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Tran, Huy Cuong

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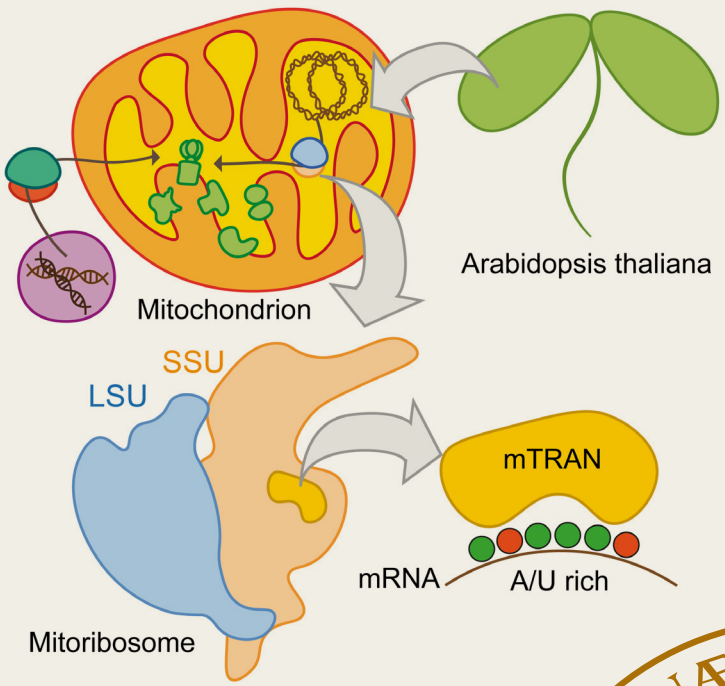

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Science at Lund University to be publicly defended on the 5th of May at 13.00 in Biology Lecture Hall, Department of Biology, Sölvegatan 35, Lund, Sweden

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Title: Characterization of land plant-specific proteins required for mitochondrial translation initiation.

Abstract: Plant mitochondria produce the majority of adenosine triphosphate (ATP) – the cellular energy currency for metabolic reactions needed for plant growth, development, and maintenance. Despite a relatively thorough understanding of basic mitochondrial functions, many mitochondrial proteins and processes remain poorly understood. The aims of this thesis are to i) review and compare the mitochondrial unfolded protein response (UPR^{mt}) and related signalling across eukaryotic kingdoms, to ii) describe an efficient isolation method of Arabidopsis mitochondria using continuous Percoll density gradients, and to iii) characterize two *Arabidopsis thaliana* genes, *AT4G15640* and *AT3G21465*.

Paper I summarizes the current knowledge of UPR^{mt} across eukaryotic kingdoms, and describes a meta-analyis of UPR^{mt} regulators and target genes. UPR^{mt} is a mitochondria-to-nucleus "retrograde" response that regulates nuclear gene expression during mitochondrial dysfunction to maintain mitochondrial homeostasis. Although UPR^{mt} has been extensively studied in animals, relatively little is known about the plant UPR^{mt} and only few regulators have recently been identified. In yeast, very few unfolded protein responses that seem to be related to UPR^{mt} have been described. Here, the UPR^{mt} in animals, yeast and plants are compared. Our study indicates that each kingdom has evolved their own specific regulators, which however induce very similar groups of target genes. Our meta-analysis identifies homologs of known UPR^{mt} regulators and responsive genes across eukaryotic kingdoms.

Paper II describes a strategy for efficient purification of Arabidopsis mitochondria using continuous Percoll density gradients. By using this method, the purity of isolated mitochondria is greatly improved. Obtained mitochodria can be either used for assays requiring highly intact and functional mitochondria, e.g. import assay or respiration measurement, or be stored for later use, e.g. BN-PAGE or western blot.

Paper III describes the characterization of two *Arabidopsis thaliana* genes, *AT4G15640* and *AT3G21465*. Here, AT4G15640 and AT3G21465 are shown to be land plant-specific mitochondrial proteins that are critical for plant performance, but they are unlikely to be adenyl cyclases as previously annotated. Interestingly, knocking out both *AT4G15640* and *AT3G21465* results in low abundance and activity of mitochondrial oxidative phosphorylation complexes. Using co-immunoprecipitation, AT4G15640 and AT3G21465 are confirmed to be part of the mitoribosome small subunit (mtSSU). Therefore, AT4G15640 and AT3G21465 were named as mitochondrial TRANslation factor 1 (mTRAN1) and 2 (mTRAN2), respectively. *In organello* protein synthesis and polysome fractionation assays suggest that mTRANs are required for mitochondrial translation initiation. Moreover, the RNA electrophoretic mobility shift assays suggest that mTRAN1 binds to the A/U-rich regions in the 5'-untranslated regions of mitochondrial mRNAs to mediate mtSSU-mRNA binding to initiate translation. Finally, our mitoribosome profiling suggests that mTRAN proteins are universal mtSSU-embedded translation initiation factors. Together, these findings suggest that mitochondrial translation initiation occurs via a unique mechanism in plants, as compared to in bacteria, yeast and mammals.

Key words: Mitochondrial unfolded protein response, Arabidopsis mitochondrial isolation, mitoribosome, mitochondrial translation initiation, mitoribosome small subunit, mTRAN, mTRAN-mRNA interaction.

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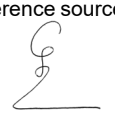
Signature  Date 2023-03-13

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To my family (Gửi tới gia đình)

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Paper I

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Paper II

Huy Cuong Tran, Olivier Van Aken (2022). Purification of leaf mitochondria from *Arabidopsis thaliana* using Percoll density gradients. *Methods in Molecular Biology* 2363: 1-12.

Paper III

Huy Cuong Tran, Vivian Schmitt, Sbatie Lama, Chuande Wang, Alexandra Launay-Avon, Katja Bernfur, Kristin Hofmann, Kasim Khan, Benoit Castandet, Fredrik Levander, Allan G. Rasmusson, Hakim Mireau, Etienne Delannoy, Olivier Van Aken. Mitochondrial translation in plants is initiated by a unique mTRAN-mRNA interaction mechanism. *In revision for Science*.

My contributions to the papers

Paper I

I participated in planning the project and performing the meta-analysis. I wrote the manuscript with input from my supervisor.

Paper II

I participated in planning the project and collecting data. I wrote the manuscript with input from my supervisor.

Paper III

I participated in conceiving and planning the project. I performed most of the experiments (with input from my supervisor), including phylogenetic analysis, confocal microscopy analysis, plant phenotyping, plant cAMP measurement, BN-PAGE following by Coomassie staining and activity measurement, oxygen consumption measurement, immunoblotting, co-immunoprecipitation, *in organello* protein synthesis, and polysome profiling. I also produced and collected the samples for RNA-seq and Ribo-seq. I co-supervised Vivian Schmitt doing her internship and master's thesis, in which she performed several immunoblot analysis, the bacterial cAMP synthase complementation assays, qRT-PCR on mitochondrial retrograde signalling genes, motif analysis of the 5'UTRs of mitochondrial mRNAs, recombinant protein expression and purification, and REMSAs. My supervisor and I wrote the manuscript with input from all co-authors.

List of additional papers not included in this thesis

Essam Darwish, Ritesh Ghosh, Abraham Ontiveros-Cisneros, **Huy Cuong Tran**, Marcus Petersson, Liesbeth De Milde, Martyna Broda, Alain Goossens, Alex Van Moerkercke, Kasim Khan and Olivier Van Aken (2022). Touch signaling and thigmomorphogenesis are regulated by complementary CAMTA3- and JA-dependent pathways. *Science Advances* 8 (20): eabm2091.

List of abbreviations

5'UTR	5' untranslated region
A	Adenine
AA	Antimycin A
ABI4	Abscisic acid-insensitive 4
Acetyl-CoA	Acetyl-Coenzyme A
AC	Adenyl cyclase
ADP	Adenosine diphosphate
ANAC017	Arabidopsis NAC domain containing 017
AOX	Alternative oxidase
ATP	Adenosine triphosphate
BLRP	Biotin ligase recognition peptide
BN-PAGE	Blue Native Polyacrylamide Gel-Electrophoresis
C	Cysteine
cAMP	3′,5′-cyclic adenosine monophosphate
CI-V	Complex I-V
C. elegans	*Caenorhabditis elegans*
cpUPR	Chloroplast unfolded protein response
Cryo-EM	Cryogenic electron microscopy
D-GAP	D-glyceraldehyde-3-phosphate
Dox	Doxycycline
DXP	1-deoxy-D-xylulose-5-phosphate
DXS	1-deoxy-D-xylulose-5-phosphate synthase
ER	Endoplasmic reticulum
$FADH_2$	Flavin adenine dinucleotide
G	Guanine
IF	Initiation factor
IMM	Inner mitochondrial membrane
IMS	Intermembrane space
IMTACT	Isolation of Mitochondria TAgged in specific Cell Types
Fe–S	Iron-sulfur
MAPK	Mitogen-activated protein kinase
MB	MitoBlock-6
MEP	Methylerythritol phosphate
MORF	Multiple Organellar RNA editing Factor
mRNA	Messenger RNA
mrpl1-1	Mitochondrial ribosomal protein L1
mtDNA	Mitochondrial DNA
mtETC	Mitochondrial electron transport chain
mtIF	Mitochondrial initiation factor

mtLSU	Mitoribosome large subunit
mTRAN1	Mitochondrial translation factor 1
mTRAN2	Mitochondrial translation factor 2
MTS	Mitochondrial targeting sequences
mtSSU	Mitoribosome small subunit
NADH	Nicotinamide adenine dinucleotide
NDB4	NAD(P)H dehydrogenase B4
nPG	n-propylgallate
OAA	Oxaloacetate
OM64	Outer membrane 64
OM66	Outer membrane protein of 66 kDa
OMM	Outer mitochondrial membrane
ORRM	Organellar RNA Recognition Motif
OXPHOS	Oxidative phosphorylation
PEP	Phosphoenolpyruvate
PNPase	Polynucleotide phosphorylase
PPR	Pentatricopeptide repeat
PVP	Polyvinylpyrrolidone
REMSA	RNA electrophoretic mobility shift assay
RIP-seq	RNA Immunoprecipitation Sequencing
RIP	RNA-editing factor interacting protein
ROS	Reactive oxygen species
RpoT	RNA polymerase of the T-phage type
rRNA	Ribosomal RNA
SD	Shine-Dalgarno
SHAM	Salicylhydroxamic acid
SUBA	Subcellular Localization Database for Arabidopsis Proteins
Succinyl-CoA	Succinyl-Coenzyme A
TCA	Tricarboxylic acid
TIM	Translocase of the inner membrane
TM	Transmembrane
TOM	Translocase of the outer membrane
TPR	Tetratricopeptide repeat
tRNA	Transfer RNA
U	Uridine
UGT74E2	Uridine diphosphate glycosyltransferase 74E2
UPOX1	Up regulated by oxidative stress AT2G21640 1
UPR^{mt}	Mitochondrial unfolded protein response
VDAC	Voltage-dependent anion channel

Popular scientific summary

Plants are of key importance as their photosynthesis produces a massive amount of oxygen in the atmosphere, which is mandatory for respiration of many other organisms as well as plants themselves. Similar to in other eukaryotes, plant cellular respiration is carried out by mitochondria – organelles producing cellular energy needed for plant growth, development and maintenance. Nowadays, most scientists agree with the evolutionary hypothesis suggesting that mitochondria originated from endosymbiotic bacteria approximately 1.5 billion years ago. Nevertheless, in a cell, mitochondrial operation is not independent, but closely interacts with the nucleus – the central regulator of eukaryotic cells that contains most genes encoding the genetic information of an organism. The nucleus can send signals and materials to mitochondria to regulate mitochondrial activity. Vice versa, mitochondria are also able to send signals to the nucleus and affect nuclear gene expression. Gene expression is the process by which information of a gene is passed onto a messenger RNA (mRNA) during transcription, which is then used as a template to synthesize a corresponding protein during translation. This ultimately may affect the phenotype – the final effect on an individual living organism.

Due to their bacterial origin, mitochondria have their own sets of genes and have partially retained their own machinery for gene expression, including essential components required for transcription and translation. Mitochondrial gene expression is important to maintain mitochondrial operation. Thus, one could expect that the cellular energy would be affected if mitochondrial gene expression is perturbed. To date, many aspects of mitochondrial translation in plants are still enigmatic. In this work, we use the plant "mouse-ear cress" (scientific name: *Arabidopsis thaliana*) to study the land plant-specific proteins of unknown functions that we named as mitochondrial TRANslation factors (mTRAN). For many years, mTRAN proteins have been poorly characterized. During the course of our study, two recent studies suggested that mTRAN proteins are mitochondria-targeted and are components of the plant mitochondrial ribosome (hereafter referred to as mitoribosome) – the protein-RNA complex that translates the genetic information encoded in a mRNA into a corresponding protein. To study the functions of mTRAN proteins, Arabidopsis mutants lacking functional mTRAN proteins were generated, and they displayed a severe growth phenotype, indicating that mTRAN proteins are essential for plant growth and development. Using a combination of molecular, biochemical and bioinformatic approaches, we showed that the importance of mTRAN proteins for plant performance is due to their critical role in efficient mitochondrial translation in plants. In particular, mTRAN proteins are required for the mitoribosome – RNA binding in the initiation step of translation. Of note, this unique mTRAN-RNA interaction mechanism to initiate plant mitochondrial translation is fundamentally different from bacterial translation and mitochondrial translation in other eukaryotes. In conclusion, our study not only gains a deeper

insight into mitochondrial translation in plants, but also contributes to the knowledge of mitochondrial translation in eukaryotes.

Introduction

An overview of cellular respiration in plants

Plants are autotrophs that uptake nutrients in inorganic form and produce organic molecules themselves. Plant photosynthesis is carried out by chloroplasts to synthesize carbohydrates (sucrose and/or starch) from carbon dioxide (CO_2) and water by using photon energy from sunlight. On the other hand, plant respiration consumes oxygen (O_2) as the electron acceptor and releases CO_2 as a by-product. Similar to in other eukaryotes, plants have mitochondria, where aerobic respiration partially occurs to generate adenosine triphosphate (ATP) – the cellular energy currency to fuel metabolism required for their growth, development, and maintenance. Plant respiration is composed of glycolysis, the tricarboxylic acid (TCA) cycle (also known as the Krebs cycle or the citric acid cycle) and the oxidative phosphorylation (OXPHOS) of adenosine diphosphate (ADP) to ATP by the mitochondrial electron transport chain (mtETC) and ATP synthase complex. Plant cellular respiration strongly resembles their animal counterparts, but it has several unique features. As plants are sessile, they are unable to move away from extreme environmental conditions. Therefore, plant metabolism in general and plant respiratory metabolism in particular have evolved to adapt with environmental stress (O'Leary and Plaxton, 2016). The overall regulation of plant respiration is complicated and can occur “locally” in different processes of the respiratory pathway. The widespread use of plant mutant lines, which have important enzymes of respiration knocked out, gives us a broader insight not only into the function of key respiratory components, but also into the flexibility of plant respiratory pathway. Plant respiration is of great importance for the global carbon cycle as the amount of CO_2 used by photosynthesis of terrestrial plants is returned to the atmosphere by plant respiration, representing approximately 50% of the total annual CO_2 released from terrestrial ecosystems (Gifford, 2003; O'Leary and Plaxton, 2016).

