Abstracts of Speakers

THE ENERGY HUBS OF LIVING ORGANISMS – BIOGENESIS AND SIGNALING

Lu Li[1], Wenhui Lyu[1], Cunman He[1], Xiangxiang Meng[1], Yanqiao Zhu[1,2], Chunli Mao[1,2], Meiyan Ren[1], Andreas Hartmann[1], Guangkun Yin[3], Peisheng Mao[2], Inge De Clercq[1,4], Frank Van Breusegem[4], Beata Kmiec[5], Pedro Teixeira[5], Elzbieta Glaser[5], David Day[6], Aneta Ivanova[7], Monika Murcha[7], Ricarda Jost[1], Yue Xu[1], Jennifer Selinski[1], Reena Narsai[1], Oliver Berkowitz[1], Yan Wang[1], **James Whelan[1]**

[1]La Trobe Institute for Agriculture and Food, La Trobe University, Melbourne, Australia, [2]Forage Seed Lab, China Agricultural University, Beijing, China, [3]National Crop Genebank, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China, [4]Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium, [5]Biochemistry and Biophysics Svante Arrhenius Vag, Stockholm University, Stockholm, Sweden, [6]College of Science and Engineering, Flinders University, Adelaide, Australia, [7]School of Molecular Sciences and Arc Centre of Excellence Plant Energy Biology, University of Western Australia, Perth, Australia

Plant mitochondria were first recorded over 100 years ago, with the cyanide insensitive respiration attributed to mitochondria over 50 years ago. Since then intensive studies have found many shared and specific features for mitochondria from plants, animals and fungi, such as association of cytoplasmic male sterility with plant mitochondria, differences in RNA editing and unique features in biogenesis and signaling. Mitochondria also play an essential role in carbon/energy metabolism, redox balance and biosynthesis. These roles vary between organs, tissues and cells. Yet, unlike their endosymbiotic plastid 'neighbours', mitochondria do not develop into specialised structures like chloroplasts (photosynthesis), chromoplasts (pigments) and amyloplasts (starch storage). Mitochondria achieve their diversity of functions by displaying plasticity, that is evident from whole plant studies to the molecular level.

To achieve this functional plasticity mitochondrial activity needs to be coordinated on the cellular level via anterograde and retrograde signaling pathways, that control mitochondrial biogenesis from seed germination to senescence. At a transcriptional level, the integration of these mitochondrial pathways with those in chloroplasts, hormonal and environmental signalling pathways is being uncovered. At a post-transcriptional level, the regulation of various cellular functions via controlling RNA and protein turnover, protein trafficking and enzyme activity, which also have impact mitochondrial function. Also, recent studies suggest post-translational regulation of mitochondrial retrograde signaling. Together these studies show that mitochondria are not only respond to stress, but also act as an sensors for cellular metabolism, and inputs from mitochondria re-program cellular processes from mitotic activity to cell death to optimise plant growth.

SPECIAL TALK: HUMAN MTDNA TRANSCRIPTIONAL REGULATION – NOVEL INSIGHTS FROM AN EVOLUTIONARY PERSPECTIVE

Dan Mishmar
Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

Endosymbiosis was accompanied by transfer of most mitochondrial DNA (mtDNA) genetic information to multiple nuclear chromosomal locations, and establishment of an independent transcriptional regulation for each gene. This suggests sharp separation between the transcriptional regulation of nuclear DNA (nDNA)-encoded mitochondrial genes and their mtDNA-encoded partners, which are co-transcribed in polycistrones using dedicated transcription factors and a mitochondrial RNA polymerase. However, nDNA and mtDNA-encoded gene products physically interact to maintain mitochondrial function in different cells and tissues. Therefore, we hypothesized that coordination of gene expression regulation should occur between the two genomes, especially in the frame of the oxidative phosphorylation system (OXPHOS). If this is true, one expects adaptation of the ancient prokaryote (the mitochondrion) to the regulatory system of the eukaryote host. Our recent analysis of ~8500 RNA-seq experiments from 48 different human body sites, revealed evidence for co-regulation between bi-genomic OXPHOS subunits across most tissues. Our analysis highlighted several candidate regulators of the coordinated mito-nuclear gene expression, which are known transcriptional regulators of nDNA gene expression. Using ChIP-seq and DNase-seq experiments we found that these factors bind in vivo the human mtDNA outside of the control region, within the gene sequence, suggesting that the mtDNA gene-coding sequences also harbor overlooked regulatory information. Silencing of two such regulatory candidates, i.e. CCAAT/enhancer-binding protein beta (CEBPb) and c-Jun, led to elevated mtDNA transcript levels in human cells, suggesting a transcriptional repressive role. When CEBPb-silenced glioblastoma human cells were subcutaneously injected into mice, significant reduction in tumor size was observed (as compared to control), suggesting in vivo phenotypic impact for altered mito-nuclear co-regulation. The previously overlooked mitochondrial role of known nuclear transcriptional regulators will be discussed.

MUTATIONAL DYNAMICS IN ANGIOSPERM MITOCHONDRIAL GENOMES

Daniel Sloan, Zhiqiang Wu, Gus Waneka, Amanda Broz, Joel Sharbrough
Department of Biology, Colorado State University, Fort Collins, USA

Angiosperm mitochondrial genomes exhibit a number of distinctive features, including an unusually low rate of nucleotide substitutions relative to the mitochondrial genomes of other eukaryotes. The mechanisms that maintain this high degree of sequence conservation remain unclear. We will present results from ongoing efforts to use high-fidelity DNA sequencing methods to detect new mutations present at extremely low frequencies within *Arabidopsis* tissue samples as a means to study the mechanisms of plant mtDNA mutation and repair. We will also discuss dramatic exceptions to the general rule of low mitochondrial substitution rates that have been observed within the angiosperm genus *Silene*. In this genus, massive accelerations in substitution rates have correlated with mitochondrial genome expansion and fragmentation into a highly multichromosomal genome. We will present evidence that this distinctive genome architecture has contributed to a history of sexual-like recombination in *S. noctiflora* and discuss whether this may act as a mechanism to limit the accumulation of deleterious mutations under elevated mutation pressure in this lineage.

PLANT ORGANELLAR DNA POLYMERASES ARE MULTIFUNCTIONAL ENZYMES

Luis G Brieba, Noe Baruch, Paola Garcia, Antolin Peralta
National Laboratory of Genomics, Cinvestav, Irapuato, Mexico

Although the mechanisms that mediate plant organellar DNA replication are unknown, plant organellar DNA polymerases play a predominant role during replication and DNA repair. Plant organellar genomes accumulate DNA lesions that potentially block the replication fork and need to be bypassed or repaired. The nuclear genome of flowering plants encodes two family-A DNA polymerases (DNAP) that resemble the Klenow fragment of bacterial DNAPs I. Those DNAPs are located in plant mitochondria and chloroplast and are the result of a duplication event. Organellar DNAPs from the model plant Arabidopsis thaliana (AtPolIA and AtPolIB) harbor lyase, strand-displacement, translesion synthesis (TLS), and DNA end-joining activities. Furthermore, AtPolIs assemble a functional primosome with the plant organellar DNA primase-helicase.

AtPolIs are not phylogenetically related to metazoan mitochondrial DNAPs and harbor unique amino acid insertions that decorate their polymerization domain. We have shown that those insertions are responsible for the multiple roles of AtPolIs in DNA damage tolerance. The latest example in the multifunctionally of AtPolIs is their ability to execute microhomology-mediated end-joining (MMEJ) by virtue of two specialized amino acid insertions located in their thumb subdomains. Single-stranded binding proteins (SSB) unique to plants, AtWhirly2 and organellar single-stranded binding proteins (AtOSBs), hinder MMEJ, whereas canonical mitochondrial SSB (AtmtSSB1 and AtmtSSB2) do not interfere with MMEJ. Our data predict that organellar DNA rearrangements by MMEJ are a consequence of a competition for the 3'-OH of a DSBs. If AtWhirlies or AtOSBs gain access to the single-stranded DNA (ssDNA) region of a DSB, the reaction will shift towards high-fidelity routes like homologous recombination.

Our work suggests that AtPolIs are fine-tuned molecular machines evolved to function in both DNA replication and repair.

GRAPH-BASED MODELS OF THE OENOTHERA MITOCHONDRIAL GENOME CAPTURE THE ENORMOUS COMPLEXITY OF HIGHER PLANT MITOCHONDRIAL DNA ORGANIZATION

Axel Fischer, Jana Dotzek, Dirk Walther, **Stephan Greiner**
Organelle Biology, Biotechnology and Molecular Ecophysiology, Max Planck Institute of Molecular Plant Physiology, Potsdam (Golm), Germany

Plant mitochondrial genomes display an enormous structural complexity and recombining repeat pairs lead to the generation of various sub-genomic molecules. This makes these genomes extremely challenging to assemble and difficult to visualize.
Here we present a novel approach to reconstruct mitochondrial genomes from next generation sequencing data. By applying a new assembly algorithm ISEIS (Iterative Sequence Ending Identity Search), followed by a graph-based visualization of the assemblies, we are able to identify recombining repeat pairs directly out of the assembly data. Then, a master cycle can be deduced from the graph.
We applied this approach to three mitochondria genomes of the genus *Oenothera* (the evening primrose, a model organism for cytoplasm genetics). We could show, that all identified repeat pairs are flanked by two alternative unique sequences (contigs) on both sides defining so called “forks”. This leads to four contig-repeat-contig combinations for each repeat pair. The structural model was validated by PCR and Southern blot analysis. Using the structural model as a base, we further analyzed the stoichiometric distribution between the different contig-repeat-contig combinations, employing an Illumina mate-pair library and PacBio sequencing. This data uncovered a remarkable structural dynamics of the mitochondrial genome as well as a substantial phylogenetic variation of the underlying repeat units.
The proposed model allows it to predict all potential recombination events and, thus, all theoretically possible sub-genomes that can be formed in a mitochondrial genome. In future work, our model can contribute for example to the investigation of the dynamics of sub-genome organization in different tissues.

