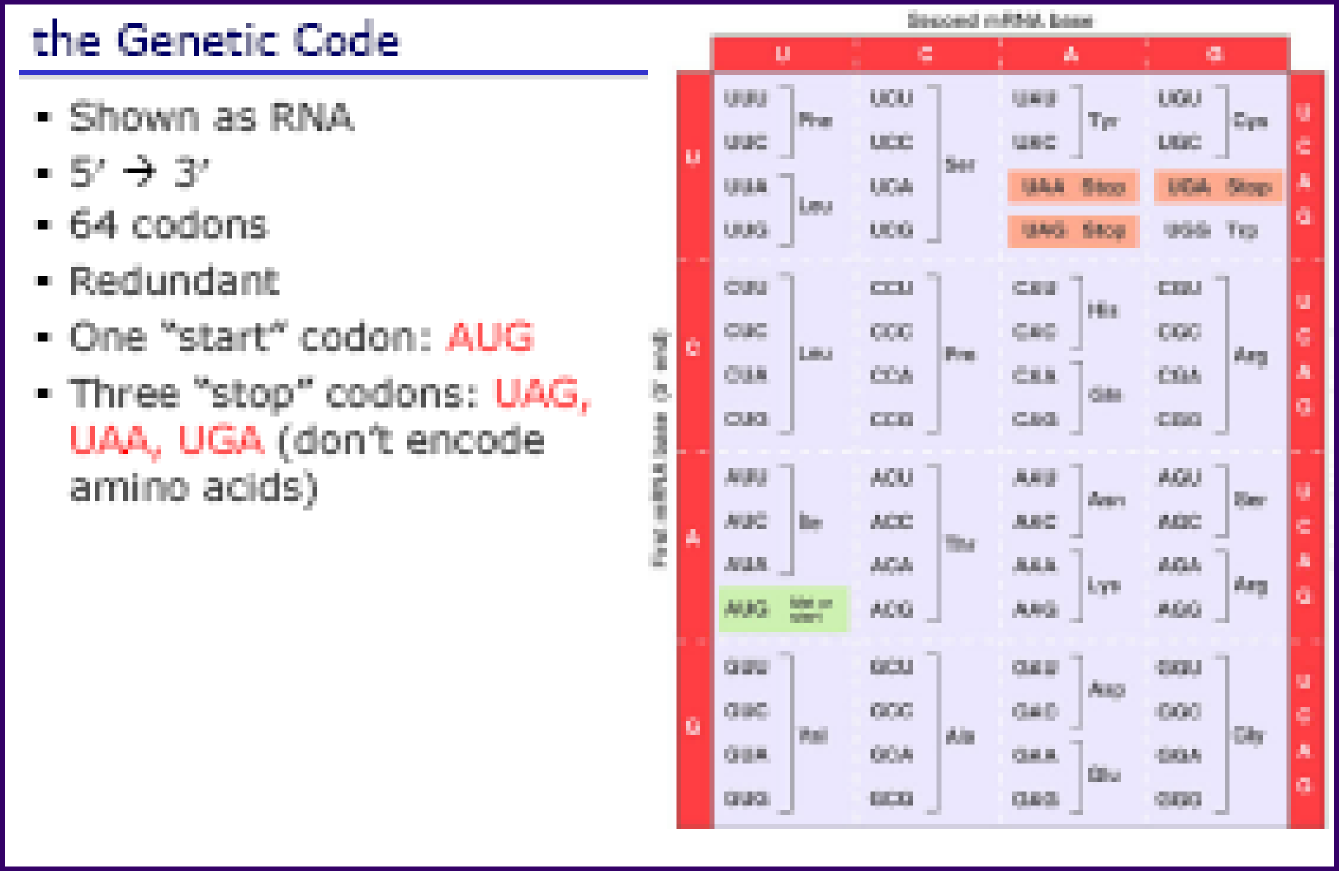
Genetic code



How can the 4 letter DNA be translated into 20 letter alphabet of amino acids, the building blocks that make up proteins.