Mitochondrial origin

In 1967, Lynn Margulis proposed that eukaryotic organelles, including the chloroplast and the mitochondrion, originated from endosymbiotic bacteria in her

famous paper “On the origin of mitosing cells” (Sagan, 1967). Later, advanced phylogenomic analysis has shown that the mitochondrial endosymbiont is related to an α-proteobacterium either within, or as a sister group to, the Rickettsiales. The host organism was likely an archaeon of the Asgard clade (Spang et al., 2015; Wang and Wu, 2014, 2015; Zaremba-Niedzwiedzka et al., 2017). The mitochondrion-containing ancestor, also known as the “last eukaryote common ancestor”, resembling the modern eukaryotes genetically and cellularly, evolved to all known eukaryotes nowadays. On one hand, the “mitochondria-early” evolutionary hypothesis proposes that the mitochondrial endosymbiotic event occurred very early in eukaryogenesis and/or even initiated the process (Martin et al., 2015). On the other hand, the “mitochondria-late” evolutionary hypothesis suggests that the mitochondrial endosymbiotic event took place when many other eukaryotic characteristics had already been established (Roger et al., 2017). Whether the former or the latter hypothesis is closer to what happened in the earliest stage of life, it is undeniable that the endosymbiotic origin of mitochondria is of key importance for the evolution of all modern eukaryotes.

A brief history of plant mitochondrial isolation

To study the structure and function of mitochondria, researchers attempted to isolate mitochondria from cells. The first attempt of mitochondrial isolation was carried out in guinea pig liver by Bensley and Hoerr in 1934 (Bensley and Hoerr, 1934) according to a recent review by Møller et al. (2021). Although mitochondria might be first described in plant cells in 1904 (Logan, 2012; Møller et al., 2021), the first plant mitochondria purification was done by Adele Millerd and her co-workers in 1951 (Millerd et al., 1951; Møller et al., 2021). At that stage, the crude mitochondria were isolated by differential centrifugations, thus they were however impure due to heavy contamination of thylakoid membranes from chloroplasts. Only in 1985, Day and his co-workers used differential centrifugations and Percoll-Polyvinylpyrrolidone (PVP) density gradients to successfully purify chlorophyll-free mitochondria from pea (*Pisum sativum*) leaves (Day et al., 1985). Also using the same approach, pure, intact and functional mitochondria of *Arabidopsis thaliana*, which became of great importance as the model dicot plant in the 1990s, were successfully purified from leaf tissues (Keech et al., 2005) and seedlings (Escobar et al., 2006). Nevertheless, this protocol of mitochondrial isolation requires a large amount of starting plant material and usually takes a few hours to accomplish. Recently, Boussardon et al. (2020) has developed a novel method of tissue-specific isolation of Arabidopsis mitochondria, named IMTACT (Isolation of Mitochondria TAgged in specific Cell Types). IMTACT, in which cell-specific biotinylated mitochondria are purified using streptavidin magnetic beads, yields pure and intact mitochondria in less than 30 min from sample preparation till final

extraction (Boussardon and Keech, 2022). This novel approach is much faster than traditional mitochondrial isolation methods using continuous or discontinuous gradients. To study plant mitochondrial compartments in depth, the protocols of isolating mitochondrial subfractions followed by biochemical approaches to study them have been developed and described in detail in the book "Plant Mitochondria: Methods and Protocols" edited by Whelan and Murcha (2015) and Van Aken and Rasmusson (2022).

Structure and basis composition of plant mitochondria

Plant mitochondria are often spherical or rod-shaped organelles that are 1-3 μm long and about 0.5 μm in diameter (Møller et al., 2021). With the availability of electron microscopes after World War II, the structure of an individual plant mitochondrion was revealed by electron micrographs (Palade, 1952). Similar to in other eukaryotes, plant mitochondria are double membranous organelles comprised of an outer mitochondrial membrane (OMM) separated from an inner mitochondrial membrane (IMM) by the intermembrane space (IMS), and the mitochondrial matrix, which is an aqueous electron-dense phase enclosed by the IMM and cristae formed by invaginations of the IMM (Figure 1). The OMM was shown to contain the voltage-dependent anion channel (VDAC), also referred as porin (Homble et al., 2012; Parsons et al., 1965; Zalman et al., 1980). Using electron tomography (available in the 1990s) to obtain 3D pictures of mitochondria, it turned out that the cristae look like narrow tubes running through the matrix (Frey and Mannella, 2000). A typical Arabidopsis mesophyll cell is comprised of many hundreds of mitochondria (Logan, 2006). Unlike mammalian and most yeast mitochondria, but resembling mitochondria in *Saccharomyces cerevisiae*, plant mitochondria move around mainly within the cytoplasm on the actin cytoskeleton (Logan, 2006; Olyslaegers and Verbelen, 1998; Van Gestel et al., 2002). Interestingly, mitochondrial fusion can occur, forming large, reticulated mitochondria. Mitochondrial fusion and fission can take place frequently to maintain the mitochondrial population in a cell homogenous (Logan, 2006; Møller et al., 2021). A recent review by Møller et al. (2021) has summarized the composition of mitochondrial compartments. In brief, the mitochondrial membranes are composed of lipids and proteins. The lipid in the mitochondrial membranes are usually phospholipids. The lipid composition of the OMM, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol, resembles that of the endoplasmic reticulum (ER) membrane. The IMM has a similar lipid composition as the OMM, but also contains a unique phospholipid, diphosphatidylglycerol (cardiolipin). The mitochondrial matrix does not contain phospholipids and has a very high concentration of proteins.

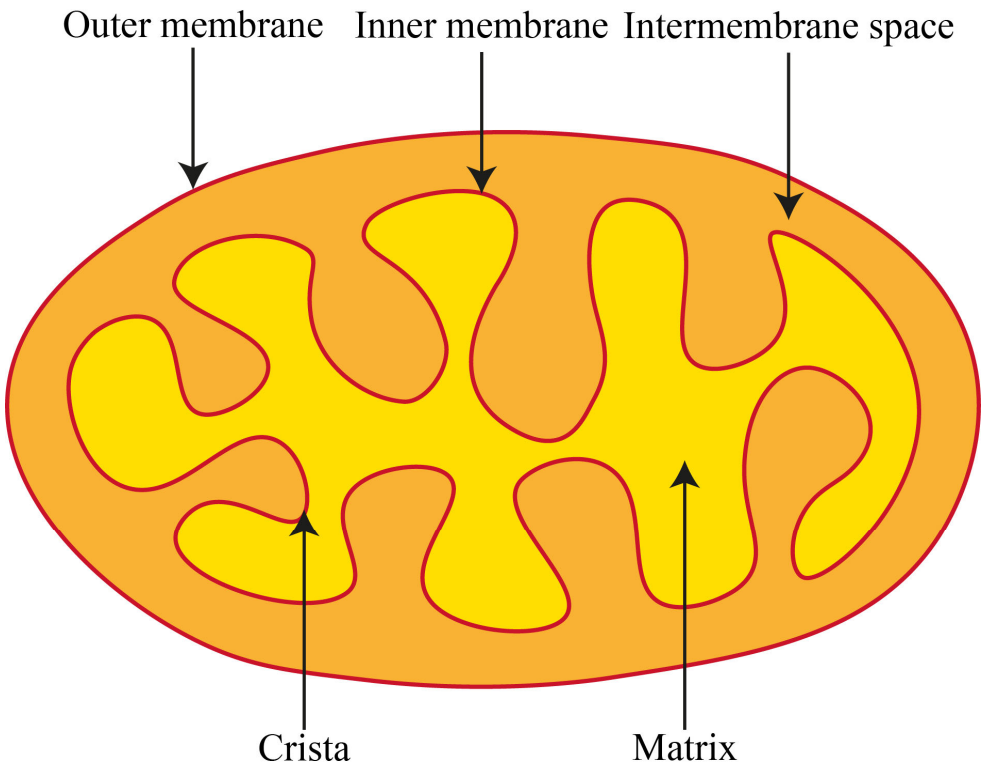

Figure 1. A simple diagram of plant mitochondrial structure.

Plant aerobic respiration partially occurs in mitochondria

As in other aerobic eukaryotes, the aerobic respiration in plants includes three processes occurring in different subcellular compartments: glycolysis, the TCA cycle and the OXPHOS system. Together, these processes produce ATP – the cellular energy currency. Mitochondria are thus considered as the cellular power houses. The following description of these three stages of plant aerobic respiration is written to a large extent based on chapter 12 “Respiration and Photorespiration” by Millar et al. (2015) in the textbook “Biochemistry and Molecular Biology of Plants” edited by Buchanan et al. (2015).

Glycolysis

Glycolysis takes place either in the cytosol or in the plastids, generating substrates that are subsequently used by the TCA cycle and the OXPHOS system, which occur within the mitochondria. Cytosolic glycolysis breaks down sucrose into glucose and fructose, which are converted into phosphoenolpyruvate (PEP). PEP can be turned either into pyruvate by pyruvate kinase and/or PEP phosphatase or into oxaloacetate (OAA) by PEP carboxylase. This glycolytic pathway generates reduced cofactor nicotinamide adenine dinucleotide (NADH) and ATP. Pyruvate is transferred directly into mitochondria whereas OAA is usually reduced to malate, which is then transported into mitochondria.

The tricarboxylic acid cycle

Once pyruvate and malate are imported into the mitochondrial matrix via carriers located in the IMM, pyruvate is oxidized and decarboxylated to generate CO_2, acetyl-Coenzyme A (acetyl-CoA) and NADH, while malate can be oxidized to either generate OAA and NADH by malate dehydrogenase or yield pyruvate, CO_2 and NADH by NAD^+-linked malic enzyme. Once the TCA cycle starts occurring within the mitochondrial matrix, acetyl-CoA and OAA combine to form citrate, which is isomerized to isocitrate that is oxidized and decarboxylated to form CO_2, 2-oxoglutarate, and NADH. 2-Oxoglutarate is further oxidized to produce CO_2, succinyl-Coenzyme A (succinyl-CoA) and NADH. Succinyl-CoA synthetase converts succinyl-CoA into succinate. Associating with this reaction, ADP is phosphorylated to ATP. Succinate is oxidized to fumarate by succinate dehydrogenase - the only IMM-localized enzyme involved in both the TCA cycle and mtETC. In the final step of the TCA cycle, fumarate is hydrated to form NADH and malate, which is oxidized to OAA that subsequently combines with another acetyl-CoA to continue the cycle (Figure 2).

The oxidative phosphorylation system

The OXPHOS system resides within the IMM and is based on the core of mtETC, which is composed of the four IMM-associated complexes: the NADH dehydrogenase (complex I), the succinate dehydrogenase (complex II), the cytochrome bc_1/cytochrome *c* reductase complex (complex III) and the cytochrome *c* oxidase (complex IV) (Figure 2). Complex I is the largest respiratory complex of the mtETC (Wirth et al., 2016). The high-resolution structure of plant complex I has recently been described by Maldonado et al. (2020) and Klusch et al. (2021). Plant complex I has an L-like shape and is comprised of the membrane arm embedded into the IMM, the peripheral arm extending into the mitochondrial matrix and another matrix-exposed domain linking to the membrane arm at the central position.

Complex I oxidizes NADH (formed by glycolysis and the TCA cycle) to regenerate NAD^+ at the peripheral arm site. Complex II, the smallest complex of four respiratory complexes (Millar et al., 2004), oxidizes succinate (generated as an intermediate in the TCA cycle) to fumarate with the concomitant oxidation of flavin adenine dinucleotide ($FADH_2$) to FAD^+ at the membrane-spanning domain site. Both complex I and complex II contain iron-sulfur (Fe–S) clusters involved in the transfer of electrons onto ubiquinone – a mobile electron transporter located within the mitochondrial IMM. The electron transport from NADH to ubiquinone causes conformational change in the membrane arm of complex I, resulting in pumping four protons from the mitochondrial matrix to the IMS. Unlike complex I, complex II cannot translocate protons across the IMM. By accepting two electrons, ubiquinone is reduced to ubiquinol, which is released from complex I and complex II and transfers electrons onto complex III – the central segment of the mtETC.

The atomic structure of plant complex III has recently been described by Maldonado et al. (2021). Plant complex III consists of 2 × 10 subunits, which can be functionally categorized as follows (Braun, 2020): subunits involved in the mtETC including the Rieske Fe-S protein, the heme-containing cytochrome c_1 and cytochrome *b* subunits (b_{566} and b_{560}); the large mitochondrial matrix-exposed domain of complex III containing the two subunits of mitochondrial processing peptidase (α-MPP and β-MPP), which cleaves the targeting peptides of nuclear-encoded mitochondrial proteins; and five small subunits QCR6-QCR10, which participate in electron transport. At complex III, electrons are transferred from ubiquinol to cytochrome *c* and protons are translocated from the matrix to the IMS by the Q cycle. During the Q cycle, ubiquinol binds to the center P of complex III, where it is oxidized and releases two electrons, one of which is transferred to cytochrome *c* via the Rieske Fe–S center and cytochrome c_1 and two protons are pumped into the IMS. The other electron is transferred by cytochrome *b*-type proteins within complex III to reduce ubiquinone (bound to the matrix side of complex III) to semiquinone at the center N. At this point, another ubiquinol attaches to the center P and passes through the same process, reduces semiquinone to ubiquinol, completes the Q cycle and pumps two more protons into the IMS. Once the Q cycle is completed, cytochrome *c*, a small heme-containing protein located on the outer surface of the IMM exposing it to the IMS, is reduced and released from complex III, carries one electron and transports it to complex IV - the terminal enzyme of the mtETC.

The molecular structure of plant complex IV has recently been described by Maldonado et al. (2021). At subunit II of complex IV, electrons from cytochrome *c* are transferred via Cu_A and cytochrome *a* then to the cytochrome a_3-Cu_B center of the subunit I, where one oxygen molecule (the final electron acceptor) is reduced by one electron to form two water molecules. This redox reaction is coupled to the translocation of two protons across the IMM. The K, D, H pathways (named after crucial amino acid residues in each pathway) were suggested to contribute to the pumping of protons by plant complex IV but it has recently been suggested that the

H pathway is not involved in this process (Maldonado et al., 2021). During one "cycle" of the mtETC, a total of 10 protons (4 from complex I, 4 from complex III and 2 from complex IV) are translocated from the mitochondrial matrix to the IMS, creating an electrochemical proton gradient, also known as the mitochondrial membrane potential. The protons in the IMS re-enter the mitochondrial matrix via the ATP synthase complex (also known as complex V) to dissipate the proton gradient. Complex V is composed of two major subcomplexes: F_o - the transmembrane subcomplex ("o" stands for oligomycin, which inhibits proton transfer through the subcomplex) and F_1 - the matrix-exposed subcomplex, are connected by a central stalk. The F_o subcomplex allows protons to pass through, causing conformational changes within the F_1 subcomplex, driving the phosphorylation of ADP to ATP. Generation of ATP – the final product of the OXPHOS system and more importantly, the cellular energy currency, is one of the prime functions of mitochondria.