PLANT MITOGENOMICS: PROGRESS AND PITFALLS

Jeffrey Mower
Center for Plant Science Innovation, University of Nebraska, Lincoln, USA

Tremendous progress has been made over the past 30 years of plant mitogenome sequencing, with hundreds of sequences now available from diverse plants and algae. Mitogenomes of land plants are particularly variable in terms of size, structure, and content. Long sequence read technology is enabling accurate genome assembly, but many hurdles remain. In particular, the reporting of large repeats and MIPTs in plant mitogenomes is generally lacking in verification. The now widespread use of RNAseq data to identify RNA edited sites is fraught with complications, resulting in false positives and negatives. The source of novel noncoding mitogenomic DNA and the mechanisms driving evolutionary diversification is still open to debate. I will present our recent research on the evolutionary diversity of plant mitogenomes and highlight some of the complications in assessing mitogenomic evolution.

A 5'-3' DNA EXONUCLEASE WITH FLAP-ENDONUCLEASE ACTIVITY IS REQUIRED FOR THE STABILITY OF THE PLANT MITOCHONDRIAL GENOME

Deborah Schatz, Frédérique Lotfi, Pierre Mercier, Khalid Iqbal Rana, André Dietrich,
José Manuel Gualberto
IBMP CNRS, Université De Strasbourg, Strasbourg, France

The pathways involved in the maintenance of plant organelles are in large part inherited from the bacterial ancestors of mitochondria and chloroplasts. In bacteria, a major player in genome maintenance is DNA Pol I, which possesses an essential 5'-3' exonuclease domain required for different replication and repair functions. Plants have two organellar DNA polymerases (POL1A and POL1B), which are derived from bacterial DNA Pol I and that are redundant for the replication of the organelle genomes. However, they lack the 5'-3' exonucleases domain.

We have identified two Arabidopsis nuclear genes coding for proteins similar to the 5'-3' exonuclease domain of DNA polI, which we named OEX1 and OEX2 (for Organellar EXonuclease 1 and 2). OEX1 is addressed to both chloroplasts and mitochondria, whereas OEX2 is only addressed to chloroplasts. We expressed and purified OEX1 and showed that it recognizes and rapidly digests double strand DNA in a 5'-3' direction, but it has almost no activity on single strand DNA. Site directed-mutagenesis confirmed that the PIN-like domain of OEX1 is essential for the activity. In addition, OEX1 has flap-endonuclease activity, a function that plays a key role in recombination and replication pathways.

A T-DNA mutant line of OEX1 shows a severe dwarf phenotype, deformed leaves and near sterility. These phenotypes aggravate in the following generations. The molecular analysis of the organellar genomes showed that these phenotypes correlate with defects in the maintenance of the mtDNA, with increased replication of subgenomes generated by recombination between small repeated sequences or microhomologies. This suggests that among other functions OEX1 might be involved in the degradation of replication intermediates of pathologic subgenomes that compromise the stability of the plant mtDNA.

WHAT PLANT MITOCHONDRIA EAT: MAPPING OUT MITOCHONDRIAL METABOLISM

Harvey Millar
Arc Centre of Excellence in Plant Energy Biology, The University of Western Australia, Perth, Australia

Mitochondrial respiration in plants provides energy for biosynthesis and its balance with photosynthesis determines the rate of plant biomass accumulation. In undertaking these roles, plant mitochondria sit at a crossroads of carbon and nitrogen metabolism. The reductant they use to drive electron transport is derived from a variety of sources, in a rhythm over the course of a day and in long arcs over the developmental lifetime of a tissue type. Much has been learnt from plant 'mitochondriology' where isolated organelle or organelle lysates are provided with exogenous substrates and the rates and dynamics of single enzymes, transporters or biochemical pathways are assessed. This work has built our understanding of plant mitochondrial enzymology, designed diagrams in textbooks of plant metabolism and metabolite transport and has identified what make plant mitochondria unique to mitochondria from other eukaryotes. Separately the study of carbon dioxide production, oxygen consumption and metabolite levels and fluxes *in vivo* has built a complex and at times perplexing presentation of what plant mitochondria consume *in vivo*. Combining these two perspectives through detailed longer term study of respiratory rates and responses *in vivo*, analysis of respiratory phenotypes using reverse genetics of mitochondrial components and exploring natural variation in respiratory rate are now providing new insights into 'what plant mitochondria eat'.

WHEN PLANTS HOLD THEIR BREATH – HOW HYPOXIA-INDUCED MITOCHONDRIAL ELECTRON TRANSPORT INHIBITION RE-ORCHESTRATES SUBCELLULAR REDOX AND ENERGY PHYSIOLOGY

Markus Schwarzländer
Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany

Low oxygen levels regularly occur in normal plant development and can be induced by the environment, for example through flooding. Then limited gas diffusion causes mitochondrial respiration to gradually deplete its key substrate, inducing hypoxia stress, which includes a cellular energy crisis. To stay alive, plant cells need to rapidly and profoundly re-orchestrate their physiology. Despite the role of the mitochondria as the major cellular oxygen consumers, recent research has mainly highlighted cytosolic and nuclear processes of the hypoxia response. In contrast, the roles of mitochondria in inducing hypoxic re-programming in plant tissues remain surprisingly poorly investigated.

To understand how plant cells respond to progressing oxygen restriction we have established a fluorescent protein sensor-based system for multiparametric monitoring of subcellular physiology in living Arabidopsis organs. By monitoring the specific dynamics of MgATP, free calcium ion concentration, pH, NAD redox status and glutathione redox status in parallel we generate an integrated picture of the dynamic physiological response to hypoxia. Pharmacological inhibition of the mitochondrial respiratory chain caused remarkably similar dynamics highlighting mitochondrial electron transport as a major determinant of the cellular consequences of hypoxia. I will discuss the current state of our efforts to map out the cell physiological context under which both mitochondrial retrograde signalling and low oxygen signalling occur.

THREE DECADES OF RNA EDITING RESEARCH

Ian Small

Arc Centre of Excellence in Plant Energy Biology, The University of Western Australia, Perth, Australia

The ability to alter nucleic acid sequences within a living cell would be a major step towards solving many of the challenges facing biologists, such as improving food production from crops or treating human genetic diseases, and thus RNA editing has always invited attention. C-to-U RNA editing was discovered in plant mitochondria 30 years ago and has intrigued (and often frustrated!) a whole generation of scientists ever since. Early biochemical studies by teams led by Axel Brennicke and Jean-Michel Grienenberger (both sadly no longer with us) demonstrated that editing proceeded by base deamination, but finding the elusive deaminase took nearly two decades, and even then the identification remained highly speculative. The discovery that pentatricopeptide repeat (PPR) proteins were the specificity factors that determined which sites were edited reinvigorated editing research, and has led to a pretty solid understanding of how editing sites are recognised. However, although many components of the editing system have been described over the years, almost all of them have been found by study of loss-of-function mutations. The reconstitution of the editing process using defined components in a simple in vitro or heterologous system would be a huge boon to researchers in this area, as well as providing the definitive proof that the editing enzyme is indeed what we think it is. Many have tried and failed to reach this goal, but at last good news is at hand, as we will hear at this conference. With what is perhaps the final breakthrough needed to fully understand the editing process now achieved, it is a good time to look back on the three decades of research that have led to this point and look at the implications of these discoveries for the future.

PLANT TYPE C-TO-U RNA EDITING IN ESCHERICHIA COLI

Mareike Schallenberg-Rüdinger, Bastian Oldenkott, Yingying Yang, Elena Lesch, Volker Knoop
Molecular Evolution, Institute for Cellular and Molecular Botany, University Bonn, Bonn, Germany

RNA editing converting cytidines (C) into uridines (U) is a hallmark of gene expression in land plant mitochondria and chloroplasts. Via RNA editing conserved codon identities are restored on transcript level to correct genetic information and to enable proper protein function. RNA binding pentatricopeptide repeat (PPR) proteins have key roles in target recognition of Cs to be edited but the functional editosome has remained elusive. With our recently established RNA editing system in the bacterium Escherichia coli, we could demonstrate that single DYW-type PPR proteins of the model moss Physcomitrella patens alone can perform efficient C-to-U editing in a heterologous system perfectly reproducing the in planta editing situation. Single amino acid exchanges in the DYW domain abolish RNA editing, confirming it as the functional cytidine deaminase. The modification of RNA targets and the identification of several off-targets in the E. coli transcriptome reveal nucleotide identities critical for recognition and cytidine conversion. First changes in single PPR motifs lead to the recognition of new targets, facing towards successful future establishment of transcript editing in other genetic environments.

CHARACTERIZATION OF THE OZ FAMILY INVOLVED IN ORGANELLE RNA METABOLISM

Stephane Bentolila[1], Andrew Gipson[1], Alexander Kehl[2], Maureen Hanson[1]
[1]Molecular Biology and Genetics, Cornell University, Ithaca, USA, [2]Plant Biology Department, University of California, Davis, USA

In land plants, organelle transcripts are modified post-transcriptionally by C-to-U editing. Typically 30 to 40 C-to-U editing events occur in the chloroplast transcriptome of flowering plants, while hundreds of C residues are edited in plant mitochondria. These extensive alterations are critical for production of mitochondrial proteins that can function properly. Our lab has actively participated in the identification of the four families of protein factors involved in organelle editing, namely, the pentatricopeptide repeat (PPR), the RIP/MORF, the ORRM and the OZ families. We identified OZ1, the founding member of the OZ family, as an essential chloroplast RNA editing factor since the mutant shows a major loss of editing extent at 14 sites. OZ1 contains two tandem zinc finger domains called RanBP2 type zinc fingers. This RanBP2 type zinc finger domain is found in three other Arabidopsis proteins, OZ2, OZ3 and OZ4.

Our subcellular localization studies demonstrate that all OZ proteins except OZ1 are targeted to mitochondria, with OZ4 being dually targeted. We recovered 3 homozygous mutant lines for *oz3* and two homozygous mutant lines for *oz4* from T-DNA collections. No homozygous mutants could be recovered from two T-DNA lines with exon insertions in *OZ2*, indicating that *oz2* mutation leads to embryo lethality. In order to study the molecular function of OZ2, the *oz2* mutant was rescued by complementing a heterozygous individual with a cDNA carrying the wild-type coding sequence under the control of the seed-specific ABSCISIC ACID-INSENSITIVE3 (ABI3) promoter. The ABI3 promoter allows mutant plants to survive to the seedling stage, when the mutant phenotype appears due to downregulation of ABI3 in later stages of development. The growth of two homozygous *oz2* mutant plants thus transformed was impaired compared to wild-type. Investigation of the molecular phenotype responsible for the growth defect in the *oz2* mutant will be presented.