Russian astronomer Gamow: the 3 letter code: $4^3=64$

The genetic code is redundant

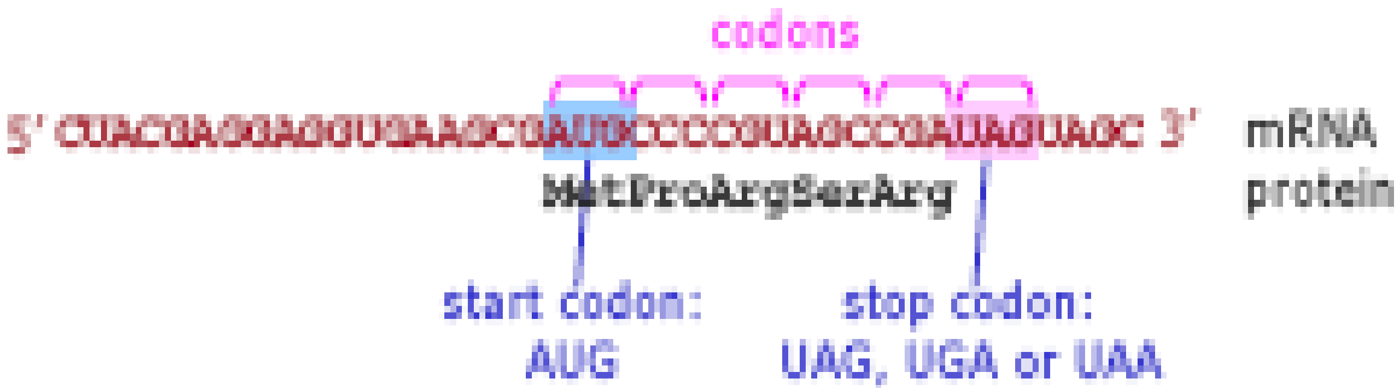


Many combinations of 3 letters can code for the same amino acid.

The genetic code is redundant

the Genetic Code

- Ribosome starts at 5′ end of mRNA (in eukaryotes)
- Protein synthesis begins at start codon, ends at stop codon



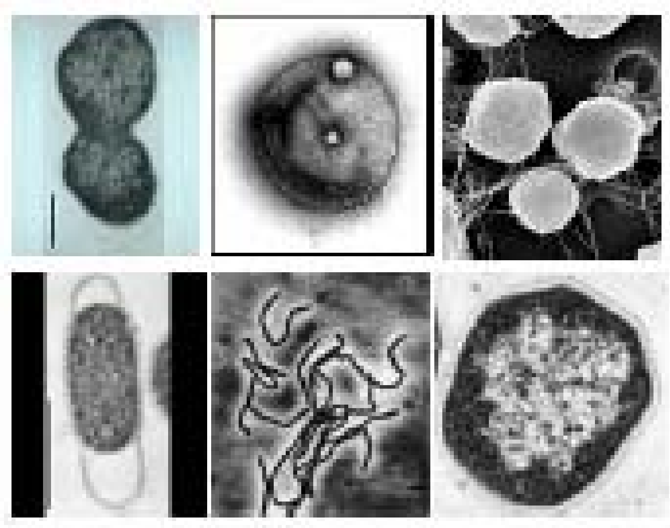
Protein synthesis starts at the start codon and ends at the stop codon.

Extremophiles

Microbial organisms living in extreme enviroments on Earth: extremophiles.

Our initial analyses focused on 7 extremophiles (hyperthermophiles) which genomes were completely sequenced and databases available in the public domain.

Hyperthermophiles are heat-loving organisms: their growth temperatures are exceeding 90 deg. C.



A-F: P. abyssi, P. horikoshii, M. jannaschii, T. maritima, M. thermoautotrophicum, A. pernix. Pictures from:Genome News Network website.

Comparisons of hyperthermophile genomes

The basis for comparisons were clusters of orthology groups (COGs). This is how the genome data is organized in the published genome sequences.

Classical sequence comparison software used in the NCBI: Blast genome and Blast protein was incorporated in our analyses. This software looks for sequence similarities on the genome and protein level.

The first step in our analyses was to exclude all the sequences which are common to other bacteria not living in extreme environments.