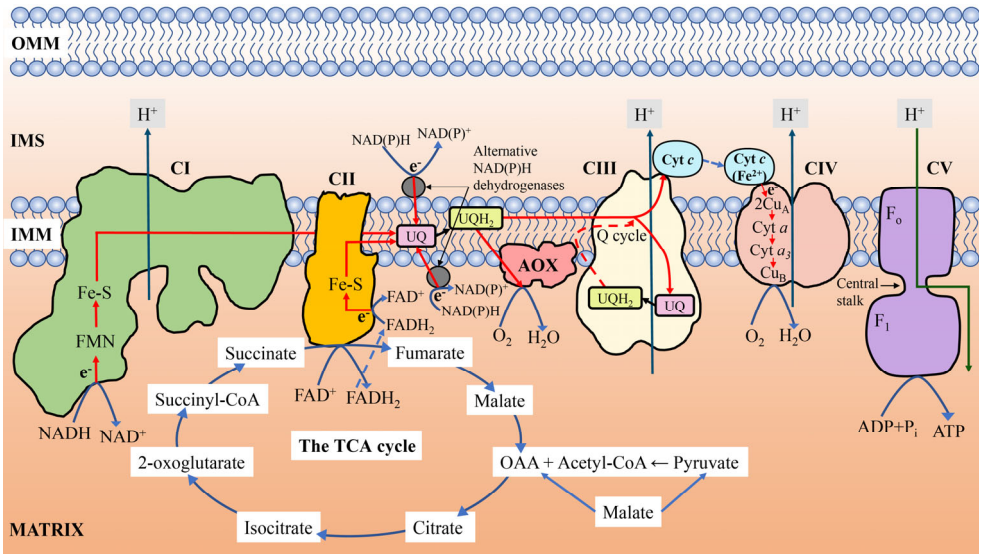

Figure 2. A diagram of the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation (OXPHOS) system in plant mitochondria. Imported pyruvate is converted into acetyl-Coenzyme A (acetyl-CoA) whereas imported malate can be used to generate either pyruvate or oxaloacetate (OAA). As the TCA cycle starts in the mitochondrial matrix, acetyl-CoA and OAA combine to generate citrate, which is subsequently isomerized to isocitrate. Isocitrate is then converted into 2-oxoglutarate, which is in turn converted into succinyl-Coenzyme A (succinyl-CoA). Succinyl-CoA is turned into succinate, which is oxidized to fumarate by the inner mitochondrial membrane (IMM)-anchored succinate dehydrogenase (complex II (CII)). Concomitantly, flavin adenine dinucleotide ($FADH_2$) is oxidized to FAD^+. Fumarate is converted into and malate, which is oxidized to OAA that subsequently combines with another acetyl-CoA to continue the cycle. The OXPHOS system occurs within the IMM. Complex I (CI) oxidizes nicotinamide adenine dinucleotide (NADH) to

generate NAD^+ at the peripheral arm site and the electrons are transferred via flavin mononucleotide (FMN) and iron-sulfur (Fe-S) clusters onto ubiquinone (UQ). CII oxidizes $FADH_2$ to FAD^+ at the membrane-spanning domain site and the electrons are transported via Fe-S clusters to UQ. During the electron transport, CI pumps protons (H^+) from the mitochondrial matrix into the mitochondrial intermembrane space (IMS) whereas CII is a non-proton pumping complex. By receiving two electrons, UQ is reduced to ubiquinol (UQH_2), which transfers electrons to complex III (CIII). At CIII, electrons are transferred from UQH_2 to cytochrome *c* (cyt *c*) and protons are translocated from the matrix into the IMS by the Q cycle. Because one cyt *c* can accept only one electron from UQH_2 at a time, the second electron is transferred to UQ bound to CIII, which is reduced to form semi-UQ. During the second half of the Q cycle, another UQH_2 enters the first half of the Q cycle, donating one electron to another cyt *c* and the second electron is transported to semi-UQ, which is fully reduced to UQH_2 and this UQH_2 will travel to the IMM and re-enter the Q cycle. Reduced cyt *c* (cyt *c* (Fe^{2+})) transfers electrons from CIII to complex IV (CIV), where electrons are transferred via Cu_A and cytochrome *a* (cyt *a*) then to the cytochrome a_3 (cyt a_3)-Cu_B center to reduce oxygen (O_2) to water (H_2O). This redox reaction is coupled to the translocation of protons from the matrix to the IMS. During mitochondrial electron transport chain (mtETC), protons are pumped from the matrix to the IMS by CI, CIII and CIV, generating an electrochemical proton gradient. The protons in the IMS re-enter the mitochondrial matrix via the subcomplex F_o of the adenosine triphosphate (ATP) synthase (complex V (CV)). As a result, the subcomplex F_1 connected to the subcomplex F_o by the central stalk changes its conformation and drives the phosphorylation of adenosine diphosphate (ADP) to ATP. In addition to CI, plant mitochondria contain the alternative nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) dehydrogenases located either on the outer surface of the IMM (exposed to the IMS) or on the inner surface of the IMM (exposed to the mitochondrial matrix). The alternative NAD(P)H dehydrogenases transport electrons from NAD(P)H to UQ, but do not pump protons across the IMM. Plants have an alternative respiratory pathway that bypasses cytochrome *c* oxidase and transfers electrons from UQH_2 to the nuclear-encoded alternative oxidase (AOX) localized in the IMM to reduce O_2 to H_2O. AOX does not pump protons, thus energy produced during the alternative respiratory pathway is lost as heat. OMM = outer mitochondrial membrane, e^- = electron. The figure is adapted from Millar et al. (2015) edited by Buchanan et al. (2015).

The respiratory complexes can associate into the respiratory supercomplexes in plants

Analyses by Blue Native Polyacrylamide Gel-Electrophoresis (BN-PAGE) have revealed that the plant respiratory complexes can associate into the respiratory supercomplexes (Figure 3) (Dudkina et al., 2006; Eubel et al., 2004; Eubel et al., 2003; Krause et al., 2004). Of note, all respiratory supercomplexes contain dimeric complex III (III_2). In supercomplexes, complex III_2 can interact either with the membrane arm of complex I or with one or two copies of monomeric complex IV or can even interact with both complex I and IV. Respiratory supercomplexes formed by complex I, III_2 and IV are referred as respirasomes because they can perform respiratory electron transport from NADH to reduce oxygen (Braun, 2020).

It is proposed that respirasomes can associate to form even larger structures, called respiratory megacomplexes, which are thought to be involved in defining the structure of the cristal membrane (Braun, 2020). Recently, the atomic structure of plant supercomplex III_2-IV has been revealed at high resolution by cryogenic electron microscopy (cryo-EM) (Maldonado et al., 2021).

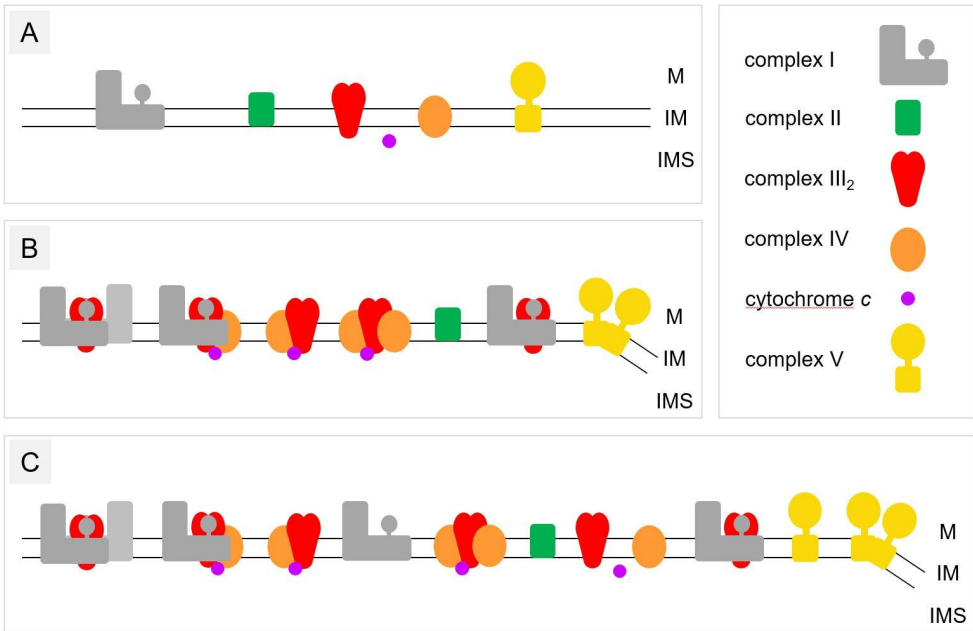

Figure 3. The respiratory complexes can associate into the respiratory supercomplexes in plants. Shapes and colors of the oxidative phosphorylation (OXPHOS) complexes and of cytochrome *c* are indicated in the legend at the top right. **A.** OXPHOS complexes in monomeric forms (except for complex III, which always occurs as a dimer). **B.** Respiratory supercomplexes in plant mitochondria. **C.** *In vivo*, monomeric OXPHOS complexes and supercomplexes are supposed to coexist within the inner mitochondrial membrane. M = matrix, IM = inner mitochondrial membrane, IMS = mitochondrial intermembrane space. The figure is taken from Braun (2020) with permission. Braun (2020) (DOI: 10.1016/j.mito.2020.04.007) is published under the terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).

Plants have an alternative electron transport pathway

The respiratory pathway described above (see "the oxidative phosphorylation system") is referred as the cytochrome *c* oxidase respiratory pathway, as it utilizes cytochrome *c* to carry electrons from complex III to complex IV. Plants have an alternative respiratory pathway that bypasses the cytochrome *c* oxidase and transfers electrons from ubiquinol to the nuclear-encoded alternative oxidase (AOX)

localized in the IMM to reduce oxygen to water. AOX is non-proton pumping, thus energy released during the alternative respiratory pathway is lost as heat. AOX is not only found in plants, but also found in many algae and fungi, animals and some protozoa (McDonald et al., 2009). The AOX pathway is resistant to classic inhibitors of complex III, such as antimycin A (AA; which inhibits the reduction of ubiquinone at the center N) and myxothiazol (which inhibits the oxidation of ubiquinol at the center P), and of complex IV, such as chemicals competing with oxygen for accepting electrons, including cyanide (CN^-), azide (N_3^-), carbon monoxide (CO) and nitric oxide (NO) (Millar et al., 2015). Nevertheless, AOX activity is inhibited by salicylhydroxamic acid (SHAM) and n-propylgallate (nPG) (Millar et al., 2015).

In addition to the NADH dehydrogenase/complex I, plant mitochondria contain the alternative (type II) nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) dehydrogenases located either on the outer surface of the IMM (exposed to the IMS) or on the inner surface of the IMM (exposed to the mitochondrial matrix) (Rasmusson et al., 2004). As the name implies, the alternative NAD(P)H dehydrogenases transport electrons from NAD(P)H to ubiquinone, but do not pump protons across the IMM (Rasmusson et al., 2004). As a result, if plants are totally dependent on the alternative NAD(P)H dehydrogenases, the theoretical number of ATP generated by complex V will be reduced. The alternative NAD(P)H dehydrogenases are insensitive to rotenone, which inhibits electron transfer from the Fe-S center of complex I to ubiquinone. Due to the presence of the alternative NAD(P)H dehydrogenases, plants can survive without complex I (Braun, 2020), although mutants defective in complex I showed severe growth and development deficiencies (Fromm et al., 2016; Kuhn et al., 2015). Interestingly, the hemi-parasitic plant, mistletoe *Viscum album*, is the first reported multicellular eukaryote that completely lacks complex I due to the absence of mitochondrially-encoded *NAD* genes and other nuclear-encoded genes that encode subunits of complex I (Maclean et al., 2018; Senkler et al., 2018). Consequently, the abundance of complex IV and V are decreased whereas the abundance of alternative dehydrogenases and oxidases are increased in *Viscum album* as compared to in the model (non-parasitic) plant *Arabidopsis thaliana* (Maclean et al., 2018).

The genome of plant mitochondria

Similar to in other eukaryotes, the plant mitochondrion has its own genome (hereafter referred as to the mitochondrial DNA (mtDNA) or the mitogenome) that originated from the endosymbiotic ancestor, an α-proteobacterium (Sagan, 1967). Through evolution, the mitochondrial genome was decreased steadily through gene loss and/or transfer to the nuclear genome (Chevigny et al., 2020). Mitochondria

have partially retained their own genomes during evolution. On one hand, it is suggested that mitochondria need local regulation of gene expression to rapidly respond to the local redox state (Allen, 2015). On the other hand, it is proposed that mitochondrially-encoded membranous ETC proteins are highly hydrophobic, which causes a problem for importing the proteins into mitochondria (von Heijne, 1986). Indeed, Bjorkholm et al. (2015) proposed that hydrophobic membrane proteins could be targeted to the ER rather than to mitochondria. Nowadays, the plant mitogenomes can be diverse in size and structure depending on plant species, but the number of mitochondrially-encoded genes, including OXPHOS and mitochondrial ribosomal genes, transfer RNA (tRNA) and ribosomal RNA (rRNA), is relatively similar (Chevigny et al., 2020). In the model plant *Arabidopsis thaliana*, the mitogenome contains 57 genes, including OXPHOS proteins, ribosomal proteins, tRNAs and rRNAs (Table 1) (Unseld et al., 1997). To date, it is reported that the size of land plant mtDNA is the largest (Gualberto and Newton, 2017). The size of mammalian mtDNA is 15-17 kilobase pairs (kb), whereas the plant mitogenome is substantially larger: 13-96 kb in algae, usually 200-700 kb in angiosperms, but can be as enormous as 11 megabase pairs (mb) (in *Silene conica*) (Gualberto and Newton, 2017; Sloan et al., 2012). This is because the plant mitogenome mostly contains non-coding sequences that are not conserved across species rather than because the plant mtDNA encodes a few more genes and several genes possess introns (Gualberto and Newton, 2017). Although the plant mitogenome has non-coding sequences originating from chloroplastic, nuclear, or viral DNA by horizontal transfer, the origin of the vast majority of non-coding sequences are unknown (Bergthorsson et al., 2003; Chevigny et al., 2020; Gualberto and Newton, 2017). In addition to the high number of non-coding sequences, the plant mtDNA also contains a high abundance of repeated sequences, which can be categorized into large repeats (> 500 bp), intermediate-sized repeats (50-500 bp) and small repeats (< 50 bp) (Arrieta-Montiel et al., 2009; Davila et al., 2011; Gualberto et al., 2014). It is generally accepted to illustrate the plant mitogenome as a single circular chromosome. For example, the mitogenomes of *Marchantia polymorpha* and *Vicia faba* were observed as single circular DNA molecules (Negruk et al., 1986; Oda et al., 1992). However, using pulse field gel electrophoresis or electron microscopy failed to detect such a single circular chromosome (Backert et al., 1997; Oldenburg and Bendich, 1996). Rather, the plant mitogenome mainly contains a collection of circular subgenomes and linear and branched DNA molecules (Sloan, 2013). Due to the structural variability, the plant mitogenomes have distinct replication mechanisms. Replication of circular DNA molecules is likely to undergo rolling circle mechanisms whereas replication of linear DNA molecules is mediated by specific proteins that covalently interact with terminal inverted repeats (Gualberto and Newton, 2017; Handa, 2008). The plant mitogenome does not exist as naked DNA in the mitochondrial matrix but as part of nucleoids, which are regarded as the heritable units of mtDNA (Dai et al., 2005;

Gilkerson et al., 2013). In spite of the massive size of mitogenomes, the number of mitochondrially-encoded genes in plants is not much higher than that of animals and yeast (about 20 additional genes) (Chevigny et al., 2020). Interestingly, the plant mitogenomes are highly stable and conserved in most higher plant species as compared to in animals because of such lower mutation rates occurring in the plant mtDNA (Wolfe et al., 1987). This could be because plant mitochondria have efficient repair mechanisms, particularly homologous recombination, which is likely to be the main mtDNA repair mechanism in plants (Chevigny et al., 2020; Gualberto and Newton, 2017). Homologous recombination fixes the damaged copy by recruiting an undamaged DNA molecule, which can be brought by the continuous mitochondrial fusion and fission (Gualberto and Newton, 2017; Møller et al., 2021). Other DNA repair pathways existing in plant mitochondria, including direct repair, mismatch repair, nucleotide excision repair and base excision repair, are described in detail by Chevigny et al. (2020).