REASSEMBLY OF COMPLEX PLANT RNA EDITOSOMES IN MITOCHONDRIA OF MARCHANTIA POLYMORPHA.

Mizuki Takenaka[1], Matthias Burger[2], Anja Jörg[2], Sascha Haag[2], Gert Weber[3]
[1]Graduate School of Science, Kyoto University, Kyoto, Japan, [2]Molekulare Botanik, Ulm University, Ulm, Germany, [3]Materialien Und Energie, Helmholtz Zentrum Berlin, Berline, Germany

RNA editing in plant organelles converts specific cytidines to uredines in mRNA. Targeted cytidines are recognized by PLS class pentatricopeptide repeat (PPR) proteins, which specifically bind upstream of the target cytidines. In the past several years, different types of proteins have been identified as RNA editing factors in angiosperms, including multiple organellar RNA editing factors/RNA editing factor interacting proteins (MORFs/RIPs), organelle RNA recognition motif (ORRM) proteins, organelle zinc-finger (OZ) proteins, a P-class PPR protein NUWA, short DYW proteins DYW1 and DYW2. Despite many key players for the assembly of editosomes having been revealed, how these components contribute editing machinery including the deaminase enzymatic activity is still unclear. To understand the function of each RNA editing factors, we are attempting to reassemble an angiosperm type RNA editing complex in mitochondria of *Marrchantia polymorpha* where the system has been lost during evolution.

POST-TRANSCRIPTIONAL GENERATION OF 5'TERMINI OF PLANT MITOCHONDRIAL RNAS

Sarah Schleicher[1], Sabine Hauber[1], Kevin Mayer[1], **Stefan Binder[2]**
[1]Ulm University, Molecular Botany, Ulm, Germany, [2]Inst. Molecular Botany, Ulm University, Ulm, Germany

In plant mitochondria, multiple post-transcriptional processes contribute to RNA maturation. For many transcript species this involves the generation of secondary 5' termini, a process whose functional importance is still largely unclear. We set out to identify and describe proteins and RNA structural elements involved in 5' end formation. Several pentatricopeptide repeat proteins, called RNA processing factors (RPFs) and two potential mitochondrial nucleases (MNU1 and MNU2) have been identified. In different Arabidopsis thaliana ecotypes, the impact of the different RPFs on distinct RNAs are highly diverse ranging from subtle differences at the 5' termini of nad3-rps12 mRNA to extremely different pattern of ccmC transcripts. Accordingly the precise function of individual RPFs in transcript end maturation is still puzzling. Predictions suggest binding of these proteins upstream of processing sites, which has to be experimentally confirmed. Electrophoretic mobility shift assays are used to further define binding requirements. Since it seems to be clear that RPFs do not have intrinsic nuclease function additional proteins are required for 5' processing, two of them being the candidate proteins MNU1 and MNU2. These proteins contain a Nedd4-BP1, YacP Nuclease (NYN) metallonuclease domain and several putative RNA binding domains. Our current efforts concentrate on the characterization of the nuclease activity as well as the RNA binding properties of MNU1.

TARGETED CLEAVAGE OF MITOCHONDRIAL TRANSCRIPTS INDUCED BY MODIFIED PENTATRICOPEPTIDE REPEAT PROTEINS

Catherine Colas des Francs-Small[1], Lilian Vincis Pereira Sanglard[1], Fei Yang[1,2], Ian Small[1]
[1]Arc Centre of Excellence in Plant Energy Biology, The University of Western Australia, Perth, Australia, [2]State Key Laboratory for Conservation and Utilization of Bio Resources, Yunnan Agricultural University, Kunming, China

PPR proteins are sequence-specific RNA-binding proteins involved in post-transcriptional stages of organelle transcript maturation. Their modular and predictable interactions with RNA make them amenable to custom modification and design. We are exploring the potential of PPR proteins as tools to deliberately alter the expression of targeted mitochondrial genes.
We have used variants of the PPR protein RNA PROCESSING FACTOR 2 (RPF2) to induce cleavage and degradation of several mitochondrial transcripts including nad6and atp1. The cleavage occurs near to the intended binding site and is sufficiently effective to almost eliminate expression of the targeted transcript. In this talk, I will describe the possible mechanism of action of RPF2 and discuss the potential issue of off-target binding.
This approach opens a way to reverse genetics studies on mitochondrial gene functions and to potential applications in agriculture, such as for example creating new restorer-of-fertility alleles to facilitate the breeding of hybrid crops.

TARGETED DISRUPTION OF MITOCHONDRIAL GENES ASSOCIATED WITH CYTOPLASMIC MALE STERILITY IN RICE AND RAPESEED.

Tomohiko Kazama[1], Chie Koizuka[2], Nobuhiro Tsutsumi[3], Kinya Toriyama[1], Nobuya Koizua[2], **Shin-ichi Arimura[3]**

[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan, [2]College of Agriculture, Tamagawa University, Tokyo, Japan, [3]Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan

A lack of methods for transforming plant mitochondrial genomes has hampered our understanding of mitochondrial genes. Some of these genes are involved in the agronomically important trait of cytoplasmic male sterility (CMS). Here we attempted to edit CMS-related genes (orf79and orf125) in their mitochondrial genomes of male-sterile lines, BTA of rice Oryza sativaand SW18 (kosena, a kind of Ogura-type) of rapeseed Brassica napus by using transcription activator-like effector nucleases (TALENs) with mitochondrial localization signals (mitoTALENs). Targeted disruption of these genes led to deletions ranging from ca. 100 bp to 5 kb and restored fertility. The sequences adjacent to the deletions did not reconnect each other but connected to distant loci by homologous recombination. These results collectively suggest that the configurations of the mitochondrial genomes after treatment of mitoTALENs changed without lacking essential genes. Fine structures of the treated mitochondrial genomes are now under analysis. mitoTALENs appear to be an effective tool for analyzing and modifying plant mitochondrial genomes.

COMPLEXITIES OF RNA SPLICING IN PLANT MITOCHONDRIA

Linda Bonen
Biology, University of Ottawa, Ottawa, Canada

Mitochondrial introns in flowering plants virtually all belong to the group II family of ribozyme mobile retroelements, however some of them lack structural features needed for classical lariat splicing. To learn more about mechanisms of cis- and trans-splicing in vivo, we determined the physical forms of excised introns in wheat mitochondria, and examined intron RNA populations by direct sequencing as well as clone analysis. We observed diverse splicing behaviours among introns, and interestingly, for certain poorly-conserved degenerate introns, we saw differences in splicing biochemistry between orthologous introns in grasses and legumes. When wheat seedlings or embryos were subjected to growth in the cold, some introns showed a shift in the complexity and form of excised products, consistent with an environmental effect on intron RNA folding and conformational flexibility. Variation was also seen in the stability of excised cis-introns, and trans-spliced 5' half-introns were unexpectedly stable. The relatively high levels of precursor RNAs in cold-treated embryos have also been helpful in tracking other RNA processing events such as editing and mRNA end-maturation. Although RNA editing is typically an early event in the production of mature mRNAs, some sites only undergo editing after splicing, pointing again to the importance of RNA structure in expression events. Our studies illustrate the plasticity of group II intron splicing pathways and the complexity of RNA processing in plant mitochondria. Supported by NSERC Canada.

DIFFERENT TYPES OF CA DOMAINS ARE PRESENT IN COMPLEX I FROM IMMATURE SEEDS AND ADULT PLANTS IN ARABIDOPSIS THALIANA

Eduardo Julián Zabaleta, Juan-Pablo Córdoba, Marisol Fassolari, Fernanda Marchetti, Débora Soto, Gabriela Pagnussat

Conicet Universidad Nacional De Mar Del Plata, Instituto De Investigaciones Biológicas, Mar Del Plata, Argentina

Mitochondrial NADH dehydrogenase complex is the first complex of the mitochondrial electron transfer chain. In plants and in a variety of eukaryotes except Opisthokonta, complex I contains an extra spherical domain called Carbonic Anhydrase (CA) domain. This domain is thought to be composed of trimers of gamma type CA and CA-like subunits. In Arabidopsis, the CA gene family contains five members (CA1, CA2, CA3, CAL1, and CAL2). The CA domain appears to be crucial for complex I assembly and is essential for normal embryogenesis. As CA and CA-like proteins are arranged in trimers to form the CA domain, it is possible for the complex to adopt different arrangements that might be tissue-specific or have specialized functions. In this work, we show that the proportion of specific complex I changes in a tissue specific manner. In immature seeds, complex I assembly may be indistinctly dependent on CA1, CA2 or CA3. However, in adult plant tissues (or tissues derived from stem cells, as cell cultures), CA2-dependent complex I is clearly the most abundant. This difference might account for specific physiological functions. We present evidence suggesting that CA3 does not interact with any other CA family member and that may play a role in assembly and stability of complex I.

COMPOSITION AND ARCHITECTURE OF ARABIDOPSIS MITOCHONDRIAL RIBOSOME

Florent Waltz[1], Hakim Mireau[2], Yaser Hashem[3], **Philippe Giegé[1]**

[1]Ibmp Cnrs, University of Strasbourg, Strasbourg, France, [2]Ijpb, Inra, Versailles, France, [3]Iecb, Inserm, Bordeaux, France

Mitochondria are responsible for energy production through aerobic respiration and represent the powerhouse of eukaryotic cells. Their metabolism and gene expression processes combine bacterial-like features and traits that evolved in eukaryotes. Among mitochondrial gene expression processes, translation remains the most elusive. In plants, while numerous pentatricopeptide repeat (PPR) proteins are involved in all steps of gene expression, their function in mitochondrial translation remains unclear.

We present the biochemical characterisation of Arabidopsis mitochondrial ribosomes and identify their protein subunit composition. Complementary biochemical approaches identify 19 plant specific mitoribosome proteins, among which 10 are PPR proteins. The knock out mutations of ribosomal PPR (rPPR) genes result in distinct macroscopic phenotypes including lethality or severe growth delays. The molecular analysis of rppr1 mutants using ribosome profiling as well as the analysis of mitochondrial protein levels reveal that rPPR1 is a generic translation factor, which is a novel function for PPR proteins.