Exclusion of common bacterial gene signatures

E. coli K12, a common laboratory strain was used to exclude all COGs common to non-hyperthermophiles. The first comparison was between E. coli and P. abyssi. This reduced the number of COGs to 484.

We were looking for a minimal gene signature, therefore we kept repeating the process by comparing the rest 6 hyperthermophile genomes looking for only those in common to all of them.

Hyperthermophile-specific signature

The comparison of E. coli and P. abyssi excluded 484 common COGs and further comparisons of COGs from P. horikoshii through M. thermoautotrophicum led to discovery of 29 COGs specific for all 6 hyperthermophiles.

Obstacles to life at extreme temperatures

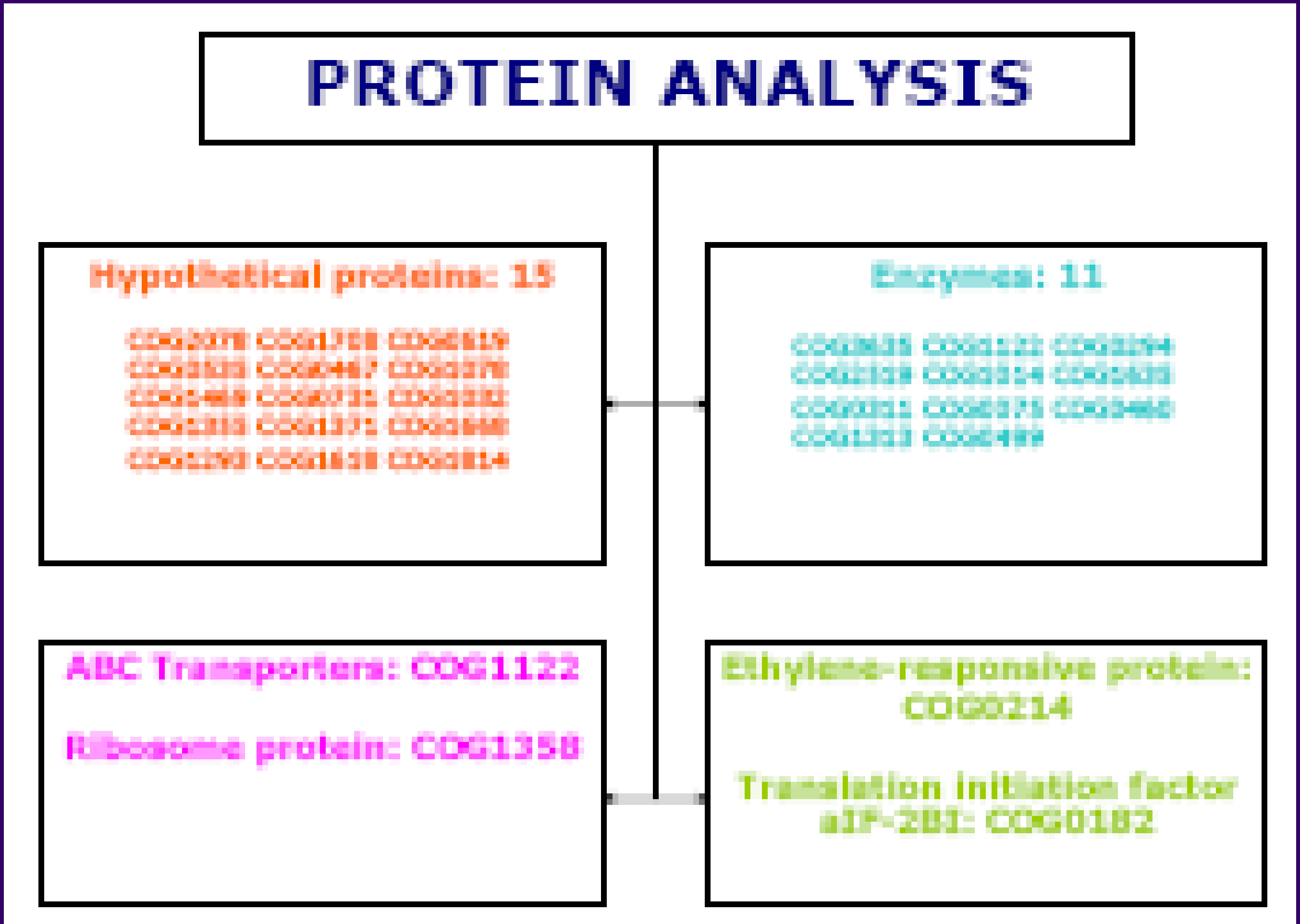
At extreme heat proteins are expected to loose their tertiary strucure, denature due to coagulation.