Table 1. The mitogenome of *Arabidopsis thaliana* contains 57 genes, encoding 25 proteins involved in the OXPHOS system (subunits of complex I, III, IV and V, and cytochrome *c* biogenesis), 7 ribosomal proteins, 22 transfer RNAs, and 3 ribosomal RNAs (5S, 18S, and 26S). The table is modified from Unseld et al. (1997). mtSSU = mitoribosome small subunit. mtLSU = mitoribosome large subunit.

Gene	Description
nad1	Subunit of complex I
nad2	
nad3	
nad4	
nad4L	
nad5	
nad6	
nad7	
nad9	
cob	Subunit of complex III
cox1	Subunit of complex IV
cox2	
cox3	
atp1	Subunit of complex V
atp4	
atp6-1	
atp6-2	
atp8	
atp9	
ccmB	Cytochrome *c* biogenesis
ccmF$_C$	
ccmFN$_1$	
ccmC	
ccmFN$_2$	

rpL2	mtLSU protein
rpL5	
rpL16	
rpS3	mtSSU protein
rpS4	
rpS7	
rpS12	
rrn18	mtSSU ribosomal RNA
rrn26	mtLSU ribosomal RNA
rrn5	
22 transfer RNAs	

The proteome of plant mitochondria

Proteomic studies have shown that the number of mitochondrial proteins is diverse across eukaryotic organisms, e.g. ~1800 proteins in mammals (Palmfeldt and Bross, 2017), ~1000 proteins in yeast (Schmidt et al., 2010) and 2000-3000 proteins in plants, of which 98-99% are nuclear-encoded, translated by cytosolic ribosomes and imported into mitochondria (Rao et al., 2017). Of note, plants possess 200-300 mitochondrial pentatricopeptide repeat (PPR) proteins (Møller et al., 2021), which are substantially more than in other eukaryotes: yeast and human have only 12 and 7 mitochondrial PPR proteins, respectively (Lightowlers and Chrzanowska-Lightowlers, 2013). Plants contain 20-40 mitochondrially-encoded proteins (Møller et al., 2021; Mower, 2020). About 70% of nuclear-encoded mitochondrial proteins have N-terminal mitochondrial targeting sequences (MTS). MTS are removed and degraded once nuclear-encoded mitochondrial proteins are imported into different sub-mitochondrial compartments via the translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) channels (Chacinska et al., 2009; Kmiec et al., 2014; Prokisch et al., 2004; Schleiff and Becker, 2011). The remaining 30%, e.g. mitochondrial proteins associating with the IMM and subunits of OXPHOS, do not contain canonical MTS (Huang et al., 2009; Kmiec et al., 2014; Schleiff and Becker, 2011; Senkler et al., 2017a). Of note, mitochondrial fusion and fission could mix individual mitochondrial proteomes (Fuchs et al., 2020; Møller, 2016; Møller et al., 2021). In order to find out if a protein is potentially targeted to mitochondria, information on its subcellular localization can be found in the Subcellular Localization Database for Arabidopsis Proteins – SUBA5 (suba.live), which is the central resource for Arabidopsis protein subcellular location data (Hooper et al., 2017). SUBA5 suggested that 2562 proteins are targeted to mitochondria by either large scale proteomics or fluorescent localization (accessed 4th January 2023).

From genes to proteins in plant mitochondria

Mitochondrial transcription

As the mitochondrion originated from an α-proteobacterial ancestor, mitochondria have partially retained machinery for expression of their own genomes. Similar to nuclear-encoded genes, mitochondrially-encoded genes undergo transcription and translation, which however occur within mitochondria. In *Arabidopsis thaliana*, the 367 kb mtDNA encodes 25 OXPHOS proteins, 7 ribosomal proteins, 3 rRNAs (5S, 18S, and 26S) and 22 tRNAs (Table 1) (Unseld et al., 1997). Mitochondria have nuclear-encoded phage-type RNA polymerases, which are thought to have taken over from the original bacterial-type RNA polymerase (Cermakian et al., 1996; Gaspari et al., 2004; Gray and Lang, 1998; Greenleaf et al., 1986; Masters et al., 1987; Tiranti et al., 1997; Weihe, 2004; Weihe et al., 1997). Organellar phage-type RNA polymerases are highly similar to the bacterial T7 RNA polymerases (Liere and Börner, 2011). Plant phage-type RNA polymerases are encoded by RpoT gene family (RNA polymerase of the T-phage type) (Liere and Börner, 2011). The RpoT gene family of Arabidopsis contains mitochondria-targeted RpoTm, plastid-targeted RpoTp and dual-targeted RpoTmp (Hedtke et al., 1997, 2000; Hedtke et al., 2002; Hedtke et al., 1999; Kobayashi et al., 2002; Kobayashi et al., 2001). Kuhn et al. (2009) showed that RpoTm acts as the fundamental RNA polymerase in Arabidopsis mitochondria and is mandatory for the transcription of most, if not all, mitochondrially-encoded genes. Identified mitochondrial promoters in Arabidopsis contain the CRTA-type consensus motifs, loosely-unusual sequences for the tetra-nucleotide core-motif (e.g. ATTA and RGTA) and promoters without consensuses (Kuhn et al., 2005). Of note, the study by Kuhn et al. (2009) also showed that RpoTmp activity is gene specific and promoter independent, suggesting that there is an RpoTmp-dependent transcriptional mechanism allowing mitochondria to regulate the expression of specific mitochondrially-encoded genes.

Post-transcriptional processing of primary mitochondrially-transcribed RNA

Following transcription, mitochondrially-transcribed messenger RNA (mRNA), tRNA and rRNA are processed. According to Binder et al. (2011), processing of most 5’ and 3’ ends of mRNAs are accomplished via post-transcriptional modification, in which the mature 5’ ends and 3’ ends are achieved by endonucleolytic and/or exonucleolytic processing. Maturation of 5’ and 3’ ends of tRNAs recruits two nucleases RNAse P and RNAse Z, and tRNA processing is accomplished by the addition of 5’-CCA-3’, by RNA editing (cysteine (C)-to-uridine (U)) and by normal base modification. Processing of the 5’ ends of 18S and

5S rRNAs is likely to be done by endonucleolytic cleavage, however, very little is known about processing the 5' and 3' ends of rRNAs. The steady-state levels of functional mitochondrial RNAs are controlled by the rates of transcribing and degrading RNA. Plant mitochondrial RNAs can be polyadenylated for degradation and the Polynucleotide phosphorylase (PNPase) is a major player for RNA degradation (Holec et al., 2006).

Similar to primary nuclear-transcribed RNAs, mitochondrially-transcribed RNA precursors contain introns, which undergo splicing processes. Organellar introns in plants are categorized into group I and group II (Bonen, 2008; Bonen and Vogel, 2001; Lambowitz and Zimmerly, 2004; Michel et al., 1989; Zimmerly et al., 2001). In angiosperms (particularly vascular plants), most mitochondrial introns are group II introns, which are likely to be spliced by trans-acting cofactors (Brown et al., 2014). Identified proteins facilitating splicing of mitochondrial group II introns include a mitochondrially-encoded maturase MatR, nuclear-encoded maturases (nMATs), CRM-domain proteins (mCSF1 and mCSF2), a DEAD-box RNA-helicase (PMH2), PPR proteins (OTP43, BIR6 and ABO5), a PORR-domain protein (WTF9) and a RCC-domain protein RUG3 (Brown et al., 2014).

Another mitochondrial post-transcriptional modification is RNA editing – a process altering bases in transcript sequences relative to their corresponding genes (Knoop, 2011). About 80% of the plant mitochondrial transcripts have editing sites that are edited in high efficiencies (Bentolila et al., 2013). The most frequent RNA editing activity in plant organelles (both mitochondria and plastids) is C-to-U substitution in mRNAs (Chateigner-Boutin and Small, 2010; Small et al., 2020). Reverse U-to-C editing is however less frequent and has been observed in a few plant lineages, e.g. hornworts, lycophytes and ferns (Chateigner-Boutin and Small, 2010; Small et al., 2020). C-to-U editing also takes place in tRNAs and rRNAs (Binder et al., 1994; Fey et al., 2002; Grewe et al., 2011; Hecht et al., 2011; Marechal-Drouard et al., 1996; Marechal-Drouard et al., 1993; Schuster et al., 1991), in introns and in 5'- and 3'-untranslated regions of many mitochondrial mRNAs (Borner et al., 1995; Carrillo and Bonen, 1997; Farre et al., 2012; Lippok et al., 1994; Shi et al., 2017; Sutton et al., 1991; Wissinger et al., 1991; Zanlungo et al., 1995). RNA editing not only changes the amino acid sequence of a protein, but also influences the RNA structure, consequently affecting RNA splicing and stability and RNA-protein interaction (Bonen, 2008; Chateigner-Boutin and Small, 2010; Oldenkott et al., 2014; Small et al., 2020). Specific editing sites of plant organelles are recognized by site-specific binding of RNA-binding cofactors - PPR proteins containing repeats of a tandem domain of 35 amino acid motifs (a helix-turn-helix structure), the PPR domains (Small and Peeters, 2000).

Several studies on the structures of PPR protein–RNA complexes showed that PPR proteins bind to single-stranded RNAs in a sequence-specific manner (Coquille et al., 2014; Gully et al., 2015; Ke et al., 2013; Shen et al., 2016; Yin et al., 2013). Within one certain repeat, the amino acids at the 5th and 35th positions, which

correspond to residues 6 and 1' in Barkan et al. (2012) and residues 4 and ii in Yagi et al. (2013), specifically recognize RNA bases (Yan et al., 2019). These di-residues are termed as the PPR code. Several PPR codes have been identified: threonine and asparagine (TN), threonine and aspartic acid (TD), asparagine and serine (NS), and asparagine and aspartic acid (ND) at the 5^{th} and 35^{th} positions recognize the corresponding nucleotides adenine (A), guanine (G), cytosine (C) and uracil (U), respectively (Barkan and Small, 2014). Notably, the 5^{th} position mainly determines the RNA base specificity. The presence of asparagine at this position prefers pyrimidine-binding whereas serine or threonine prefers purine-binding (Barkan and Small, 2014). The 35^{th} position is the second main determinant. The presence of asparagine at this position prefers to bind to base A or C while aspartate results in preference of binding to base G or U (Barkan and Small, 2014). Recently, Yan et al. (2019) has further explored the correlation between PPR codes and RNA bases, which enriched the pool of PPR codes. Based on the results, Yan and co-workers developed the online PPRCODE web server (http://yinlab.hzau.edu.cn/pprcode/) to facilitate the prediction of PPR protein-RNA binding sites.

PPR proteins are categorized into two subclasses: the PPR-P subclass present in many eukaryotes, and the PPR-PLS subclass, which are land plant-specific and have long 35 or 36 amino acids and short 31 or 32 amino acids variants of the canonical 35 amino acid PPR motif (P) (Cheng et al., 2016; Lurin et al., 2004). The PPR-P subclass has been proposed to be involved in RNA transcription, splicing, editing, cleavage, stability and in mitochondrial translation (Barkan and Small, 2014; Haili et al., 2016; Rugen et al., 2019; Schmitz-Linneweber and Small, 2008; Uyttewaal et al., 2008; Waltz and Giege, 2020; Waltz et al., 2019; Waltz et al., 2020b). The PPR-PLS subclass is specific to C-to-U RNA editing (Barkan and Small, 2014; Small et al., 2020). The PPR-PLS proteins contain highly conserved domains E1, E2 and DYW (named after amino acid residues of the domain). E1 and E2 domains resemble PPR or tetratricopeptide repeat (TPR) motifs (Cheng et al., 2016) but their exact functions remain elusive. The DYW domain is likely to possess a cytidine deaminase activity that performs the nucleotide exchange process (Small et al., 2020). However, it remains unclear how many RNA-editing PPR proteins that do not contain the DYW domain can still perform editing (Small et al., 2020). In addition to the PPR-PLS subclass, non-PPR proteins, including Multiple Organellar RNA editing Factors (MORFs) (also known as RNA-editing factor Interacting Proteins (RIPs)), Other RNA Recognition Motif (ORRM) (e.g. ORRM4 and ORRM5) and NUWA-related proteins (a P-class PPR protein), are also found to be involved in plant mitochondrial RNA-editing (Andres-Colas et al., 2017; Bentolila et al., 2012; Guillaumot et al., 2017; Shi et al., 2017; Shi et al., 2016; Takenaka et al., 2012).

Mitochondrial translation

Similar to nuclear gene expression, the last step of mitochondrial gene expression is translation, a process in which the mRNA sequence is used as a template to synthesize a corresponding protein chain by the mitochondrial ribosome (hereafter referred as to the mitoribosome). Translation is composed of four phases: initiation, elongation, termination and ribosome recycling. Like the cytosolic ribosome, a mitoribosome is also composed of rRNAs and two main subunits: the small subunit, which binds to mRNA and reads the genetic information, and the large subunit, which has tRNA aminoacyl (A), peptidyl (P) and exit (E) sites to synthesize the protein chain. In contrast to plastid translation, very little is known about plant mitochondrial translation. However, recent studies on mitoribosome structures of eukaryotes, including plants, help us to predict some aspects of plant mitochondrial translation.

Using cryo-EM, the structure of bacterial ribosome and mitoribosome structures of mammals, yeast, trypanosomes and plants were described at very high resolution (Figure 4) (Amunts et al., 2015; Desai et al., 2017; Greber et al., 2015; Noeske et al., 2015; Ramrath et al., 2018; Waltz et al., 2020b; Watson et al., 2020). As compared to bacterial ribosomes, mitoribosomes from eukaryotic kingdoms are protein-rich and 1.2-1.5 time larger (Waltz et al., 2020a; Waltz and Giege, 2020). This is because the mitoribosome small subunit (mtSSU) is substantially larger, and even surpasses the size of the mitoribosome large subunit (mtLSU) in trypanosomes and plants (Ramrath et al., 2018; Rugen et al., 2019; Waltz et al., 2019). In addition to the size difference, the structure and composition of eukaryotic mitoribosomes are also very different from the bacterial counterparts (Waltz and Giege, 2020). The diversity of mitoribosomes across eukaryotes suggests that eukaryotic mitoribosomes have evolved to adapt to particular niches during evolution (Waltz et al., 2020a). The composition of plant mitoribosome was revealed by Waltz et al. (2019) and Rugen et al. (2019), identifying 19 plant-specific mitoribosome proteins, of which half are PPR proteins. The study by Waltz et al. (2020b) described the atomic structure of the plant mitoribosome. Briefly, the plant mitoribosome is extended in rRNAs and protein contents. Most of plant-specific PPR proteins bind to the plant-specific rRNA expansion segments, which leads to reshaping the overall structure of plant mitoribosome. The central protuberance of plant mitoribosomes, where the mtLSU inter-connects with the mtSSU, consists of a 5S rRNA (like the bacterial counterpart) and mitochondrial plant-specific proteins. This specific structure thus contributes to increasing and reshaping the overall volume of the plant ribosome central protuberance. Similar to mammalian and yeast mitoribosomes, the plant mitoribosome is likely to be attached to the IMM and to interact with the insertase OXA1 through linker(s) (possibly protein(s)) (Waltz et al., 2020a; Waltz et al., 2020b).