Finally, single particle cryo-electron microscopy reveals the unique structural architecture of Arabidopsis mitoribosomes, characterised by a very large small ribosomal subunit, larger than the large subunit, bearing an additional RNA domain grafted on the head.

Overall, results show that Arabidopsis mitoribosomes are substantially divergent from bacterial and other eukaryote mitoribosomes, both in terms of structure and of protein content. This contributes to unravel the diversity of translation systems across eukaryotes.

Reference

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THE TRANSLATIONAL LANDSCAPE OF PLANT MITOCHONDRIA AND CONTROL BY PPR PROTEINS

Tan-Trung Nguyen[1], Noelya Planchard[1], Pierre Bertin[2], Martine Quadrado[1], Céline Dargel-Graffin[1], Isabelle Hatin[3], Olivier Namy[3], **Hakim Mireau[2]**

[1]Institut Jean Pierre Bourgin, Inra, Versailles, France, [2]Intitut Jean Pierre Bourgin, Inra, Versailles, France, [3]Institute for Integrative Biology of the Cell, Cnrs, Orsay, France

Translation in plant mitochondria is a complex process that is still poorly understood at the molecular level. Growing evidence indicates though that mitochondrial translation differs from its bacterial counterpart in many key aspects. To better understand how mitochondrial translation is orchestrated and regulated in plants, we have used the ribosome profiling technology to generate genome-wide views of the mitochondrial translatome in different plant genotypes. This approach led us to reveal that most plant mitochondrial ribosome footprints measure 27 and 28 bases. Quantification of ribosome footprints along transcripts revealed that mRNAs have highly divergent ribosome densities, suggesting a tight control of translation initiation or elongation in plant mitochondria. To better understand the basis of this control, we identified and have been characterizing mitochondria-targeted pentatricopeptide repeat (PPR) proteins specifically involved in translation. I will present our most recent advances on the characterization of these PPR proteins.

RIBOSOMAL REGULATION OF PLANT MITOCHONDRIAL TRANSLATION

Hanna Elzbieta Janska
Faculty of Biotechnology, Molecular Biology of the Cell Dept, University of Wroclaw, Wroclaw, Poland

Ribosomes are not simple non-selective translation machines, but may also function as regulatory elements choosing appropriate set of transcripts to meet current demands of the cell. The basis of ribosome filter hypothesis will be presented with the emphasis on plant mitochondrial translation system. We found that silencing of the nuclear *RPS10* gene encoding mitochondrial ribosomal protein S10 in *Arabidopsis* disturbed biogenesis of mitoribosomes and affects several steps of mitochondrial genome expression. To adjust mitochondrial proteome to a continuously generated deficit of the S10 protein mitoribosomes characteristic for *rps10* translate the mRNA pool differentially compared with wild-type. The wild type mitoribosomes preferentially synthesize OXPHOS proteins while *rps10* mitoribosomes are less selective and translation efficiency of transcripts encoding ribosomal proteins, maturase and the TatC protein is substantially increased. The translational efficiency of transcripts encoding OXPHOS proteins is also modulated but not so strong and for majority of transcripts this metric is reduced. The contribution of transcriptional control in the production of proteins in *rps10* will be also shown. The predominance of translation control over transcriptional was observed for majority of subunits of complex I. Recent results concerning distinctive features of translation in *rps10* will be also discussed.
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POST-TRANSLATIONAL PROTEIN MODIFICATIONS REGULATE MITOCHONDRIAL METABOLISM

Ian Max Møller[1], Abir U. Igamberdiev[2], Natalia V. Bykova[3], Allan G. Rasmusson[4], Iris Finkemeier[5], Markus Schwarzländer[5]

[1]Department of Molecular Biology and Genetics, Aarhus University, Slagelse, Denmark, [2]Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, [3]Morden Research and Development Centre, Agriculture and Agri Food Canada, Morden, Manitoba, Canada, [4]Department of Biology, Lund University, Lund, Sweden, [5]Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany

The metabolism of plant mitochondria is redox regulated via post-translational modifications (PTMs) of mitochondrial proteins. The most prominent PTMs are oxidative modifications - thiol/disulfide interconversions, nitrosylation and methionine oxidation - but also others, such as the classical phosphorylation and the rapidly emerging acetylation, are important. Recent progress in targeted proteomics and use of in vivo fluorescent redox probes have contributed greatly to our understanding of this regulation. The PTMs are mostly enzyme catalyzed and reversible so they are regulated in turn. Two PTMs can even interact to regulate enzyme activity, a case in point being methionine oxidation and phosphorylation. This regulation acts to rapidly adjust mitochondrial metabolism to suit the cellular requirements on the seconds-to-minutes time scale, before retrograde signaling can activate genetic regulation.

UNIQUE FEATURES OF CHLAMYDOMONAS MITOCHONDRIAL GENOME EXPRESSION

Thalia Salinas-Giegé[1], Claire Remacle[2], Laurence Maréchal-Drouard[1], Olivier Vallon[3]
[1]Institut De Biologie Moléculaire Des Plantes (Ibmp), Cnrs, Strasbourg, France, [2]Institute of Botany, University of Liège, Liège, Belgium, [3]Institut De Biologie Physico Chimique (Ibpc), Cnrs, Paris, France

Recent years have brought important insights into mitochondrial (mt) gene expression. Nonetheless, many fundamental questions remain unanswered regarding the post-transcriptional modifications that stabilize mt mRNAs and prepare them for translation.
In this context, we initiated the characterization of mt transcripts in *Chlamydomonas reinhardtii*, a unicellular green alga that, in the recent years, has emerged as a powerful model to study mt molecular processes. Using complementary approaches, we evidenced peculiar features on these mRNAs. First, they lack a 5' untranslated region (UTR), similar to mammalian mt ones, and start at the AUG initiation codon. Second, around 35% of mRNAs possess post-transcriptionally added tails at the 3' extremity of their short 3'-UTR. While some tails are short and rich in A and U residues, most of them are predominantly Cytidine-rich. This is the first and only example of such modification to date. Such C-rich tails are very rare on mt rRNAs, and cannot be detected neither in plastidial nor in cytosolic mRNAs. A phylogenetic study also demonstrated that polycytidylation of mt mRNAs is a hallmark of Chlorophyceae group, which Chlamydomonas belongs [1]. This finding raises fascinating questions as to what roles these C-rich tails may play in mt gene expression.
From our analysis, we can observe that a single endoribonucleolytic cleavage usually releases both the 3' end of a mRNA and the 5' extremity of the downstream transcript. Strikingly, most of the C-rich tails lie at or very near this cleavage site, suggesting that these tails are added immediately after processing. Thus, the endoribonucleolytic processing and the addition of C-tails must be studied in conjunction. In order to shed light on the function of the C-tails, we are currently analyzing two type of protein families: the RNA-dependent nucleotidyl-transferases (NTRs) proteins that could be involved in mRNA polycytidylation and the OctotricoPeptideRepeat (OPR) proteins that could be implicated in endonucleolytic processing. Biochemical and genetic studies are underway to determine if members of these protein families are indeed involved in Chlamydomonas mitochondrial mRNA maturation.
[1] Salinas-Giegé T., Cavaiuolo M., *et al*. (2017) Polycytidylation of mitochondrial mRNAs in *Chlamydomonas reinhardtii*. Nucleic Acids Res., 22, 12963-73.

MITORIBOSOME SPECIFICITIES AND DIVERGENCE ACROSS EUKARYOTES, AND THE ROLES OF PPR PROTEINS

Florent Waltz[1,2], Philippe Giege[1]
[1]Cnrs, Ibmp, Strasbourg, France, [2]Inserm, Iecb, Bordeaux, France

Ribosomes are the molecular machines performing protein biosynthesis. In eukaryotes they are found in the cytosol, where they take in charge nucleus-encoded mRNAs, but specific ribosomes, mitoribosomes, are also found in mitochondria. The latter translate the small subset of mRNAs encoded by the mitochondrial genome.
Recently, our laboratory described the unusual structure and composition of the *Arabidopsis thaliana* mitoribosomes. Using complementary biochemical approaches, 19 plant specific r-proteins were identified, among which 10 are PPR proteins (rPPR), a family of proteins particularly prevalent in plants. Moreover, using single particle cryo-electron microscopy the unique structural architecture of Arabidopsis mitoribosomes was revealed. The most striking feature being the large small subunit, where most of the PPRs locate, characterized by a novel head domain.
These results emphasize the fact that mitoribosomes strongly diverged during eukaryote evolution. Indeed, recent analyses on human, yeast and trypanosoma showed that their composition and structures are completely different from each other. Still, a major feature is that, with the exception of yeast, all mitoribosomes have recruited PPR proteins for their activity. In yeast, although PPRs are not core mitoribosome proteins, they do play a central role as activator of translation. Here, the composition and structural heterogeneity of mitoribosomes will be presented and the possible function(s) of PPR proteins as part of these specialized ribosomes and for translation in general will be discussed.
Keywords: Translation, mitochondria, Arabidopsis, pentatricopeptide repeat

NON-PROTEIN AMINO ACIDS TARGETING ORGANELLAR TRANSLATION

Oren Ostersetzer-Biran[1], Hagit Zer[1], Ruth Van Oss Pinhasi[2], **Liron Klipcan[2]**

[1]Department of Plant & Environmental Sciences, The Alexander Silberman Institute of Life Sciences, the Hebrew University of Jerusalem, Jerusalem, Israel, [2]Department of Plant Sciences, Gilat Research Center, Agricultural Research Organization, Gilat, Israel

Plants contain an extraordinary variety of modified or non-protein amino acids (NPAAs), and up to date, more than 500 have been isolated. Recently a natural derivative of phenylalanine called L-meta-tyrosine (m-Tyr) was proposed as a new potential herbicide. We have recently demonstrated that several synthetic m-Tyr derivatives are highly effective in inhibiting seed-germination and plant development. We show that Arabidopsis plants treated with low concentrations of m-Tyr, exhibit short roots, and retarded growth phenotypes. Electron microscopy (EM) analyses of Arabidopsis plants treated with m-Tyr indicate to altered chloroplasts and mitochondria morphologies. Blue-Native (BN) PAGE analysis further showed that m-Tyr affects the accumulation of native organellar (plastid and mitochondria) complexes. Moreover, the addition of sucrose to the growth media reduces the effects of m-Tyr on the plants' growth and development. These results strongly imply that organellar biogenesis was strongly affected by the addition of m-Tyr to the growth media. Our data suggest that m-Tyr is incorporated to the organellar proteomes by the phenylalanyl-tRNA synthetase (PheRS) enzyme (dually targeted to the mitochondria and plastids), whereas the activity of the cytosolic enzyme is not affected by this analog. The data we obtained imply two central modes of actions by the m-Tyr analoges: (i) NPAA analogues of Phe are killing plants by specifically affecting proteins synthesis in the chloroplasts/mitochondria and/or (ii) by altering the biosynthesis of aromatic amino acids (shikimate pathway), possibly by inhibiting key enzymes of the pathway.