Plasma membrane is not expected to retain its semi-liquid state.

Functioning of enzymes at these temperatures is another challenge that needs to be overcome.

We have identified proteins dealing with these issues.

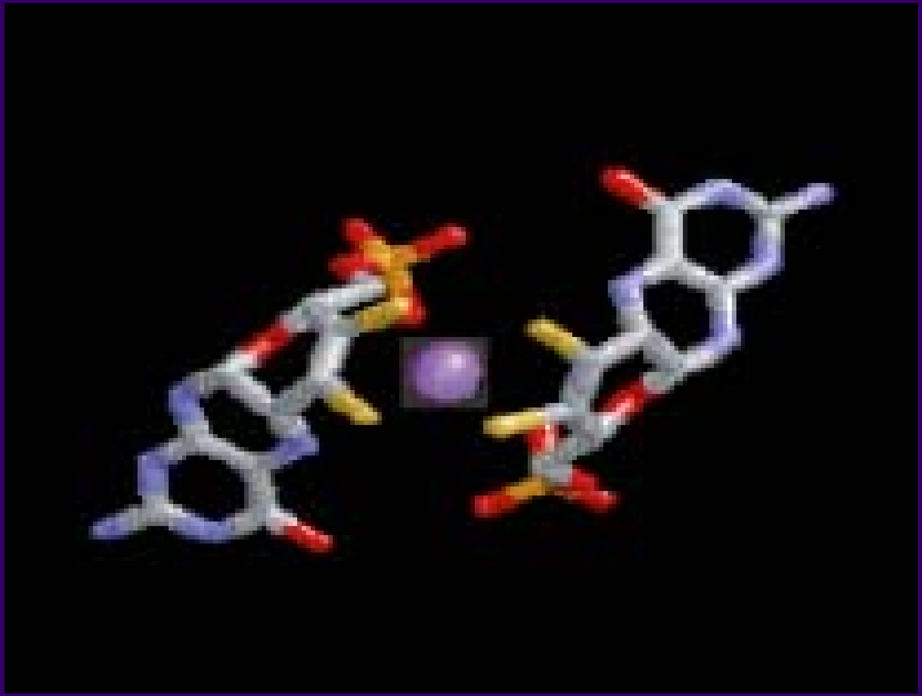
Results:


PROTEIN ANALYSIS
Hypothetical proteins: 15
Enzymes: 11
ABC Transporters: COG1122
Ribosome protein: COG1358
Ethylene-responsive protein:
COG0214
Translation initiation factor
aIF-2B1: COG0182

The group of enzymes

ENZYMES	
COG3635	Phosphonopyruvate decarboxylase, putative
COG0294	DIHYDROPTEROATE SYNTHASE
COG2519	PROTEIN-L-ISOASPARTATE METHYLTRANSFERASE HOMOLOG
COG1014	2-ketoglutarate ferredoxin oxidoreductase, subunit gamma (korG-1)
COG0311	Imidazoleglycerol-phosphate synthase, subunit H, putative
COG0075	SERINE-GLYOXYLATE AMINOTRANSFERASE related (EC2.6.1.45) (SERINE-GLYOXYLATE AMINOTRANSFERASE)
COG0460	Homoserine dehydrogenase (hom)
COG0467	RECOMBINASE related
COG0535	Tungsten-containing aldehyde ferredoxin oxidoreductase COFACTOR MODIFYING PROTEIN
COG1313	Pyruvate formate-lyase activating enzyme (pflX)
COG0499	Adenosylhomocysteinase (ahcY)

Structure of Tungsten-containing enzyme



Tungsten cofactor from aldehyde ferredoxin oxidoreductase from P. furiosus. Picture obtained from Tatur J. website.