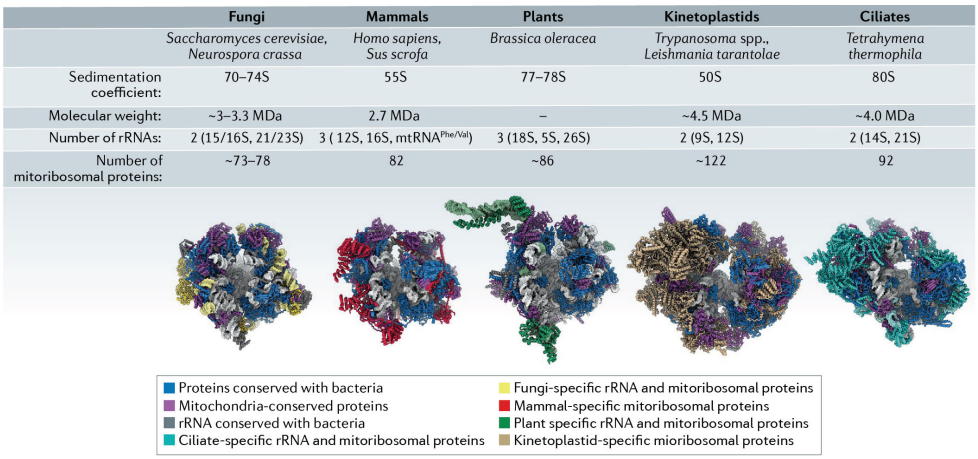

Figure 4. Advanced cryo-electron microscopy studies have recently revealed the structure of mitoribosomes and their diversification in fungi, mammals, plants and protozoans (kinetoplastids and ciliates). The figure is taken from Kummer and Ban (2021) (DOI: 10.1038/s41580-021-00332-2) with permission from Springer Nature.

In eukaryotes, including yeast, mammals and plants, mitochondrial mRNAs do not have Shine-Dalgarno (SD) sequences – the ribosomal binding site in bacterial mRNAs (Ayyub and Varshney, 2020; Derbikova et al., 2018; Hazle and Bonen, 2007). Of note, yeast mitochondrial mRNAs have very long 5' untranslated regions (5'UTRs) while mammalian mitochondrial mRNAs have very short 5'UTRs (maximum three-nucleotide length) or even do not have 5'UTRs (Ayyub and Varshney, 2020; Derbikova et al., 2018). Therefore, mitochondrial translation initiation in yeast and mammals strongly relies on translation initiation factors and/or activators (Ayyub and Varshney, 2020; Derbikova et al., 2018). A unique feature of mitochondrial translation initiation in mammals has recently been described by Kummer et al. (2018). Translation initiation in mammalian mitochondria is mediated by initiation factors, IF2 and IF3, and the mtSSU PPR protein mS39, which binds to an U-rich domain in the mRNA downstream of its start codon AUG (Kummer et al., 2018). Such U-rich regions are conserved and found downstream of codon 7 (after AUG) in 11 mRNAs (out of 13) of mammalian mitochondria (Amunts et al., 2015; Bieri et al., 2018; Kummer et al., 2018). In yeast, mitochondrial translation initiation seems to recruit universal translation initiation factors and specific translation activators to help the mtSSU interact with the 5'UTRs of mRNAs (Derbikova et al., 2018). Plant mitochondrial mRNAs also contain long 5'UTRs without SD sequences and plant mitochondrial rRNAs do not have anti-SD sequences either (Waltz et al., 2020b). To date, the mechanism of translation initiation in plant mitochondria is still enigmatic. The structure study of plant mitoribosome by Waltz et al. (2020b) suggested that mS83/rPPR10 is a PPR protein sitting in a cleft where the incoming mRNAs may be recruited. Waltz et al.

(2020b) also hypothesized that the PPR motifs of mS83/rPPR10 recognize AxAAA-related motifs in the 5'UTRs (located about 19 nucleotides upstream of the start codon) of 17 (out of 33) mitochondrial mRNAs, thus acting as a SD–anti-SD-like recognition system.

Mitochondrial retrograde signalling

Although mitochondria likely evolved from endosymbiotic α-protobacteria, mitochondria do not function independently, but their activities are integrated with other cellular organelles. To operate efficiently, a continuous flow of information between the nucleus and the mitochondrion is required, and it is described as anterograde and retrograde signalling (Ng et al., 2014). Anterograde signals from the nucleus are transmitted to the mitochondrion, as the nuclear regulation of the mitochondrial activity. Retrograde signals derived from the mitochondrion are sent to the nucleus and affect nuclear gene expression. In plants, mitochondrial retrograde signalling was first discovered by inhibition of mitochondrial function, resulting in the upregulation of *AOX1* transcript in tobacco (*Nicotiana tabacum*) (Vanlerberghe and McIntosh, 1996). Subsequently, the promoter region of *AOX1a* has been used as a "classical" bait for identification of mitochondrial retrograde signalling regulators in plants, including ABSCISIC ACID-INSENSITIVE 4 (ABI4) (Giraud et al., 2009), WRKY transcription factors (Van Aken et al., 2013; Vanderauwera et al., 2012), and MYB transcription factors (Ivanova et al., 2014; Zhang et al., 2017a; Zhang et al., 2017b). Nevertheless, the most well-studied and specific transcriptional regulators of mitochondrial retrograde signalling belong to the ARABIDOPSIS NAC DOMAIN CONTAINING (ANAC) protein family, including ANAC017 functioning as the main regulator and ANAC013 contributing as a downstream target in positive feedback loops (De Clercq et al., 2013; Ng et al., 2013; Van Aken et al., 2016). Of note, the study by Ng et al. (2013) showed that ANAC017 is a dual-targeted protein. ANAC017 is anchored in the ER membrane via a C-terminal transmembrane (TM) domain containing a consensus rhomboid protease cleavage site, which is suggested to be truncated proteolytically by rhomboid-related proteases during mitochondrial dysfunction. This allows ANAC017 to be released from the ER while its C-terminal TM domain stays in the ER, leading to the translocation of the N-terminal fragment of ANAC017 into the nucleus to activate gene expression (Ng et al., 2013). However, the exact mechanism of ANAC017 translocation remains enigmatic. In the nucleus, ANAC017 stimulates expression of mitochondrial retrograde signalling marker genes, including *AOX1a*, *UP REGULATED BY OXIDATIVE STRESS AT2G21640 1* (*UPOX1*), *URIDINE DIPHOSPHATE GLYCOSYLTRANSFERASE 74E2* (*UGT74E2*), *NAD(P)H DEHYDROGENASE B4* (*NDB4*), *At12CYS-2*, *H_2O_2 RESPONSE GENE 1* (*HRG1*)

and *OUTER MEMBRANE PROTEIN OF 66 KDA* (*OM66*) (Van Aken et al., 2016; Van Aken et al., 2007). Reactive oxygen species (ROS) generated by the mtETC and peptides from degraded proteins possibly trigger the ANAC017 pathway (Møller and Sweetlove, 2010; Ng et al., 2014; Vestergaard et al., 2012), but the molecular signals from a dysfunctional mitochondrion and how they initiate this signalling pathway remain elusive. Surprisingly, when mitochondrial function was sufficiently impaired, a consistent downregulation of chloroplast-encoded transcripts was observed (Adamowicz-Skrzypkowska et al., 2020; Zubo et al., 2014), suggesting that an as yet unknown (possibly retrograde) signalling pathway apparently can reduce chloroplast transcription.

Mitochondrial unfolded protein response

The mitochondrial unfolded protein response (UPR^{mt}) is described as the transcriptional response that regulates nuclear gene expression during mitochondrial malfunction to maintain mitochondrial homeostasis by inducing the mitochondrial protein quality control (chaperones and proteases). The UPR^{mt} can be triggered by mtDNA mutations (Lin et al., 2016), malfunctioning mitochondrial membrane potential (Rolland et al., 2019), imbalance between nuclear- and mitochondrially-encoded proteins (Houtkooper et al., 2013) or overloading concentration of unfolded/misfolded proteins in mitochondria (Zhao et al., 2002). Recent studies have gained insight into the plant UPR^{mt}. The study by Moullan et al. (2015) suggested that the plant UPR^{mt} is at least partially conserved to the animal UPR^{mt}. Wang and Auwerx (2017) showed that the UPR^{mt} in *Arabidopsis thaliana* is activated by a transient oxidative burst, resulting in the activation of mitogen-activated protein kinases (MAPKs) and hormonal signalling (ethylene, auxin and jasmonate) to recover mitochondrial proteostasis. Kacprzak et al. (2020) showed that ANAC017 – the main transcriptional regulator of mitochondrial retrograde signalling, induces many classes of genes that are classic targets of UPR^{mt} in response to AA treatment. Of note, ANAC017 induces "classical" mitochondrial retrograde signalling marker genes that have been also suggested as UPR^{mt} marker genes by Moullan et al. (2015) and Wang and Auwerx (2017). This thus suggests that "classical" mitochondrial retrograde signalling and UPR^{mt} in plants are most likely one and the same response (Kacprzak et al., 2020; Tran and Van Aken, 2020).

Adenyl cyclases in plants

Adenyl cyclases (ACs), also known as adenylyl cyclases or adenylate cyclases, are enzymes that convert ATP into 3′,5′-cyclic adenosine monophosphate (cAMP), which is a secondary messenger that can affect different physiological and biochemical processes in the cells. The important roles of cAMP as well as identification of ACs have been well-established in bacteria, animals and fungi. In plants, cAMP is involved in ion homeostasis, stomatal opening, pollen tube development, seed germination and cell cycle progression (Blanco et al., 2020). Nevertheless, comprehensive knowledge of plant cAMP and ACs is still missing. This could be due to two reasons. Firstly, it could be difficult to detect plant cAMP because the cAMP concentration is lower in plants than in animals. Reported cAMP concentration in plants is less than 40 pmol g^{-1} fresh weight in rye grass endosperm cell cultures, *Torenia* stem segments, and cultured cells of *Phaseolus vulgaris* and *Nicotiana tabacum*, 40-170 pmol g^{-1} fresh weight in *Daucus carota*, *Triticum aestivum*, and *Lemna paucicostata*, 200–250 pmol g^{-1} fresh weight in *Citrus*, *Lactuca*, *Hordeum*, and *Zea* (Sabetta et al., 2016). On the contrary, reported cAMP level in animals is usually greater than 250 pmol g^{-1} wet weight (Gehring, 2010). Secondly, it could be challenging to identify plant ACs because plant ACs seem not to have conserved catalytic domains (Gehring, 2010). However, Gehring (2010) proposed that plant ACs might have the conserved motif [RKS]X[DE]X(9,11)[KR]X(1,3)[ED] in their catalytic domains. To date, experimentally identified ACs in plants are PsiP in *Zea mays* (Moutinho et al., 2001), HpAC1 in *Hippeastrum x hybridum* (Swiezawska et al., 2014), AtKUP7, ATPPR-AC, AtClAP and LRRAC1 in *Arabidopsis thaliana* (Al-Younis et al., 2015; Bianchet et al., 2019; Chatukuta et al., 2018; Ruzvidzo et al., 2013), NbAC in *Nicotiana benthamiana* (Ito et al., 2014), and MpCAPE in *Marchantia polymorpha* (Kasahara et al., 2016). Of note, these plant ACs, except HpAC1 and MpCAPE, appeared to have the AC-motif proposed by Gehring (2010). Therefore, it seems to be difficult to identify plant ACs if just based on this motif search. It is also noticeable that *AT4G15640* and *AT3G21465* are annotated as ACs on The Arabidopsis Information Resource (TAIR), but there is no experimental confirmation.

The aims of this thesis

The mitochondrial unfolded protein response (UPR^{mt}) is described as the transcriptional response that regulates nuclear gene expression during mitochondrial malfunction to maintain mitochondrial homeostasis. The aim of paper I is to review and compare UPR^{mt}-related signalling across eukaryotic kingdoms, including animals, fungi and plants.

The study of plant mitochondria usually requires isolation of pure, intact and functional mitochondria from plant tissues. The aim of paper II is to present a strategy for efficient isolation of mitochondria from leaf tissues of *Arabidopsis thaliana* by using continuous Percoll density gradients.

Despite a relatively thorough understanding of basic mitochondrial functions, many mitochondrial proteins and processes remain poorly understood. The aim of paper III is to characterize the two *Arabidopsis thaliana* genes, *AT4G15640* and *AT3G21465*, which are predicted as mitochondria-targeted proteins and have been poorly annotated as adenyl cyclases on TAIR.

Summary of papers

Paper I summarizes the current knowledge of UPR^{mt} across eukaryotic kingdoms, including animals, fungi and plants. UPR^{mt} has been extensively studied in animals, whereas very few related-UPR^{mt} have been described in yeast. In plants, little is known about the UPR^{mt} and only few regulators have recently been identified. In this work, we compare the UPR^{mt} across kingdoms. Our study indicates that each kingdom has evolved their own specific regulators, which however induce very similar groups of target genes. Our meta-analysis identifies homologs of known UPR^{mt} regulators and their responsive genes across eukaryotic kingdoms, which could be of interest for future research.

Paper II describes two strategies for plant growth (in soil and in liquid half-strength Murashige and Skoog media) and an efficient method of mitochondrial isolation from leaf tissues of *Arabidopsis thaliana*. By using continuous Percoll density gradients, isolated mitochondria are relatively free from chloroplast contamination. Isolated Arabidopsis mitochondria obtained by this method can be either used for assays requiring intact mitochondria, e.g. import assays or respiration measurements, or be stored at -80°C for later use, e.g. BN-PAGE or western blot.