LESSONS FROM MISTLETOE MITOCHONDRIA

Jennifer Senkler, Nils Rugen, Holger Eubel, Lucie Schröder, **Hans-Peter Braun**
Leibniz Universität Hannover, Institute of Plant Genetics, Hannover, Germany

European mistletoe (*Viscum album*) is an obligatory hemiparasitic flowering plant, which grows on branches of various trees. It is supplied with water, minerals and organic compounds from the host. At the same time, *V. album* carries out photosynthesis and produces energy rich compounds. The mitochondrial genome of *V. album* is exceptional because it lacks all genes encoding subunits of complex I of the respiratory chain. A project to biochemically investigate *V. album* mitochondria was therefore initiated. First results were published recently (Senkler et al. 2018). V. album is the first example of a multicellular species that naturally lacks mitochondrial complex I, the main entrance point for electrons into the respiratory chain. At the same time, complexes II and V are of low abundance. Complexes III and IV form a remarkably stable supercomplex and alternative respiratory enzymes are prominent. Furthermore, the nucleic acid metabolism of *V. album* mitochondria is altered. Several questions remain to be addressed: How can *V. album* survive with a considerably reduced capacity to produce ATP in mitochondria? Are some of the so-called accessory subunits of complex I present in mistletoe mitochondria and in which locations? How does the absence of complex I affect photosynthesis in *V. album*? We will present new directions of research.

Senkler, J., Rugen, N., Eubel, H., Hedermann, J. and Braun, H.P. (2018) Absence of complex I implicates rearrangement of the respiratory chain in European Mistletoe. Current Biology 28, 1606-1613.

THE UNUSUAL RESPIRATION OF VISCUM ALBUM.

Andrew Maclean[1], Alexander Hertle[2], Joanna Ligas[2], Ralph Bock[2], Janneke Balk[1],
Etienne Meyer[3,2]

[1]Department of Biological Chemistry, John Innes Centre, Norwich, United Kingdom,
[2]Organelle Biology and Biotechnology, Max Planck Institute of Molecular Plant Physiology, Potsdam Golm, Germany, [3]Institute of Plant Physiology, Martin Luther University, Halle Wittenberg, Halle (Saale), Germany

Cellular respiration is a set of metabolic reactions converting biochemical energy from nutrients into ATP. ATP can be produced either during the catabolic reactions (substrate level phosphorylation) or during the last step of cellular respiration: oxidative phosphorylation (OXPHOS). In multicellular eukaryotes, the bulk of ATP is produced by the OXPHOS system located in the mitochondrial inner membrane. However, in some situations, ATP production through substrate level phosphorylation can become essential. This is observed under anoxic conditions or in cancer cells where the metabolic switch from oxidative phosphorylation to substrate level phosphorylation has been called the Warburg effect. In plants, we have previously observed a Warburg-like effect in Arabidopsis mutants lacking the activity of complex I, the first enzyme of the OXPHOS system.

Recently, the mitochondrial genomes of several *Viscum* species were shown to lack genes encoding complex I subunits, raising the issues of the presence of complex I and of the respiratory metabolism utilized by these hemiparasitic plant species to produce ATP. We isolated mitochondria from the European mistletoe (*Viscum album*) and analyzed their composition using biochemical and proteomics tools. We could not detect complex I and we found that all the other complexes of the OXPHOS system, including the ATP synthase, were low abundant. These observations suggest that the mitochondria of *Viscum album* lost most of their capacity for oxidative phosphorylation and therefore ATP production. We measured fluxes through the respiratory pathway and observed elevated glycolytic fluxes, suggesting that *Viscum album* adapted its metabolism to produced ATP outside mitochondria.

THE UNUSUAL ELECTRON TRANSPORT CHAIN IN MISTLETOE MITOCHONDRIA

Gitte Petersen[1], Ian Max Möller[2], Athanasios Zervas[3], Ole Seberg[4]
[1]Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden, [2]Department of Molecular Biology and Genetics, Aarhus University, Slagelse, Denmark, [3]Department of Environmental Science, Aarhus University, Roskilde, Denmark, [4]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

Mistletoe species (*Viscum* spp.) have been reported to have a unique loss of Complex I genes from their mitochondrial genomes. Simultaneously, most of the remaining mitochondrial genes show substitution rates much higher than most other plants. Recently, biochemical studies confirmed the lack of Complex I and presence of Complexes II-V.
New evidence shows that the loss of mitochondrial Complex I genes is not unique to the genus Viscum, but possible to a clade within the order Santalales. Transcriptome studies have confirmed the absence of mitochondrial Complex I transcripts, but also the active transcription and RNA editing of the highly deviating mitochondrial complex II-V genes. Preliminary analyses of substitution rates and expression patterns of the nuclear genes encoding the mitochondrial electron transport components reveal – as expected – lack of Complex I transcripts, however, with the notable exception of genes encoding two very interesting proteins, carbonic anhydrase 1 and L-galactono-1,4-lactone dehydrogenase. Additionally, we find transcription of a number of alterative NAD(P)H dehydrogenases and oxidases. For nuclear Complex II-V genes, transcription appears normal, and the genes do not show high substitution rates as their mitochondrial counterparts.

THE ROLE OF THE PLANT MITOCHONDRIAL TAT PATHWAY

Renuka Kolli, Kerstin Schäfer, Jürgen Soll, **Chris Carrie**
Botany, Ludwig Maximilians Universität München, Munich, Germany

The OXPHOS system of plant mitochondria is composed of large multiprotein complexes of dual genetic origin. How the individual subunits are assembled together to form these complexes is a central question in understanding plant mitochondrial biogenesis. Recently we have uncovered several plant specific pathways for the assembly of complexes III and IV from the respiratory chain. Firstly, it could be demonstrated that plant mitochondria contain a Twin arginine transport (Tat) pathway for the assembly of complex III. Complementation with a C-terminal truncated TatB subunit lead to plants with severe growth defects and male sterility. It was determined that these phenotypes were caused by a failure to insert the Rieske Fe/S protein into dimeric complex III. Secondly, two members of the YidC/Oxa1/Alb3 family of proteins named Oxa2A and Oxa2B which both contain plant specific C-terminal TPR domains were essential for the assembly of complexes III and IV, respectively. It was shown that deletion of the TPR domain of Oxa2B led to plants which fail to correctly insert Cox2 into the inner membrane resulting in a loss of complex IV. Deletion of the TPR domain of Oxa2A produced plants with no apparent phenotypic consequences. However, using a semi-complementation approach, it was observed that plants lacking Oxa2A resulted in a large reduction in complex III. At this point in time, the exact substrate of Oxa2A is unknown but it is predicted that it may be responsible for the insertion of the Cyt c1 subunit. Our recent results in studying the roles and functions of Oxa proteins and the mitochondrial Tat pathway in plant mitochondrial biogenesis will be discussed.

NEO-FUNCTIONALISATION OF MITOCHONDRIAL PROTEINS AND INCORPORATION INTO SIGNALING NETWORKS IN PLANTS

Sbatie Lama[1], Martyna Broda[2], Zahra Abbas[2], Dries Vaneechoutte[3], Katharina Belt[2], Torbjörn Säll[1], Klaas Vandepoele[4], **Olivier Van Aken[1]**

[1]Department of Biology, Lund University, Lund, Sweden, [2]Arc Centre of Excellence in Plant Energy Biology, University of Western Australia, Crawley, Australia, [3]Vib Department of Plant Systems Biology, Ghent University, Ghent, Belgium, [4]Vib Department of Plant Systems Biology, Lund University, Lund, Sweden

Mitochondria and chloroplasts are crucial for plant viability and communicate information on their functional status to the cellular nucleus via retrograde signalling, thereby affecting gene expression. Retrograde signalling can occur in response to stresses, but also during organelle biogenesis. Although mitochondrial retrograde signalling occurs in a wide range of eukaryotic taxa such as yeast, animals and plant, the different taxa appear to have evolved their own specific signalling pathways and target often lineage-specific genes. Using next generation sequencing, we have identified a set of novel plant proteins with an uncharacterised conserved domain, which respond to mitochondrial dysfunction. Surprisingly, phylogenetic analysis revealed that these proteins represent a unique example of de novo mitochondrial functionalisation, and have only been incorporated into the retrograde signalling pathway relatively recently. This shows that new stress response strategies are still being created in very recent plant evolutionary history. The very rapid expansion of paralogs of this protein group suggests that significant evolutionary pressure exists to create unique variants for different cellular organelles

FUNCTION OF MITOCHONDRIAL EXTERNAL NAD(P)H DEHYDROGENASES IN PLANTS

Mengshu Hao[1], Anna Podgórska[2], Monika Ostaszewska-Bugajska[2], Bozena Szal[2], **Allan G. Rasmusson[1]**

[1]Department of Biology, Lund University, Lund, Sweden, [2]Institute of Experimental Plant Biology and Biotechnology, University of Warsaw, Warsaw, Poland

Plant mitochondria contain several external dehydrogenases (DHs) oxidising cytosolic NADPH or NADH. The NADPH DH function is generally carried by an NDB1-type protein (numbered according to *Arabidopsis thaliana*), which is present throughout most eukaryotes and is evolutionarily ancestral to the NDB2-type proteins present in dicots and monocots (1). External mitochondrial NADH DH in potato is activated by sub-µM concentrations of calcium ions. In contrast, NDB1 is rapidly activated by calcium ions in the µM range, and the activation depends on pH (2). After bacterial overexpression and purification, both potato StNDB1 and *A. thaliana* AtNDB2 were found to reside in solution as oligomers of homodimer units. StNDB1 displayed rapid calcium-activation of NAD(P)H DH activity whereas NADH oxidation by AtNDB2 was less affected by calcium ions. Purified StNDB1 also displayed rapid calcium ion-dependent changes in protein fluorescence, indicating conformational changes being induced (Hao et al., unpublished).