Adaptations to life in extreme cold

- Most bodies of our Solar System are "Frozen Worlds" where prevailing temperature remains at or below freezing.
- Even on Earth there are vast permanently frozen regions of permafrost, polar ice sheets and glaciers, and the deep oceans and deep-sea marine sediments have remained at 2-4C for eons.
- Cold environments are actually more prominent in the world the hot ones. Oceans are cover half of Earth and are at 1-3C constantly.

Psychrophiles: cold-loving organisms

- Psychrophiles possess enzymes that function at low temperatures and are denatured at moderate temperatures.
- One of the features of their adaptations is the presence of polyunsaturated lipids in their membranes.
- A huge area of Earth that was once thought to be uninhabitable, is now showing the presence of these organisms.
- Psychrophiles are interesting because they represent analogs of microbial life that might inhabit such habitats in space.

Cold habitats in space potentially suitable for life

- One can imagine that some of the habitats in space might be suitable for life of cold-loving microbes. Such examples include:

- Ice sheets and permafrost of Mars
- Comets
- Ice/water interfaces or deep sediments beneath the icy crust of Europa, Callisto, or Ganymede.
- Cryopreserved microorganisms can remain viable (in a deep anabiotic state) for millions of years frozen in permafrost and ice.

Cold habitats in space: temperatures potentially suitable for life

- Psychrophiles can survive for thousands of years at the temperatures of -40C. One of the sturdiest psychrophiles is Colwellia. There are claims that it can metabolize at -196C, although these should be taken with reservations

- This possibility of extreme cold tolerance and longevity increases the odds of life in the solar system. For example: Mars polar caps vary in temperature between -120 to -40C, theoretical temperatures for Europa oceans are -90 to -10C, while Titan at -180C may already be out of range.

Genetic adaptations of microorganisms to cold environments

This is a topic of multi-disciplinary international initiative which will be re-submitted for funding.

The multi-disciplinary approach to study these adaptations involves a team gathered from the fields of microbiology, molecular biology, genetics, mathematics and astronomy.

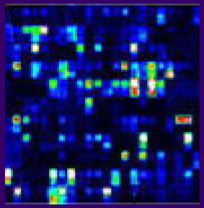
Genetic adaptations of microorganisms to cold environments

The aims of the study:

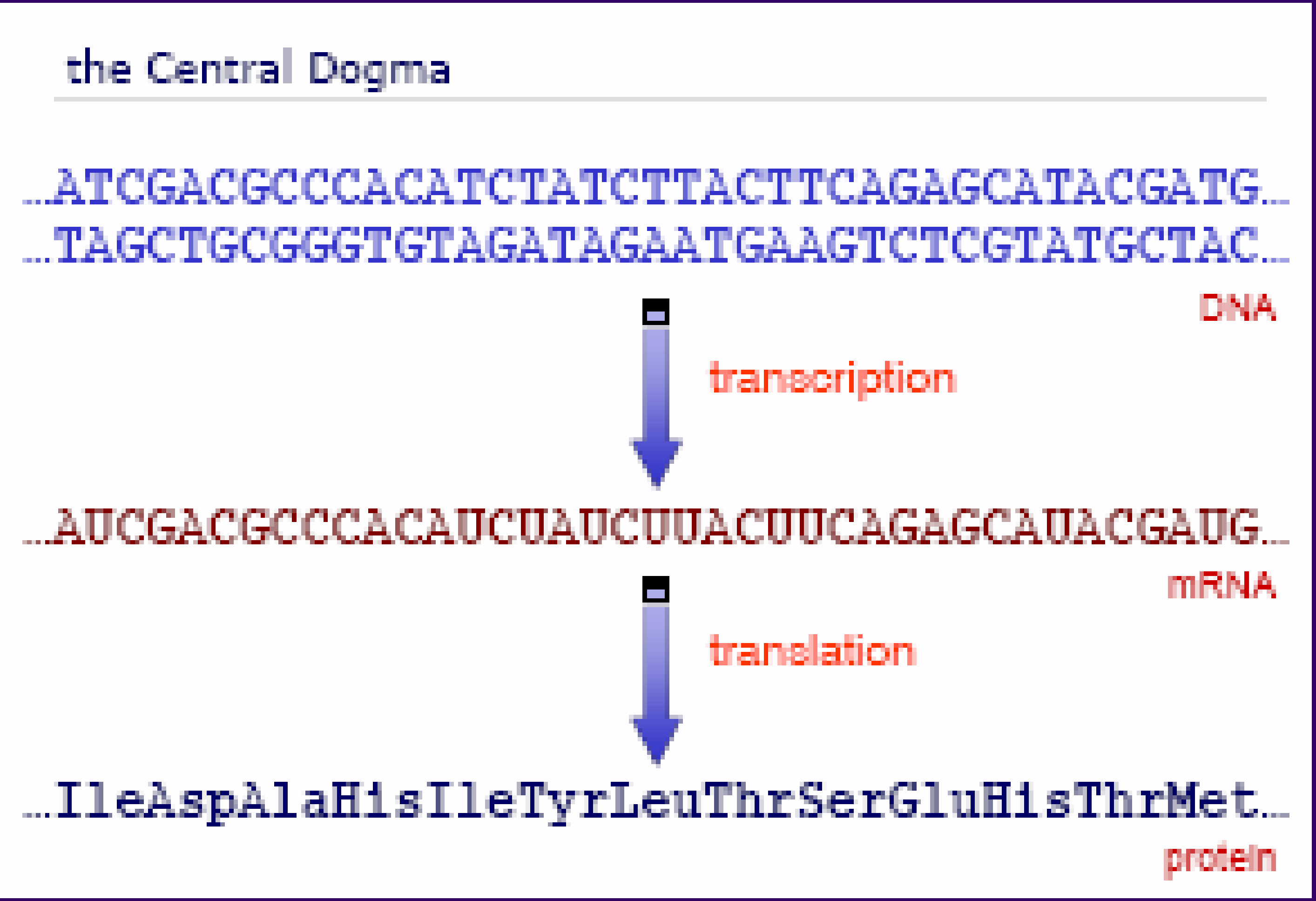
1) Computational identification of genes responsible for adaptation to cold.
2) Construction of microarrays and use them for screening of 20 psychrophiles from Danish collection of these microbes.
3) Test the limits of survival of these microbes which had the cold adaptation signature and identify a candidate for sequencing
4) Construction of Atlas of planetary conditions potentially suitable for life

Microarray design

Computationally determined protein sequences responsible for adaptation to cold environments will be traced back to conserved regions in DNA. These will be used for synthesis of short oligonucleotide probes which will be printed on DNA chips. These chips will be used for testing of 20 of the psychrophiles present in the Danish collection for the presence of unique psychrophile gene signature, thereby confirming the computational findings.



Central dogma



Mars simulation laboratory

Collaboration with Mars simulation laboratory:

- Because they developed a chamber which simulates conditions on Mars, therefore perfect for testing if a microorganism would survive such conditions
- Because they possess a collection of over 120 psychrophiles, most of them poorly defined.

Mars simulation laboratory

http://www.marslab.dk/

Survival, activity and diversity of microbial communities under Martian conditions

Heterogenous processes in the Martian atmosphere. UV-oxidation, oxidation products and activation energies. Reactive molecules and surfaces.

Aerodynamics modelling and magnetic capture. Particle adhesion, cohesion and charging. Particle velocity and particle size determination methods under low pressure conditions.

Mars analogue mineralogy. Mineral synthesis and identification. CEMS Mössbauer spectroscopy. Mössbauer- and magnetic instrument tests, and interpretation of backscatter Mössbauer results from magnetic captured material. Study of Martian conditions on XRF analyses.

Conclusions

Understanding genetic basis for adaptations to extreme environments helps us better understand life on Earth, the limits of life in space and opens the possibility for terraforming processes

Astrobiology is a new field which requires multidisciplinary research and international collaboration is very important

We started as a team of 1 astronomers and 1 biologist, a hobby project, and on the next stage evolved in an international team adding microbiologist, mathematician, geneticist and more astronomers.

Interest in astrobiology and funding opportunities are on the rise.