Paper III describes the characterization of two *Arabidopsis thaliana* genes, *AT4G15640* and *AT3G21465*. We show that AT4G15640 and AT3G21465 are unlikely to be ACs as previously annotated on TAIR. Instead, AT4G15640 and AT3G21465 are land plant-specific mitochondrial proteins that are essential for plant performance. Knocking out both *AT4G15640* and *AT3G21465* results in low abundance and activity of mitochondrial OXPHOS complexes, thus reducing capacity of oxygen consumption. Our immunoblotting and tandem mass spectrometry (MS/MS) analysis using isolation mitochondria show a severe perturbation of the mitochondrial proteome and reduction of many OXPHOS components due to loss of *AT4G15640* and *AT3G21465*. Using co-immunoprecipitation, we confirm that AT4G15640 and AT3G21465 are part of the mtSSU, in line with two proteomic studies of Arabidopsis mitoribosome during the course of our study. Therefore, we named AT4G15640 and AT3G21465 as mitochondrial TRANslation factor 1 (mTRAN1) and 2 (mTRAN2), respectively. Transcriptome profile of *mtran1 mtran2* mutant resembles most two mutants defective in a mitoribosomal protein and shows no clear effects on mitochondrial splicing and editing, thus indicating mitochondrial translation defects. *In organello* protein synthesis and polysome fractionation assays suggest that mTRAN proteins

are required for mitochondrial translation initiation. Our motif analysis suggests that the 5'UTRs of mitochondrial mRNAs contain two potential mitoribosome binding sites that are A/U-rich regions (CUUUxU and AAGAAx/AxAAAG). Moreover, the RNA electrophoretic mobility shift assays show that mTRAN1 binds to these A/U-rich domains in the 5'UTRs of mitochondrial mRNAs to initiate translation. Finally, our Ribo-seq analysis shows that not only a subset, but all mitochondrial mRNAs have lower ribosome loading levels in the *mtran* mutants compared to in the wild type, suggesting that mTRAN proteins are universal mtSSU-embedded translation initiation factors.

Discussion

In paper I, the current knowledge of UPR^{mt}-related signalling across eukaryotic kingdoms, including animals, fungi and plants, was summarized and compared. The comparison indicates that each kingdom has evolved their own specific regulators, which however induce very similar groups of target genes, such as mitochondrial (co-)chaperones and proteases. Homologs of known UPR^{mt} regulators and responsive genes are identified across eukaryotic kingdoms, which might be interesting for future research. The authors hypothesized that such different UPR^{mt} pathways occurring across kingdoms could be due to major evolutionary events, such as rise of multicellularity or colonization of land. Indeed, the ANAC017 group – one of the main UPR^{mt} regulators in land plants, is suggested to be evolved from ancestral NACs (found in at least some green algae) during the period of land colonization by plants (Khan and Van Aken, 2022). In plants, "classical" mitochondrial retrograde signalling and UPR^{mt} appear to be the same response during mitochondrial dysfunction. In Arabidopsis, UPR^{mt} is activated in response to mitochondrial malfunction by treatment of Doxycycline (Dox) - a mitochondrial ribosome inhibitor, MitoBlock-6 (MB) - an inhibitor of protein import into mitochondria and AA, which inhibits electron transfer at the center N of complex III leading to an increase in ROS. However, the molecular signals from a dysfunctional mitochondrion and how they initiate this signalling pathway remain enigmatic.

In paper II, we described an efficient method of mitochondrial isolation from leaf tissues of *Arabidopsis thaliana* by using continuous Percoll-PVP density gradients. The most advantage of this protocol is that freshly isolated mitochondria are intact and pure (relatively free form chloroplast contamination), which is of great importance for following assays requiring functional mitochondria, e.g. *in organello* protein synthesis (Kwasniak-Owczarek et al., 2022), import assays (Zhang et al., 2012) and oxygen consumption measurement (Keech et al., 2005). However, this method usually requires a large amount of starting plant material and takes a few hours to complete. Another method of plant mitochondrial isolation, IMTACT (Boussardon et al., 2020), in which transgenic Arabidopsis carrying mitochondrial OUTER MEMBRANE 64 (OM64) - biotin ligase recognition peptide (BLRP) placed under tissue-specific promoters, enables cell-specific biotinylated mitochondria to be purified with streptavidin magnetic beads in less than 30 min (Boussardon and Keech, 2022). As compared to IMTACT, our described

mitochondrial isolation protocol is slower (timewise) and requires much more plant material. Nevertheless, IMTACT requires generation of transgenic plants, which would take time to produce a desired plant line. For instance, if we want to apply IMTACT on the *mtran* double mutants for mitochondrial isolation, we must cross the *mtran* double mutant line with the OM64-BLRP line, which would take at least several months to get a homozygous *mtran* double mutant line expressing OM64-BLRP. Depending on the purposes plus time management, we recommend you to wisely choose which method of Arabidopsis mitochondrial isolation will be suitable for your project.

In paper III, the two *Arabidopsis thaliana* genes, *AT4G15640* and *AT3G21465*, were characterized. It turned out that these genes are unlikely to be ACs as annotated on TAIR. The study shows that AT4G15640 and AT3G21465 are land-plant specific mitochondrial proteins that are critical for plant growth. Strikingly, AT4G15640 and AT3G21465 are shown to be part of the mtSSU and are of great importance for efficient mitochondrial translation initiation. Therefore, AT4G15640 and AT3G21465 are suggested to be annotated as mitochondrial TRANslation factor 1 (mTRAN1) and mitochondrial TRANslation factor 2 (mTRAN2), respectively. Of note, comparison of transcriptomic data (the *mtran1 mtran2* mutant versus *rps10* mutants, which have reduced expression of mtSSU ribosomal protein *RPS10*) showed that UPR^{mt} was activated, leading to induction of target genes in ANAC017 pathway in both mutants. Wang and Auwerx (2017) showed that in the Arabidopsis *mitochondrial ribosomal protein L1* (*mrpl1-1*) mutant, UPR^{mt} was activated, which in turn induced ethylene responses as a systemic signal. Together, these suggest that UPR^{mt} is activated by impairment of mitochondrial translation. Such a UPR^{mt} activation was not only observed in plants, but also in animals, for instance, knockout of mitochondrial ribosomal protein triggered mitonuclear protein imbalance, which activated the UPR^{mt} in *Caenorhabditis elegans* (*C. elegans*) (Houtkooper et al., 2013). Therefore, imbalance between nuclear- and mitochondrially-encoded proteins probably induces the plant UPR^{mt} during malfunctioning mitochondrial translation. Upon UPR^{mt} activation, ANAC017 and ethylene signalling pathways are independently induced. This suggests that there are possibly specific signal molecules inducing different signalling responses. However, the specific signals from mitochondria defective in translation and how they induce the UPR^{mt} in plants remain elusive.

Knockout of *mTRAN1* and *mTRAN2* showed a reduction in OXPHOS complex abundance and activity, which resulted in a strong decrease in capacity of the cytochrome *c* oxidase respiratory pathway. Such a defect caused *mtran1 mtran2* mutants to rely on the alternative respiration. Rolland et al. (2019) proposed that the UPR^{mt} in *C. elegans* was activated when most mitochondrial processes required for the maintenance of mitochondrial homeostasis, e.g mtETC, were compromised. A general signal that possibly triggers UPR^{mt} is a decrease in mitochondrial membrane potential, which results in a decrease in mitochondrial protein import, likely leading

to accumulation of non-imported mitochondrial proteins (Tran and Van Aken, 2020). As loss of *mTRAN1* and *mTRAN2* impaired mtETC, one could expect that mitochondrial membrane potential was possibly reduced though it was not confirmed experimentally, causing activation of UPR^{mt}. In addition, *mtran1 mtran2* mutants showed a defect in complex I and III, which could lead to an increase in the rate of superoxide production (Cadenas et al., 1977; Møller, 2001; Murphy, 2009). Although ROS measurement was not carried out in the study, superoxide – a signal that could trigger the plant UPR^{mt} (Tran and Van Aken, 2020), could be expected to be increased and activate UPR^{mt} due to loss of *mTRAN1* and *mTRAN2*.

Surprisingly, the RNA-seq data of *mtran1 mtran2* mutant showed a downregulation of 117 chloroplast-encoded transcripts, which was also observed in two previous studies (Adamowicz-Skrzypkowska et al., 2020; Zubo et al., 2014). Of note, the chloroplast transcriptome of *mtran1 mtran2* mutant showed that all chloroplast-encoded tRNAs were significantly downregulated, indicating that chloroplast gene expression was likely to be decreased, which could lead to a decrease in photosynthesis and an increase in ROS production in chloroplast (Arsova et al., 2010; Kindgren et al., 2012; Myouga et al., 2008). ROS can damage proteins inside the chloroplasts, causing activation of chloroplast unfolded protein response (cpUPR) (Kessler and Longoni, 2019). Indeed, *ClpB3* – a cpUPR marker gene (Llamas et al., 2017), was upregulated in the *mtran1 mtran2* mutant. ClpB3 is a nuclear-encoded chloroplastic Clp protease that helps refolding unfolded/misfolded/aggregated 1-deoxy-D-xylulose-5-phosphate synthase (DXS) – an enzyme that converts pyruvate and D-glyceraldehyde-3-phosphate (D-GAP) into 1-deoxy-D-xylulose-5-phosphate (DXP) in the first step of the methylerythritol phosphate (MEP) pathway occurring within the chloroplasts (Pulido et al., 2016). DXS aggregation was formed after chloroplast translation was specifically inhibited by lincomycin even though DXS transcript levels remained unchanged and only its protein levels increased (Llamas et al., 2017). The *mtran1 mtran2* mutant had all chloroplast-encoded tRNAs downregulated, suggesting that chloroplast translation was likely to be perturbed. Therefore, one could expect that DXS aggregation would occur, leading to the upregulation of *ClpB3*. Taken together, we suggest that loss of *mTRAN* genes not only induces UPR^{mt} but also activates cpUPR.

All in all, compromised mitochondrial translation in plants could result in (i) mitonuclear protein imbalance, (ii) a decrease in mitochondrial membrane potential leading to accumulation of non-import protein in mitochondria, and (iii) impaired mtETC causing an increase in ROS production in mitochondria. These consequences could activate UPR^{mt} and another as yet known (probably retrograde) signalling pathway that apparently can reduce chloroplast transcription, possibly leading to a decrease in chloroplast gene expression, which causes activation of cpUPR. Another hypothesis is that there is possibly a crosstalk between mitochondria and chloroplasts (but not via the nucleus), in which mitochondrial

gene expression could somehow affect transcription and/or even gene expression in chloroplasts.

To date, the full mechanism of mitochondrial translation in plants remains elusive. The atomic structure of cauliflower mitoribosomes revealed by Waltz et al. (2020b) helps predicting how plant mitoribosome initiates translation. Waltz and his co-workers suggested that mTRAN1 (named as mS83/rPPR10 by the authors) is a PPR protein, which functions as a RNA-binding protein. However, the PPR codes of mTRAN proteins could not be predicted by the PPRCODE web server (Yan et al., 2019). mTRAN1 resides in a cleft of the mtSSU, where its PPR domains might recognize and bind to AxAAA-related motifs of mRNAs to initiate translation because plant mitochondrial mRNAs do not contain SD sequences nor do plant mitochondrial rRNAs have anti-SD sequences. These AxAAA-related motifs were found in the 5'UTRs of 17 out of 33 mitochondrially-encoded mRNAs (Waltz et al., 2020b). In contrast, our polysome profiling showed that mTRAN1 and mTRAN2 are required for mitoribosome binding/translation initiation of not only mitochondrial mRNAs containing such an AxAAA 5'UTR motif (*NAD9*, *COX2* and *ATP9*) but also mitochondrial mRNAs that do not appear to contain this binding site (*NAD7*, *COB*, *ATP1* and *ATP8*). Moreover, the Ribo-seq analysis of mitochondrial genes further supports that mTRAN proteins are universal mtSSU-embedded translation initiation factors, which could recognize the motif CUUUxU/AxAAAG/AAGAAx in the 5'UTRs of mitochondrial mRNAs to initiate translation based on our motif analysis and RNA electrophoretic mobility shift assays (REMSAs). To verify the interaction between mTRAN1 and mitochondrial mRNAs *in vivo*, we will perform RNA Immunoprecipitation Sequencing (RIP-seq) using Arabidopsis cell culture transformed with mTRAN1-GFP and/or isolated mitochondria from a stably transformed Arabidopsis line expressing mTRAN1-GFP. Transcripts interacting with mTRAN1-GFP complexes will be purified using GFP-affinity purification and subsequently sequenced to find out if mTRAN1 interacts with the vast majority of mitochondrial mRNAs *in planta*, further supporting our finding that mTRAN1 is a universal translation initiation factor of mtSSU. In addition, RIP-seq could also allow us to identify the mitoribosomal binding sites to validate our REMSA results.

As mitochondria originated from the ancient endosymbiotic bacteria, mitochondrial translation could be expected to resemble bacterial translation, at least partially. However, comparison of bacterial ribosomes versus mitoribosomes of mammals, yeast and plants showed the diversity of mitoribosome structure, indicating that eukaryotic mitoribosomes have evolved to adapt to particular niches during evolution (Waltz et al., 2020a). This suggests that mitochondrial translation in general and mitochondrial translation initiation in particular could also be diverse across eukaryotic kingdoms. In bacteria, the ribosomal small subunit binds to the SD motif of mRNA using an anti-SD sequence of 16S rRNA (Shine and Dalgarno, 1974). In addition, bacterial translation initiation requires initiation factor (IF) 1, 2

and 3. Unlike bacteria, eukaryotes, including yeast, mammals and plants, do not have SD sequences in the 5'UTRs of mitochondrially-encoded mRNAs. In mammals (at least in humans), most of mitochondrial mRNAs do not have 5'UTRs (Montoya et al., 1981). Kummer et al. (2018) suggested that the PPR protein mS39, which is located in the mtSSU, binds to the U-rich motif downstream the start codon of mRNAs to initiate translation. Notably, the PPRCODE prediction tool–Yin Lab (Yan et al., 2019) predicts that mS39 has four PPR motifs that bind to RNA bases (G)(C>U)(C>U>A)(-) with low scores. Thus, it is possible that both mS39 and mTRAN proteins are PPR-like proteins that do not obey the "classical" PPR code rule. Mammals lack mitochondrial IF1 (mtIF1) and require mtIF2 and mtIF3 for translation initiation (Ayyub and Varshney, 2020). In yeast, the mtSSU contains a platform formed by mS35, mS46 and extensions of uS3m and uS5m, which is suggested to be the mtSSU-mRNA interaction site during translation initiation (Itoh et al., 2020). Mitochondrial translation initiation in yeast seems to recruit universal mtIFs and specific translation activators that are likely to recognize specific 5'UTRs of mitochondrial mRNAs (Derbikova et al., 2018). Therefore, the mtSSU-mRNA interaction site proposed by Itoh et al. (2020) is expected to act as a platform for mtIFs and translation activators to bind to the 5'UTRs of mitochondrial mRNAs to initiate translation. In fact, several translation activators were co-purified with the yeast mitoribosome (Desai et al., 2017). In plants, mTRAN1 is likely to be a PPR-like protein acting as a mRNA-binding factor of the mtSSU. Of note, both yeast and plant mitochondrial mRNAs have long 5'UTRs (Derbikova et al., 2018; Waltz et al., 2020a). Therefore, one could speculate that yeast and plant mitochondrial translation initiation/mtSSU-mRNA binding could somehow use a comparable mechanism. Our findings suggest that mTRAN proteins act as universal recognition factors for mitochondrial mRNAs, however, there is a high possibility that mtIFs and/or mitochondrial translation activators required for recognition of different specific motifs of mRNAs exist in plants. To date, very little is known about mtIFs in plants, e.g. only MTL1 and RFL8 have recently been identified as specific proteins mandatory for translation of *NAD7* and *ccmFN*$_2$, respectively (Haili et al., 2016; Nguyen et al., 2021). We attempted to perform a BLAST search in *Arabidopsis thaliana* to identify proteins that are similar to bacterial translation IFs (unpublished data). The putative subcellular localization of identified proteins was searched in SUBA5 (Hooper et al., 2017). Bacterial translation IF1 has a similar protein, AT4G11175, which is targeted to chloroplasts and enables mRNA-binding (Bach-Pages et al., 2020; Millen et al., 2001; Trotta et al., 2019). Bacterial translation IF2 has 64 similar proteins with low scores of similarities, of which, 11 are targeted to mitochondria. Bacterial translation IF3 resembles 11 proteins, of which, AT1G34360 annotated as a translation IF3 family protein, is targeted to mitochondria (Senkler et al., 2017b). It is worth to mention that AT1G34360 did not give a high score of similarity by BLAST, but it is probably because the length of bacterial IF3 protein sequence (180 amino acids) is shorter as compared to that of AT1G34360 (520 amino acids). Atkinson et al. (2012) attempted to find functional

orthologues of mtIF3 in yeast by a sensitive PSI-Blast across eukaryotes, as BlastP searches alone could not detect mtIF3 homologues from distantly related eukaryotes. They found that Aim23p in yeast was a "missing" orthologue similar to mtIF3 in other eukaryotes and successfully showed that it functioned as the mtIF3 in yeast. Surprisingly, their phylogenetic analysis also revealed AL6G45350 as an orthologue of mtIF2 in *Arabidopsis lyrata*, which is a homologue to AT4G11160 in *Arabidopsis thaliana*. AT4G11160 was also found in our BLAST search but with a low score of similarity. According to SUBA5, it is predicted to be localized in cytosol, chloroplasts and mitochondria and no experimental studies have confirmed its subcellular localization. Atkinson et al. (2012) also identified SMO171G0119 as an orthologue of mtIF3 in *Selaginella moellendorffii* (an early vascular plant), which is a homologue to AT1G34360 in *Arabidopsis thaliana*.