Suppression of AtNDB1 decreases growth rate and affect respiration and cellular redox homeostasis under normal growth conditions (3). In contrast, novel data indicates that under ammonium toxicity-associated oxidative stress in *A. thaliana*, AtNDB1-suppressed plants grew faster than wild type. The faster growth was associated with decreased mitochondrial ROS production and elevated levels of glutathione-associated antioxidants (4). Regulation of NDB1 in association with stress metabolism will be discussed.

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FLEXIBILITY OF MITOCHONDRIAL RESPIRATION IS REQUIRED TO ALLEVIATE REDUCTIVE STRESS IN PLANTS

Philippe Fuchs[1,2], Stephan Wagner[1,2], Berivan Mansuroglu[2,3], Cristina Ruberti[1], Andreas Meyer[2], Markus Schwarzländer[1,2]

[1]Institute for Plant Biology and Biotechnology, University of Münster, Münster, Germany,
[2]Institute for Crop Science and Resource Conservation, University of Bonn, Bonn, Germany,
[3]Department of Biology, Lund University, Lund, Sweden

The flexibility of electron transport is a characteristic feature of plant mitochondria. It supports survival of plants in adverse environments and prevents photoinhibition in the chloroplast for instance. Mechanistically, the function of electron transport as an *on-demand* reductant sink is mediated by partial uncoupling of electron flux to oxygen from the phosphorylation of ADP. Yet, our understanding when exactly uncoupling is important and how different uncoupling strategies are integrated in plant mitochondria remains highly incomplete. We genetically targeted two mechanistically independent uncoupling mechanisms — Alternative Oxidase (AOX) and Uncoupling Protein (UCP) — in Arabidopsis. While phenotypically unaffected under most conditions, including high light and drought, we found pronounced phenotypes of *aox* and *ucp* plants under thiol-mediated reductive stress. Combining analyses in isolated mitochondria and *in vivo* we observe that mitochondrial electron transport acts in the detoxification of thiol reductant. We expand the current model of plant mitochondria as flexible cellular safe-guards against metabolic redox imbalance by adding thiol-based reductive stress. The implications of our findings for inter-organellar crosstalk and plant stress responses will be discussed.

Keywords: respiration, uncoupling, AOX, UCP, thiols, reductive stress

INTERORGANELLAR NETWORK INFLUENCE ON PLANT PHENOTYPIC PLASTICITY

Xiaodong Yang, Hardik Kundariya, Jesus Beltran, Robersy Sanchez, **Sally Mackenzie**
Department of Biology, The Pennsylvania State University, University Park, USA

Seed plants inhabit a multitude of environments, and during their expansion on land have developed mechanisms for phenotypic plasticity to cope with environmental variation. These mechanisms can be subdivided to a means of environmental sensing, components of signaling, and gene network and epigenomic responses to condition phenotype. We investigate a plant-specific environmental sensing, signaling and response system that integrates both mitochondrial and plastid responses to variable environmental cues, and provides important phenotypic plasticity that is demonstrably adaptive. The system began with the identification of MSH1, a plant-specific gene involved in mitochondrial genome stability, but has expanded to encompass much broader pathway integrations. Evolutionarily, the system emphasizes the importance of protein multifunctionalization that has accompanied the post-endosymbiosis process; functionally, the system offers valuable insight into environmental adaptation mechanisms that are more comprehensive than previously suspected. Controlled spatio-temporal regulation of a number of genes, including MSH1, combined with promoter-mediated induction and suppression of expression in response to distinct environmental cues, results in an array of phenotypic responses and heritable epigenetic memory. We have attempted to dissect these mechanisms with respect to their adaptive and agricultural potential.

MITOCHONDRIAL PERSULFIDE METABOLISM IN HORMONE SIGNALING

Björn Heinemann, Christin Lorenz, Saskia Brandt, **Tatjana Hildebrandt**
Plant Proteomics, Leibniz University Hannover, Hannover, Germany

The mitochondrial sulfur dioxygenase ETHE1 oxidizes persulfide groups to sulfite, which is rapidly converted to the less reactive and thus non-toxic sulfur intermediate thiosulfate by the action of a sulfurtransferase. These reactions are part of a cysteine degradation pathway and essential for reduced sulfur homeostasis. Knockout of the sulfur dioxygenase is embryo-lethal in Arabidopsis, indicating an important function during early embryo development. The knockdown line *ethe1-1* is less tolerant against carbohydrate starvation than the wild type showing cell death and early senescence under light limiting growth conditions. Characterization of the mutant plants revealed that ETHE1 has a function in hormone signaling during embryogenesis as well as in the establishment of pathogen defense reactions. We detected an imbalance in the levels of the phytohormones abscisic acid (ABA) and jasmonic acid (JA) and a decrease in ABA sensitivity during early seedling establishment in *ethe1-1* compared to the wild type indicating a block in ABA signal transduction. In addition, sulfur dioxygenase deficient plants accumulate the immune signals salicylic acid and pipecolic acid leading to constitutive induction of defense proteins and strongly increased resistance against the biotrophic pathogen *Pseudomonas syringae*. Our results demonstrate that regulation of persulfide homeostasis is an important function of plant mitochondria and persulfide signaling is most likely involved in several physiological processes during plant development such as embryogenesis, germination, and biotic stress resistance.

A NOVEL MITOCHONDRIAL LYR PROTEIN IS REQUIRED FOR COMPLEX I ASSEMBLY IN ARABIDOPSIS.

Aneta Ivanova[1], Mable Gill-Hille[1], Shaobai Huang[1], Rui Branca[2], Beata Kmiec[3], Pedro Teixeira[3], James Whelan[5], **Monika Weronika Murcha[4]**

[1]School of Molecular Sciences & Arc Centre of Excellence Plant Energy Biology, The University of Western Australia, Perth, Australia, [2]Clinical Proteomics Mass Spectrometry, Department of Oncology Pathology, Science for Life Laboratory and Karolinska Institutet, Stockholm, Sweden, [3]Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, [4]School of Molecular Sciences and Arc Centre of Excellence Plant Energy Biology, The University of Western Australia, Perth, Australia, [5]Department of Animal, Plant and Soil Science & Arc Centre of Excellence Plant Energy Biology, La Trobe University, Bundoora, Australia

Building a functional Complex I requires the expression of nuclear and mitochondrial located genes, translation, import, co-factor biosynthesis and the assembly of at least 44 subunits. This requires assembly factors that interact with subunits of Complex I but are not part of the final holocomplex. We show that a plant specific mitochondrial matrix protein which we term COMPLEX I ASSEMBLY FACTOR 1 (CIAF1), contains a LYR domain and is required for Complex I activity. T-DNA insertion mutants of CIAF1 lack Complex I and the Supercomplex I+III. Biochemical characterisation shows that the assembly of Complex I is stalled at 650 and 800 kDa intermediates in mitochondria isolated from ciaf1 mutant lines. Yeast-2-Hybrid interaction assays indicates that CIAF1 specifically interacts with matrix facing subunits of Complex I. Together, these data show that CIAF1 plays an essential role in the assembly of the peripheral matrix arm Complex I subunits to form the holoenzyme Complex I. Quantitative proteomics revealed that there is an extensive up-regulation of components required for mitochondrial biogenesis in ciaf1 mutants, suggesting that a mitochondrial retrograde signalling pathway is activated and executed in response to the lack of Complex I.

MINTACT: LIPIDOMICS, METABOLOMICS AND PROTEOMICS OF PLANT ORGANELLES AT THE CELL TYPE LEVEL

Uwe Heinig[1], Jedrzej Szymanski[1], Iris Tal[2], Carmel Gilda[1], Hadar Kedem[1], Maxim Itkin[3], Drew Sturtevant[4], Amanda Phelps Bowman[5], Sergey Malitsky[3], Guido Verbeck[5], Kent Chapman[4], Eilon Shani[2], Asaph Aharoni[1]

[1]Plant and Environmental Sciences, Weizmann Institute, Rehovot, Israel, [2]Department of Molecular Biology and Ecology, Tel Aviv University, Tel Aviv, Israel, [3]Life Science Core Facilities, Weizmann Institute, Rehovot, Israel, [4]Department of Biological Sciences, University of Northern Texas, Denton, Texas, USA, [5]Department of Chemistry, University of Northern Texas, Denton, Texas, USA

Research at the subcellular level resolution is key to the study of metabolism and metabolic engineering efforts since metabolic pathways are typically spread over two and likely more cell compartments. Whereas data for protein localization is partially available, metabolite localization at the organelle level is only poorly resolved. We developed mINTACT as a new and efficient system for the isolation a single organelle type from specific tissues and cell types. This procedure is based on adding a molecular tag onto the outer envelope of compartments and exploiting the tags to purify organelles of choice using magnetic beads. Here we present lipidomics, metabolomics and deep proteomics analysis of Arabidopsis organelles down to a single organelle resolution. We employed the mINTACT technique for isolating four different organelles, namely, chloroplasts, peroxisomes, mitochondria and nuclei in root and green tissues. Moreover, nuclei were purified from plant lines expressing the tag-protein in 5 different root cell types. In a typical mINTACT organelle preparation, we were able to detect more than 2.000-3.000 proteins and 100s of lipid species, demonstrating unique organelle protein composition as well as lipid distribution profiles resembling the biosynthetic origin of the compounds. This study represents the first subcellular lipidome map of plants and the analysis of organelle composition at the cell type level. Finally, we established single organelle lipidomics analysis using a combined approach of mINTACT, nano-manipulation and MALDI Imaging Mass Spectrometry, demonstrating the detection of lipids from a single chloroplast.

A NOVEL MECHANISM EXPLAINS THE EVOLUTION OF MITOCHONDRIAL NAD-MALIC ENZYME FROM A TCA CYCLE-ASSOCIATED ENZYME TO A C4 DECARBOXYLASE

Meike Hüdig[1], Marcos Tronconi[2], **Veronica G. Maurino[1]**

[1]Developmental and Molecular Biology of Plants, Heinrich Heine University, Düsseldorf, Germany, [2]Centro De Estudios Fotosintéticos Y Bioquímicos, Universidad Nacional De Rosario, Rosario, Argentina

In plants, NAD-malic enzyme (NAD-ME) is exclusively present in mitochondria, where it is involved in malate respiration. In species performing a specific type of C4 photosynthesis, NAD-ME was co-opted to decarboxylate malate in bundle sheath cell mitochondria, thereby facilitating CO2 concentration in close proximity to RubisCO. Surprisingly, a specific C4 NAD-ME was never identified. We use the model system Cleomaceae to study the molecular evolution of NAD-ME. Transcriptional analysis did not identify a C4 candidate, as all three genes encoding NAD-ME proteins in the C4 Cleome Gynandrosis gynandra (GgNAD-Meα, GgNAD-MEβ1 and GgNAD-MEβ2) are expressed at higher levels than their C3 orthologs in Tarenaya hassleriana. We found that GgNAD-MEβ1 is catalytically inactive. Biochemical and proteomic analyses showed that a unique NAD-ME entity exists exclusively in photosynthetic tissues of G. gynandra. Strikingly, this heterodimeric NAD-ME is composed of GgNAD-MEα and GgNAD-MEβ1 and exhibits both high affinity towards malate and high catalytic activity. We conclude that GgNAD-MEβ1 likely evolved to mediate the C4 function. In addition to the GgNAD-MEα/GgNAD-MEβ1 heterodimer, an NAD-ME entity composed of all three subunits exists in G. gynandra photosynthetic and heterotrophic tissues. In all tissues of T. hassleriana, we also identified an NAD-ME entity composed of all three subunits, with similar kinetic properties as the enzyme composed of three subunits in C4 Cleome. Accordingly, we propose that the NAD-MEs composed of three subunits is involved in malate respiration in different C3 and C4 Cleome organs and cellular types. Our results lead to the conclusion that in Cleome C4 species, the functions of NAD-ME as a mitochondrial respiratory enzyme and as a specific photosynthetic decarboxylase are performed by separated enzymatic entities formed through a differential combination of subunits.

PEPTIDOLYTIC NETWORK IN MITOCHONDRIA AND CHLOROPLASTS

Elzbieta Glaser
Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

The great majority of mitochondrial proteins are synthesized in the cytosol as precursor proteins containing N-terminal targeting peptides, TPs, which are cleaved off inside the organelle by the mitochondrial processing peptidase, MPP, after completed import. TPs have potential toxic effects as they dissipate membrane potential and cause feedback inhibition of MPP, resulting in aberrant protein processing and accumulation of uncleaved precursor proteins. Therefore, TPs need to be degraded. Our work resulted in identification of oligopeptidases (Presequence Protease PreP and Organellar OligoPeptidase OOP), which in combination with aminopeptidases form a proteolytic network for a stepwise degradation of peptides to amino acids in plant mitochondria and chloroplasts. Recent studies revealed that the lack of PreP and OOP oligopeptidases results in the accumulation of free peptides in organelles. mRNA-seq analysis and deep coverage proteomics in knockout plants uncovered a peptide-stress response resulting in the activation of the classical plant defense pathways, however, in the absence of a pathogen. Our results indicate that the accumulated organellar peptides are perceived as pathogenic effectors activating the signaling pathways of plant-defense response.

In human mitochondria PreP was shown not only to degrade TPs, but also to be responsible for degradation of Amyloid-beta peptide, Abeta, associated with Alzheimer´s disease, AD. PreP activity is reduced in brain samples of AD patients and overexpression of PreP in AD mice models reverses the phenotype. Furthermore, mouse heterozygous for PreP revealed the accumulation of Abeta aggregates in the brain further suggesting a role for PreP in Abeta metabolism. Human homologue of OOP, neurolysin, was found to complement PreP in the degradation of TPs as well as in degradation of Abeta.

UNEXPECTED NEWS FROM A CRYPTIC CYTOPLASMIC MALE STERILITY

Noémie Dehaene[1,2], Matthieu Simon[1], Stéphanie Durand[1], Anthony Ricou[1], Christine Camilleri[1], **Françoise Budar[1]**

[1]Institut Jean Pierre Bourgin, Inra, Versailles, France, [2]Université Paris Sud, Université Paris Saclay, Orsay, France

The discovery of a cryptic cytoplasmic male sterility (CMS) in crosses from natural variants of *Arabidopsis thaliana* (Gobron et al, 2013) has opened up new opportunities for the exploration of genetic and physiological aspects of mitochondrial function during pollen development.

CMS is induced by the cytoplasm of Shahdara (Sha), from Tadjikistan, and by those of several other natural variants, all originating from Central Asia or Russia. All these variants are fully fertile, and no gynodioecious population was reported so far in this species. As a matter of fact, most *A. thaliana* natural variants that were tested carry nuclear genes that restore fertility (Rf genes), even though they do not carry the sterilizing cytoplasm. The genetic architecture of fertility restoration is variable among natural genotypes, from a unique strong Rf to multiple Rf loci with moderate individual effects on the phenotype (Simon et al 2016). Deciphering one of these loci revealed an unexpected role for a PPR protein in CMS.

As the *A. thaliana* CMS is gametophytic, meaning the primary cause of pollen abortion occurs inside the pollen during its development, the following of mitochondrial physiological parameters during pollen development will be instrumental to better understand how mitochondria manage to kill the pollen. Results obtained using cytological approaches combined with pollen-expressed fluorescent sensors bring some relevant cues on this question.

Gobron, N., Waszczak, C., Simon, M., Hiard, S., Boivin, S., Charif, D., et al. (2013). A Cryptic Cytoplasmic Male Sterility Unveils a Possible Gynodioecious Past for Arabidopsis thaliana. PLoS ONE 8, e62450. doi:10.1371/journal.pone.0062450.

Simon, M., Durand, S., Pluta, N., Gobron, N., Botran, L., Ricou, A., et al. (2016). Genomic Conflicts that Cause Pollen Mortality and Raise Reproductive Barriers in Arabidopsis thaliana. Genetics 203, 1353–1367. doi:10.1534/genetics.115.183707.

B13 SUBUNIT OF ELECTRON TRANSPORT COMPLEX I IS RELATED TO THE METABOLIC INTERACTION BETWEEN THE TCA CYCLE AND ELECTRON TRANSPORT CHAIN

Toshihiro Obata[1], Etienne Meyer[2], Alisdair Fernie[3]

[1]Department of Biochemistry and Center for Plant Science Innovation, University of Nebraska Lincoln, Lincoln, United States, [2]Department 3, Max Planck Institute of Molecular Plant Physiology, Potsdam Golm, Germany, [3]Department 1, Max Planck Institute of Molecular Plant Physiology, Potsdam Golm, Germany

B13 protein is a subunit of the mitochondrial electron transport complex I located in the matrix arm. The mammalian analogue NDUFA5 functions in the assembly/stability of the complex I. B13 protein is one of a few complex I subunits detected as a soluble matrix protein in Arabidopsis mitochondria, suggesting additional functions of this subunit in plants. In this study, B13 protein was detected as an interactor of mitochondrial tricarboxylic acid (TCA) cycle enzyme, citrate synthase 4, in illuminated Arabidopsis leaves. In order to analyze the metabolic functions of this protein, B13 knock-down (KD) lines were generated. One Arabidopsis line harboring a T-DNA insertion in the promotor region of B13 gene and two constitutive RNAi lines (i2 and i14) showed reduced levels of B13 mRNA. These lines contained less amount of complex I, confirming the function of B13 in assembly and/or stabilization of complex I. These B13 KD lines showed common phenotypes with other complex I mutants, including growth retardation, high relative respiratory flux into the TCA cycle, and the accumulation of amino acids. However, there are also remarkable differences between B13 KD lines and other mutants. First, the early growth phenotype of the B13 KD lines was not rescued in the presence of sucrose and their seeds germinated normally in the absence of sucrose. Second, the mitochondria isolated from the B13 KD lines consumed less O2 than wild type mitochondria when malate and glutamate were supplied as respiratory substrate. Third, general accumulation of amino acids was observed only in i14 line which showed the most severe growth phenotype. Aspartate and threonine, amino acids synthesized from oxaloacetate, were significantly reduced in all KD lines. These results suggest that the B13 protein functions in assembly and/or stabilization of complex I and also in the metabolic interaction between the TCA cycle and complex I.

MITOCHONDRIAL AUGMENTATION THERAPY OF HEMATOPOIETIC STEM CELLS IN MITOCHONDRIAL DELETION SYNDROMES: PRELIMINARY SAFETY AND EFFICACY DEMONSTRATED IN FIRST-IN-HUMAN STUDY

Natalie Yivgi Ohana[1], Elad Jacoby[2], Moriya Blumkin[3], Noa Sher[3], Nira Varda-Bloom[4], Julia Pansheen[4], Omer Bar-Yosef[5], Noah Gruber[6], Einat Lahav[7], Michal Besser[8], Jacob Schachter[8], Yair Anikster[9], Amos Toren[2]

[1]R&D, Minovia Therapeutics Ltd, Rehovot, Israel, [2]Pediatric Hemato Oncology, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel, [3]Research, Minovia Therapeutics Ltd, Tirat Hacarmel, Israel, [4]Bmt Unit, Sheba Medical Center, Ramat Gan, Israel, [5]Pediatric Neurology, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel, [6]Pediatric Endocrinology, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel, [7]Pediatric Nephrology, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel, [8]Ella Lemelbaum Institute Fro Cancer Research, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel, [9]Pediatric Metabolism, The Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel

Mitochondrial diseases caused by mtDNA deletions or mutations are debilitating and life-threatening, yet to date no effective pharmacologic treatments are available and all treatment is symptomatic. Several decades ago, the capacity of mitochondria to enter living cells and augment endogenous mitochondrial metabolic activity was demonstrated; intercellular transfer of mitochondria has more recently been demonstrated in vitro and in vivo. In preclinical models of mitochondrial and lysosomal disorders, hematopoietic stem and progenitor cells (HSPCs) have been shown capable of carrying and transferring normal organelles into diseased tissues, thereby altering disease phenotype. We demonstrate the enrichment of patient-derived HSPCs with wild-type mitochondria derived from the mother, a process termed mitochondrial augmentation therapy. We report on first-in-man study in four patients with Pearson and Kearn-Sayrne Syndromes (PS and KSS) treated with autologous HSPCs following ex-vivo mitochondrial augmentation.