Taken together, our findings suggest that AT4G11160 and AT1G34360 in *Arabidopsis thaliana* could function similarly to mtIF2 and mtIF3 in other eukaryotes, although AT4G11160 is only predicted to be targeted to mitochondria. If it is true, eukaryotic mitochondria seem to have lost mtIF1 and retain mtIF2 and mtIF3 for translation initiation during evolution. Mammalian mtIF2 has an extension domain (37 amino acids long) that functionally compensates for the absence of mtIF1 (Gaur et al., 2008; Yassin et al., 2011). This domain prevents tRNA from binding to the ribosomal A site, thus facilitating the fMet-tRNA to bind to the ribosomal P site during translation initiation (Kummer et al., 2018; Yassin et al., 2011). However, the conservation of this extension domain is limited to vertebrates (Atkinson et al., 2012). The role of mammalian mtIF3 is not clear. It is suggested that the mtSSU associates with mtIF2 and mtIF3 to form a translation initiation complex, ensuring that mRNA is stably bound to mtSSU prior to tRNA recruitment and before mtSSU and mtLSU assemble into a monosome (Kummer and Ban, 2021). In yeast, mtIF3 seems not to be a core component for translation of all mRNAs, but mitochondrial translation lacking mtIF3 is strongly imbalanced (Derbikova et al., 2018; Kuzmenko et al., 2016). In fact, yeast mitochondria are likely to recruit specific translation activator complexes to bind to the 5'UTRs of mRNAs to initiate translation (Derbikova et al., 2018). Therefore, it is difficult to predict whether plant mtIF2 and mtIF3 (if any) function in a comparable way as in other eukaryotes. In this study, we propose that mTRANs are PPR-like proteins that do not follow the "classical" PPR code rule and bind to the A/U-rich domains in the 5'UTRs of mRNAs to initiate translation. Perhaps due to their substantial length, the plant mitochondrial mRNA's 5'UTRs might form either the 2D or 3D structures to bring the binding sites physically closer to the start codon. Universal mtIFs and/or specific mtIFs/translation activators possibly help the mtSSU find the correct binding site and/or stably bind to mRNAs during the initiation step.

Conclusion

In conclusion, the thesis work suggests that compromised mitochondrial translation results in the UPR^{mt} activation in plants. In addition, we show that mTRAN proteins act as universal recognition factors of mtSSU for mitochondrial mRNAs to initiate translation. This indicates that translation initiation by mitochondrial ribosomes occurs in a fundamentally different way in plants as compared to in bacteria, yeast and mammals. mtSSU-mRNA binding in plants may also require mitochondrial translation IFs, which could be at least partially similar to in other eukaryotes. Therefore, further studies, such as characterizing Arabidopsis mutant(s) lacking functional mtIF2 and/or mtIF3 (see discussion), are required to demonstrate this hypothesis and to gain a broader insight into the mechanism of mitochondrial translation in plants.

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References

Adamowicz-Skrzypkowska, A., Kwasniak-Owczarek, M., Van Aken, O., Kazmierczak, U., and Janska, H. (2020). Joint inhibition of mitochondrial complex IV and alternative oxidase by genetic or chemical means represses chloroplast transcription in Arabidopsis. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences *375*, 20190409.

Al-Younis, I., Wong, A., and Gehring, C. (2015). The *Arabidopsis thaliana* K^+-uptake permease 7 (AtKUP7) contains a functional cytosolic adenylate cyclase catalytic centre. FEBS letters *589*, 3848-3852.

Allen, J.F. (2015). Why chloroplasts and mitochondria retain their own genomes and genetic systems: Colocation for redox regulation of gene expression. Proceedings of the National Academy of Sciences *112*, 10231-10238.

Amunts, A., Brown, A., Toots, J., Scheres, S.H.W., and Ramakrishnan, V. (2015). The structure of the human mitochondrial ribosome. Science *348*, 95-98.

Andres-Colas, N., Zhu, Q., Takenaka, M., De Rybel, B., Weijers, D., and Van Der Straeten, D. (2017). Multiple PPR protein interactions are involved in the RNA editing system in Arabidopsis mitochondria and plastids. Proceedings of the National Academy of Sciences of the United States of America *114*, 8883-8888.

Arrieta-Montiel, M.P., Shedge, V., Davila, J., Christensen, A.C., and Mackenzie, S.A. (2009). Diversity of the Arabidopsis mitochondrial genome occurs via nuclear-controlled recombination activity. Genetics *183*, 1261-1268.

Arsova, B., Hoja, U., Wimmelbacher, M., Greiner, E., Ustun, S., Melzer, M., Petersen, K., Lein, W., and Bornke, F. (2010). Plastidial thioredoxin z interacts with two fructokinase-like proteins in a thiol-dependent manner: evidence for an essential role in chloroplast development in *Arabidopsis* and *Nicotiana benthamiana*. The Plant Cell *22*, 1498-1515.

Atkinson, G.C., Kuzmenko, A., Kamenski, P., Vysokikh, M.Y., Lakunina, V., Tankov, S., Smirnova, E., Soosaar, A., Tenson, T., and Hauryliuk, V. (2012). Evolutionary and genetic analyses of mitochondrial translation initiation factors identify the missing mitochondrial IF3 in *S. cerevisiae*. Nucleic Acids Research *40*, 6122-6134.

Ayyub, S.A., and Varshney, U. (2020). Translation initiation in mammalian mitochondria - a prokaryotic perspective. RNA Biology *17*, 165-175.

Bach-Pages, M., Homma, F., Kourelis, J., Kaschani, F., Mohammed, S., Kaiser, M., van der Hoorn, R.A.L., Castello, A., and Preston, G.M. (2020). Discovering the RNA-binding proteome of plant leaves with an improved RNA interactome capture method. Biomolecules *10*, 661.

Backert, S., Lynn Nielsen, B., and Börner, T. (1997). The mystery of the rings: structure and replication of mitochondrial genomes from higher plants. Trends in Plant Science *2*, 477-483.

Barkan, A., Rojas, M., Fujii, S., Yap, A., Chong, Y.S., Bond, C.S., and Small, I. (2012). A combinatorial amino acid code for RNA recognition by pentatricopeptide repeat proteins. PLoS Genetics *8*, e1002910.

Barkan, A., and Small, I. (2014). Pentatricopeptide repeat proteins in plants. Annual Review of Plant Biology *65*, 415-442.

Bensley, R.R., and Hoerr, N.L. (1934). Studies on cell structure by the freezing-drying method VI. The preparation and properties of mitochondria. The Anatomical Record *60*, 449-455.

Bentolila, S., Heller, W.P., Sun, T., Babina, A.M., Friso, G., van Wijk, K.J., and Hanson, M.R. (2012). RIP1, a member of an Arabidopsis protein family, interacts with the protein RARE1 and broadly affects RNA editing. Proceedings of the National Academy of Sciences of the United States of America *109*, E1453-1461.

Bentolila, S., Oh, J., Hanson, M.R., and Bukowski, R. (2013). Comprehensive high-resolution analysis of the role of an Arabidopsis gene family in RNA editing. PLoS Genetics *9*, e1003584.

Bergthorsson, U., Adams, K.L., Thomason, B., and Palmer, J.D. (2003). Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature *424*, 197-201.

Bianchet, C., Wong, A., Quaglia, M., Alqurashi, M., Gehring, C., Ntoukakis, V., and Pasqualini, S. (2019). An *Arabidopsis thaliana* leucine-rich repeat protein harbors an adenylyl cyclase catalytic center and affects responses to pathogens. Journal of Plant Physiology *232*, 12-22.

Bieri, P., Greber, B.J., and Ban, N. (2018). High-resolution structures of mitochondrial ribosomes and their functional implications. Current Opinion in Structural Biology *49*, 44-53.

Binder, S., Hölzle, A., and Jonietz, C. (2011). RNA processing and RNA stability in plant mitochondria. In Plant Mitochondria, F. Kempken, ed. (Springer, New York, NY), pp. 107-130.

Binder, S., Marchfelder, A., and Brennicke, A. (1994). RNA editing of $tRNA^{Phe}$ and $tRNA^{Cys}$ in mitochondria of *Oenothera berteriana* is initiated in precursor molecules. Molecular and General Genetics *244*, 67-74.

Bjorkholm, P., Harish, A., Hagstrom, E., Ernst, A.M., and Andersson, S.G. (2015). Mitochondrial genomes are retained by selective constraints on protein targeting. Proceedings of the National Academy of Sciences of the United States of America *112*, 10154-10161.

Blanco, E., Fortunato, S., Viggiano, L., and de Pinto, M.C. (2020). Cyclic AMP: A polyhedral signalling molecule in plants. International Journal of Molecular Sciences *21*, 4862.

Bonen, L. (2008). Cis- and trans-splicing of group II introns in plant mitochondria. Mitochondrion *8*, 26-34.

Bonen, L., and Vogel, J. (2001). The ins and outs of group II introns. Trends in Genetics *17*, 322-331.

Borner, G.V., Morl, M., Wissinger, B., Brennicke, A., and Schmelzer, C. (1995). RNA editing of a group II intron in *Oenothera* as a prerequisite for splicing. Molecular and General Genetics *246*, 739-744.

Boussardon, C., and Keech, O. (2022). Cell type-specific isolation of mitochondria in Arabidopsis. Methods in Molecular Biology *2363*, 13-23.

Boussardon, C., Przybyla-Toscano, J., Carrie, C., and Keech, O. (2020). Tissue-specific isolation of Arabidopsis/plant mitochondria - IMTACT (isolation of mitochondria tagged in specific cell types). The Plant Journal *103*, 459-473.

Braun, H.P. (2020). The oxidative phosphorylation system of the mitochondria in plants. Mitochondrion *53*, 66-75.

Brown, G.G., Colas des Francs-Small, C., and Ostersetzer-Biran, O. (2014). Group II intron splicing factors in plant mitochondria. Frontiers in Plant Science *5*, 35.

Buchanan, B.B., Gruissem, W., and Jones, R.L. (2015). Biochemistry and molecular biology of plants, 2nd edition. John Wiley and Sons.

Cadenas, E., Boveris, A., Ragan, C.I., and Stoppani, A.O. (1977). Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. Archives of Biochemistry and Biophysics *180*, 248-257.

Carrillo, C., and Bonen, L. (1997). RNA editing status of *nad7* intron domains in wheat mitochondria. Nucleic Acids Research *25*, 403-409.

Cermakian, N., Ikeda, T.M., Cedergren, R., and Gray, M.W. (1996). Sequences homologous to yeast mitochondrial and bacteriophage T3 and T7 RNA polymerases are widespread throughout the eukaryotic lineage. Nucleic Acids Research *24*, 648-654.

Chacinska, A., Koehler, C.M., Milenkovic, D., Lithgow, T., and Pfanner, N. (2009). Importing mitochondrial proteins: machineries and mechanisms. Cell *138*, 628-644.

Chateigner-Boutin, A.L., and Small, I. (2010). Plant RNA editing. RNA Biology *7*, 213-219.

Chatukuta, P., Dikobe, T.B., Kawadza, D.T., Sehlabane, K.S., Takundwa, M.M., Wong, A., Gehring, C., and Ruzvidzo, O. (2018). An Arabidopsis clathrin assembly protein with a predicted role in plant defense can function as an adenylate cyclase. Biomolecules *8*, 15.

Cheng, S., Gutmann, B., Zhong, X., Ye, Y., Fisher, M.F., Bai, F., Castleden, I., Song, Y., Song, B., Huang, J., *et al.* (2016). Redefining the structural motifs that determine RNA binding and RNA editing by pentatricopeptide repeat proteins in land plants. The Plant Journal *85*, 532-547.

Chevigny, N., Schatz-Daas, D., Lotfi, F., and Gualberto, J.M. (2020). DNA repair and the stability of the plant mitochondrial genome. International Journal of Molecular Sciences *21*, 328.

Coquille, S., Filipovska, A., Chia, T., Rajappa, L., Lingford, J.P., Razif, M.F., Thore, S., and Rackham, O. (2014). An artificial PPR scaffold for programmable RNA recognition. Nature Communications *5*, 5729.

Dai, H., Lo, Y.S., Litvinchuk, A., Wang, Y.T., Jane, W.N., Hsiao, L.J., and Chiang, K.S. (2005). Structural and functional characterizations of mung bean mitochondrial nucleoids. Nucleic Acids Research *33*, 4725-4739.

Davila, J.I., Arrieta-Montiel, M.P., Wamboldt, Y., Cao, J., Hagmann, J., Shedge, V., Xu, Y.Z., Weigel, D., and Mackenzie, S.A. (2011). Double-strand break repair processes drive evolution of the mitochondrial genome in Arabidopsis. BMC Biology *9*, 64.

Day, D., Neuburger, M., and Douce, R. (1985). Biochemical characterization of chlorophyll-free mitochondria from pea leaves. Functional Plant Biology *12*, 219-228.

De Clercq, I., Vermeirssen, V., Van Aken, O., Vandepoele, K., Murcha, M.W., Law, S.R., Inze, A., Ng, S., Ivanova, A., Rombaut, D., *et al.* (2013). The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in Arabidopsis. The Plant Cell *25*, 3472-3490.

Derbikova, K.S., Levitsky, S.A., Chicherin, I.V., Vinogradova, E.N., and Kamenski, P.A. (2018). Activation of yeast mitochondrial translation: Who is in charge? Biochemistry (Moscow) *83*, 87-97.

Desai, N., Brown, A., Amunts, A., and Ramakrishnan, V. (2017). The structure of the yeast mitochondrial ribosome. Science *355*, 528-531.