Mitochondrial augmentation therapy was demonstrated to be safe; anemia, hypocalcemia and alkalosis, related to the CD34+ mobilization prior to administration of augmented CD34+ cells, were rapidly resolved. In patients in which mitochondrial heteroplasmy levels were discernable in peripheral blood, we observed in vivo improved heteroplasmy starting 3-4 months after cellular therapy, which was maintained throughout the follow-up period. Improvement in mitochondrial heteroplasmy and function was in line with clinical findings. Importantly, functional quality of life, as measured by the International Pediatric Mitochondrial Disease Score (IPMDS) and PEDI questionnaire, was greatly improved after treatment. It is known that there is a high variability in presentation and progression of patients; individual improvements per patient were noted.

Together, these preliminary clinical data suggest that mitochondrial augmentation therapy, in which we enrich HSPCs with mitochondria carrying non-deleted mtDNA sequence, is a potential method to diminish disease progression in patients with mtDNA deletions, and may be extrapolatable to additional indications involving mutations in the mitochondrial DNA.

STUDY OF FACTORS INFLUENCING THE ACTIVITY OF DNA IMPORT INTO ARABIDOPSIS THALIANA MITOCHONDRIA IN ORGANELLO AND IN VIVO

Yuri M Konstantinov[1], Ekaterina Klimenko[2], Tatiana Tarasenko[2], Vladislav Tarasenko[2], Milana Koulintchenko[3]

[1]The Plant Genetic Engineering Laboratory, The Siberian Institute of Plant Physiology and Biochemistry, the Siberian Branch of Russian Academy of Scineces, Irkutsk, Russia, [2]The Plant Genetic Engineering Laboratory, Siberian Institute of Plant Physiology and Biochemistry Sb Ras, Irkutsk, Russia, [3]The Plant Genetic Engineering Laboratory, Siberian Institute of Plant Physiology and Biochemistry Sb Ras, Siberian Institute of Plant Physiology and Biochemistry Sb Ras, Russia

The phenomenon of natural competence of the plant mitochondria to uptake DNA (DNA import) obviously should have a relation to horizontal gene transfer into these organelles and to maintenance of the dynamic mitochondrial genome. Wherein, mitochondrial DNA import seems to have a rather early evolutionary origin, as it was registered for different representatives of eukaryotes (higher plants, animals and yeast). However, in contrast to the protein and RNA import into mitochondria, the genetic and physiological functions of the mitochondrial import of DNA remains poorly understood. Recently, we found a stimulation of DNA import into isolated mitochondria upon addition of endoplasmic reticulum (ER) membranes. Analysis of the currently available information on the mitochondria functional bioenergetics allows to suggests that the mitochondrial import of DNA may depend not only on what channels/pores provide the transport of these macromolecules, but also on how the formation of these channels/pores is influenced by (1) morphological and functional state of mitochondria, defined by their organ, tissue or intracellular specialization, or (2) interaction of mitochondria with other cell organelles, mainly the ER. The aim of the present study is to clarify the role of structural organization of mitochondria and their interaction with the ER in the DNA import in conditions close to in vivo. Two approaches were used for the analysis of DNA import into mitochondria of the Arabidopsis wild-type plants and mutant plants with inactivated proteins presumably involved in the formation of membrane contact sites between mitochondria and ER: (1) the in organello system and (2) protoplasts. The determination of nature of cellular factors which influence DNA import into mitochondria is a necessary condition for development of cell technologies for genetic manipulation of mitochondrial genes. This work was financially supported by the Russian Fund for Basic Research (Grant 18-04-00603).

Keywords: mitochondria, DNA import, endoplasmic reticulum

3D IMAGING OF TAPETAL MITOCHONDRIA SUGGESTS THE IMPORTANCE OF PROPER MITOCHONDRIAL FISSION IN POLLEN DEVELOPMENT

Der-Fen Suen[1], Pei-Ying Chen[1], Chia-Chen Wu[1], Chung-Chih Lin[2], Wann-Neng Jane[3]
[1]Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan, [2]Department of Life Sciences and Institute of Genome Sciences, National Yang Ming University, Taipei, Taiwan, [3]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Mitochondrial fission occurs frequently in plant cells, but its biological significance is poorly understood because mutants specifically impaired in mitochondrial fission do not show obvious defect in vegetative growth. Here, we revealed that the production of viable pollen was reduced in mutants lacking DRP3A, DRP3B or ELM1, the three main proteins involved in mitochondrial fission in Arabidopsis. In drp3b and elm1, young microspores contained abnormal number of nucleus and mature pollen had aberrant accumulation of lipids in pollen coat and irregular pollen outer wall. The formation of pollen wall and coat is mainly associated with tapetal function, we therefore used 3D imaging to quantify features of cells and mitochondria in the tapetum at different stages, with isolated single tapetal cells for which the on-site morphology and volume of cells and mitochondria were preserved. Our results showed that wild-type tapetal volume gradually increased until vacuolated pollen (VP) stage and remained unchanged at binucleate (BN) and tricelullar (TC) stage, and mitochondrial density per tapetal cell increased until VP and dramatically declined at BN stage, where mitochondria became fragmented and swollen. Defect in mitochondrial fission caused the change of mitochondrial status including mitochondrial elongation, abnormal mitochondrial ultrastructure, decrease in cross-sectional area, and slight alteration of mitochondrial distribution as well as large reduction in mitochondrial density. Our studies suggest that mitochondrial fission is required for proper mitochondrial status in the tapetum and perhaps in pollen, which is important for the production of viable pollen.

ROLES OF OXA2A AND OXA2B IN PLANT MITOCHONDRIAL BIOGENESIS

Renuka Kolli[1], Jürgen Soll[1,2], Chris Carrie[1]

[1]Biology I Botany, Ludwig Maximilian University of Munich, Munich, Germany, [2]Munich Centre for Integrated Protein Science, Ludwig Maximilian University of Munich, Munich, Germany

The evolutionarily conserved YidC/Oxa1/Alb3 proteins facilitate the insertion of membrane proteins into the bacterial plasma membrane, the mitochondrial inner membrane, the chloroplast thylakoid membrane and the endoplasmic reticulum membrane. Plant mitochondrial OXA2a and OXA2b are unique in possessing a tetratricopeptide repeat (TPR) domain at their C-termini. Individual knockouts of their genes in *Arabidopsis thaliana* are embryonically lethal. Complementation plants of *oxa2a*, rescued by partial complementation using the embryo-specific ABI3 promoter, displayed a reduced growth rate due to deficiency of the respiratory complex III. Plants of *oxa2b* complemented with the N-terminal insertase domain, but lacking the TPR domain, were severely retarded in growth due to a strong reduction in the steady-state abundance of the respiratory complex IV. The defect in complex IV biogenesis was caused due to incomplete membrane insertion of cytochrome *c* oxidase subunit 2 (COX2). Hence, while OXA2a is crucial for the biogenesis of complex III, OXA2b is required for the biogenesis of complex IV in plant mitochondria.

ANALYSIS OF A NOVEL PHOSOPHODEGRON-LIKE SELECTIVITY MECHANISM IN STATIONARY PHASE MITOPHAGY

Panagiota Kolitsida[1], Jianwen Zhou[2], Jörn Dengjel[2], Micha Rackiewicz[2], **Hagai Abeliovich[1]**
[1]Biochemistry, Food Science and Nutrition, Hebrew University of Jerusalem, Rehovot, Israel ,
[2]Biology, University of Fribourg, Fribourg, Switzerland

Mitophagy, or the autophagic degradation of mitochondria, is an important housekeeping function of eukaryotic cells that prevents the accumulation of defective mitochondria due to oxidative damage and spontaneous mutations. The culling of defective mitochondria has been suggested to delay the onset of aging symptoms, and defects in mitophagy have been linked to late onset hereditary disorders such as Parkinson's disease and type II diabetes. We previously showed that different mitochondrial matrix proteins undergo mitophagy at different rates. An attractive model, supported by existing data, is that fission and fusion of mitochondria are linked to the segregation of defective mitochondrial compartments from undamaged ones, in a 'distillation' mechanism that leads to the selective turnover of defective compartments. We now demonstrate that dynamic mitochondrial matrix protein phosphorylation and dephosphorylation generate a segregation principle that would couple with mitochondrial fission and fusion to generate such a distillation process. Our data support a model wherein differences in protein-protein interactions between differentially phosphorylated proteins of the same species can drive a microscopic phase separation which, coupled with fusion-fission dynamics, may account for the observed selectivity.

LINKING MITOCHONDRIAL DYNAMICS TO MITOCHONDRIA/ER CONTACT SITES

Anna-Lena Falz[1], Finja Bohle[1], Markus Schwarzländer[2], Andreas Meyer[1],
Stefanie Mueller-Schuessele[1]
[1]Inres Chemical Signalling, University of Bonn, Bonn, Germany, [2]Ibbp, University of Münster, Münster, Germany

Mitochondria and the endoplasmic reticulum are dynamic structures that are known to transiently interact in the context of lipid transfer as well as mitochondrial fission and fusion in animals and fungi. Plant mitochondria have been described as “discontinuous whole”, as they undergo frequent and extensive mixing of mitochondrial matrix content, based on a balance between mitochondrial motility and fission/fusion events.
While membrane contact sites between mitochondria and the ER are involved in mitochondrial dynamics in animal and fungal cells, evidence for their existence in plants is mainly based on correlative movement of both organelles. This study addresses the question of the functional involvement of mitochondria/ER contacts by synthetically linking both organelles and characterizing the effects on mitochondrial motility and dynamics in the model moss *Physcomitrella patens*.
We find that while forcing mitochondria/ER contacts, mitochondrial motility is largely suppressed whereas mitochondrial fission seems to increase. These findings indicate a causal relationship between the association of ER to mitochondria and mitochondrial dynamics in plants. The future challenge will be to study the functions of these specific membrane contact sites and to identify the yet unknown endogenous proteins dynamically tethering the ER and mitochondria in plants.