Dudkina, N.V., Heinemeyer, J., Sunderhaus, S., Boekema, E.J., and Braun, H.P. (2006). Respiratory chain supercomplexes in the plant mitochondrial membrane. Trends in Plant Science *11*, 232-240.

Escobar, M.A., Geisler, D.A., and Rasmusson, A.G. (2006). Reorganization of the alternative pathways of the Arabidopsis respiratory chain by nitrogen supply: opposing effects of ammonium and nitrate. The Plant Journal *45*, 775-788.

Eubel, H., Heinemeyer, J., and Braun, H.P. (2004). Identification and characterization of respirasomes in potato mitochondria. Plant Physiology *134*, 1450-1459.

Eubel, H., Jansch, L., and Braun, H.P. (2003). New insights into the respiratory chain of plant mitochondria. Supercomplexes and a unique composition of complex II. Plant Physiology *133*, 274-286.

Farre, J.C., Aknin, C., Araya, A., and Castandet, B. (2012). RNA editing in mitochondrial trans-introns is required for splicing. PloS One *7*, e52644.

Fey, J., Weil, J.H., Tomita, K., Cosset, A., Dietrich, A., Small, I., and Marechal-Drouard, L. (2002). Role of editing in plant mitochondrial transfer RNAs. Gene *286*, 21-24.

Frey, T.G., and Mannella, C.A. (2000). The internal structure of mitochondria. Trends in Biochemical Sciences *25*, 319-324.

Fromm, S., Senkler, J., Eubel, H., Peterhansel, C., and Braun, H.P. (2016). Life without complex I: proteome analyses of an Arabidopsis mutant lacking the mitochondrial NADH dehydrogenase complex. Journal of Experimental Botany *67*, 3079-3093.

Fuchs, P., Rugen, N., Carrie, C., Elsasser, M., Finkemeier, I., Giese, J., Hildebrandt, T.M., Kuhn, K., Maurino, V.G., Ruberti, C., *et al.* (2020). Single organelle function and organization as estimated from Arabidopsis mitochondrial proteomics. The Plant Journal *101*, 420-441.

Gaspari, M., Falkenberg, M., Larsson, N.G., and Gustafsson, C.M. (2004). The mitochondrial RNA polymerase contributes critically to promoter specificity in mammalian cells. The EMBO Journal *23*, 4606-4614.

Gaur, R., Grasso, D., Datta, P.P., Krishna, P.D., Das, G., Spencer, A., Agrawal, R.K., Spremulli, L., and Varshney, U. (2008). A single mammalian mitochondrial translation initiation factor functionally replaces two bacterial factors. Molecular Cell *29*, 180-190.

Gehring, C. (2010). Adenyl cyclases and cAMP in plant signaling - past and present. Cell Communication and Signalling *8*, 15.

Gifford, R.M. (2003). Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. Functional Plant Biology *30*, 171-186.

Gilkerson, R., Bravo, L., Garcia, I., Gaytan, N., Herrera, A., Maldonado, A., and Quintanilla, B. (2013). The mitochondrial nucleoid: integrating mitochondrial DNA into cellular homeostasis. Cold Spring Harbor Perspectives in Biology *5*, a011080.

Giraud, E., Van Aken, O., Ho, L.H., and Whelan, J. (2009). The transcription factor ABI4 is a regulator of mitochondrial retrograde expression of ALTERNATIVE OXIDASE 1A. Plant Physiology *150*, 1286-1296.

Gray, M.W., and Lang, B.F. (1998). Transcription in chloroplasts and mitochondria: a tale of two polymerases. Trends in Microbiology *6*, 1-3.

Greber, B.J., Bieri, P., Leibundgut, M., Leitner, A., Aebersold, R., Boehringer, D., and Ban, N. (2015). The complete structure of the 55S mammalian mitochondrial ribosome. Science *348*, 303-308.

Greenleaf, A.L., Kelly, J.L., and Lehman, I.R. (1986). Yeast RPO41 gene product is required for transcription and maintenance of the mitochondrial genome. Proceedings of the National Academy of Sciences of the United States of America *83*, 3391-3394.

Grewe, F., Herres, S., Viehover, P., Polsakiewicz, M., Weisshaar, B., and Knoop, V. (2011). A unique transcriptome: 1782 positions of RNA editing alter 1406 codon identities in mitochondrial mRNAs of the lycophyte *Isoetes engelmannii*. Nucleic Acids Research *39*, 2890-2902.

Gualberto, J.M., Mileshina, D., Wallet, C., Niazi, A.K., Weber-Lotfi, F., and Dietrich, A. (2014). The plant mitochondrial genome: dynamics and maintenance. Biochimie *100*, 107-120.

Gualberto, J.M., and Newton, K.J. (2017). Plant mitochondrial genomes: dynamics and mechanisms of mutation. Annual Review of Plant Biology *68*, 225-252.

Guillaumot, D., Lopez-Obando, M., Baudry, K., Avon, A., Rigaill, G., Falcon de Longevialle, A., Broche, B., Takenaka, M., Berthome, R., De Jaeger, G., *et al.* (2017). Two interacting PPR proteins are major Arabidopsis editing factors in plastid and mitochondria. Proceedings of the National Academy of Sciences of the United States of America *114*, 8877-8882.

Gully, B.S., Shah, K.R., Lee, M., Shearston, K., Smith, N.M., Sadowska, A., Blythe, A.J., Bernath-Levin, K., Stanley, W.A., Small, I.D., *et al.* (2015). The design and structural characterization of a synthetic pentatricopeptide repeat protein. Acta Crystallographica Section D-biological Crystallography *71*, 196-208.

Haili, N., Planchard, N., Arnal, N., Quadrado, M., Vrielynck, N., Dahan, J., des Francs-Small, C.C., and Mireau, H. (2016). The MTL1 pentatricopeptide repeat protein is required for both translation and splicing of the mitochondrial *NADH DEHYDROGENASE SUBUNIT7* mRNA in Arabidopsis. Plant Physiology *170*, 354-366.

Handa, H. (2008). Linear plasmids in plant mitochondria: peaceful coexistences or malicious invasions? Mitochondrion *8*, 15-25.

Hazle, T., and Bonen, L. (2007). Comparative analysis of sequences preceding protein-coding mitochondrial genes in flowering plants. Molecular Biology and Evolution *24*, 1101-1112.

Hecht, J., Grewe, F., and Knoop, V. (2011). Extreme RNA editing in coding islands and abundant microsatellites in repeat sequences of *Selaginella moellendorffii* mitochondria: the root of frequent plant mtDNA recombination in early tracheophytes. Genome Biology and Evolution *3*, 344-358.

Hedtke, B., Borner, T., and Weihe, A. (1997). Mitochondrial and chloroplast phage-type RNA polymerases in Arabidopsis. Science *277*, 809-811.

Hedtke, B., Borner, T., and Weihe, A. (2000). One RNA polymerase serving two genomes. EMBO Reports *1*, 435-440.

Hedtke, B., Legen, J., Weihe, A., Herrmann, R.G., and Borner, T. (2002). Six active phage-type RNA polymerase genes in *Nicotiana tabacum*. The Plant Journal *30*, 625-637.

Hedtke, B., Meixner, M., Gillandt, S., Richter, E., Börner, T., and Weihe, A. (1999). Green fluorescent protein as a marker to investigate targeting of organellar RNA polymerases of higher plants *in vivo*. The Plant Journal *17*, 557-561.

Holec, S., Lange, H., Kuhn, K., Alioua, M., Borner, T., and Gagliardi, D. (2006). Relaxed transcription in Arabidopsis mitochondria is counterbalanced by RNA stability control mediated by polyadenylation and polynucleotide phosphorylase. Molecular Cell Biology *26*, 2869-2876.

Homble, F., Krammer, E.M., and Prevost, M. (2012). Plant VDAC: facts and speculations. Biochimica et Biophysica Acta *1818*, 1486-1501.

Hooper, C.M., Castleden, I.R., Tanz, S.K., Aryamanesh, N., and Millar, A.H. (2017). SUBA4: the interactive data analysis centre for Arabidopsis subcellular protein locations. Nucleic Acids Research *45*, D1064-D1074.

Houtkooper, R.H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R.W., and Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. Nature *497*, 451-457.

Huang, S., Taylor, N.L., Whelan, J., and Millar, A.H. (2009). Refining the definition of plant mitochondrial presequences through analysis of sorting signals, N-terminal modifications, and cleavage motifs. Plant Physiology *150*, 1272-1285.

Ito, M., Takahashi, H., Sawasaki, T., Ohnishi, K., Hikichi, Y., and Kiba, A. (2014). Novel type of adenylyl cyclase participates in tabtoxinine-beta-lactam-induced cell death and occurrence of wildfire disease in *Nicotiana benthamiana*. Plant Signaling & Behavior *9*, e27420.

Itoh, Y., Naschberger, A., Mortezaei, N., Herrmann, J.M., and Amunts, A. (2020). Analysis of translating mitoribosome reveals functional characteristics of translation in mitochondria of fungi. Nature Communications *11*, 5187.

Ivanova, A., Law, S.R., Narsai, R., Duncan, O., Lee, J.H., Zhang, B., Van Aken, O., Radomiljac, J.D., van der Merwe, M., Yi, K., *et al.* (2014). A functional antagonistic relationship between auxin and mitochondrial retrograde signaling regulates *Alternative Oxidase 1a* expression in Arabidopsis. Plant Physiology *165*, 1233-1254.

Kacprzak, S.M., Dahlqvist, A., and Van Aken, O. (2020). The transcription factor ANAC017 is a key regulator of mitochondrial proteotoxic stress responses in plants. Philosophical transactions of the Royal Society of London Series B, Biological Sciences *375*, 20190411.

Kasahara, M., Suetsugu, N., Urano, Y., Yamamoto, C., Ohmori, M., Takada, Y., Okuda, S., Nishiyama, T., Sakayama, H., Kohchi, T., *et al.* (2016). An adenylyl cyclase with a phosphodiesterase domain in basal plants with a motile sperm system. Scientific Reports *6*, 39232.

Ke, J., Chen, R.Z., Ban, T., Zhou, X.E., Gu, X., Tan, M.H., Chen, C., Kang, Y., Brunzelle, J.S., Zhu, J.K., *et al.* (2013). Structural basis for RNA recognition by a dimeric PPR-protein complex. Nature Structural & Molecular Biology *20*, 1377-1382.

Keech, O., Dizengremel, P., and Gardeström, P. (2005). Preparation of leaf mitochondria from *Arabidopsis thaliana*. Physiologia Plantarum *124*, 403-409.

Kessler, F., and Longoni, P. (2019). How chloroplasts protect themselves from unfolded proteins. Elife *8*, e51430.

Khan, K., and Van Aken, O. (2022). The colonization of land was a likely driving force for the evolution of mitochondrial retrograde signalling in plants. Journal of Experimental Botany *73*, 7182-7197.

Kindgren, P., Kremnev, D., Blanco, N.E., de Dios Barajas Lopez, J., Fernandez, A.P., Tellgren-Roth, C., Kleine, T., Small, I., and Strand, A. (2012). The plastid redox insensitive 2 mutant of Arabidopsis is impaired in PEP activity and high light-dependent plastid redox signalling to the nucleus. The Plant Journal *70*, 279-291.

Klusch, N., Senkler, J., Yildiz, O., Kuhlbrandt, W., and Braun, H.P. (2021). A ferredoxin bridge connects the two arms of plant mitochondrial complex I. The Plant Cell *33*, 2072-2091.

Kmiec, B., Teixeira, P.F., and Glaser, E. (2014). Shredding the signal: targeting peptide degradation in mitochondria and chloroplasts. Trends in Plant Science *19*, 771-778.

Knoop, V. (2011). When you can't trust the DNA: RNA editing changes transcript sequences. Cellular and Molecular Life Sciences *68*, 567-586.

Kobayashi, Y., Dokiya, Y., Kumazawa, Y., and Sugita, M. (2002). Non-AUG translation initiation of mRNA encoding plastid-targeted phage-type RNA polymerase in *Nicotiana sylvestris*. Biochemical and Biophysical Research Communications *299*, 57-61.

Kobayashi, Y., Dokiya, Y., and Sugita, M. (2001). Dual targeting of phage-type RNA polymerase to both mitochondria and plastids is due to alternative translation initiation in single transcripts. Biochemical and Biophysical Research Communications *289*, 1106-1113.

Krause, F., Reifschneider, N.H., Vocke, D., Seelert, H., Rexroth, S., and Dencher, N.A. (2004). "Respirasome"-like supercomplexes in green leaf mitochondria of spinach. The Journal of Biological Chemistry *279*, 48369-48375.

Kuhn, K., Obata, T., Feher, K., Bock, R., Fernie, A.R., and Meyer, E.H. (2015). Complete mitochondrial complex I deficiency induces an up-regulation of respiratory fluxes that is abolished by traces of functional complex I. Plant Physiology *168*, 1537-1549.

Kuhn, K., Richter, U., Meyer, E.H., Delannoy, E., de Longevialle, A.F., O'Toole, N., Borner, T., Millar, A.H., Small, I.D., and Whelan, J. (2009). Phage-type RNA polymerase RPOTmp performs gene-specific transcription in mitochondria of *Arabidopsis thaliana*. The Plant Cell *21*, 2762-2779.

Kuhn, K., Weihe, A., and Borner, T. (2005). Multiple promoters are a common feature of mitochondrial genes in Arabidopsis. Nucleic Acids Research *33*, 337-346.

Kummer, E., and Ban, N. (2021). Mechanisms and regulation of protein synthesis in mitochondria. Nature Reviews Molecular Cell Biology *22*, 307-325.

Kummer, E., Leibundgut, M., Rackham, O., Lee, R.G., Boehringer, D., Filipovska, A., and Ban, N. (2018). Unique features of mammalian mitochondrial translation initiation revealed by cryo-EM. Nature *560*, 263-267.

Kuzmenko, A., Derbikova, K., Salvatori, R., Tankov, S., Atkinson, G.C., Tenson, T., Ott, M., Kamenski, P., and Hauryliuk, V. (2016). Aim-less translation: loss of *Saccharomyces cerevisiae* mitochondrial translation initiation factor mIF3/Aim23 leads to unbalanced protein synthesis. Scientific Reports *6*, 18749.

Kwasniak-Owczarek, M., Tomal, A., and Janska, H. (2022). Assessment of protein synthesis in mitochondria isolated from rosette leaves and liquid culture seedlings of Arabidopsis. Methods in Molecular Biology *2363*, 183-197.

Lambowitz, A.M., and Zimmerly, S. (2004). Mobile group II introns. Annual Reviews of Genetics *38*, 1-35.

Liere, K., and Börner, T. (2011). Transcription in plant mitochondria. In Plant Mitochondria, F. Kempken, ed. (Springer, New York, NY), pp. 85-105.

Lightowlers, R.N., and Chrzanowska-Lightowlers, Z.M. (2013). Human pentatricopeptide proteins: only a few and what do they do? RNA Biology *10*, 1433-1438.

Lin, Y.F., Schulz, A.M., Pellegrino, M.W., Lu, Y., Shaham, S., and Haynes, C.M. (2016). Maintenance and propagation of a deleterious mitochondrial genome by the mitochondrial unfolded protein response. Nature *533*, 416-419.

Lippok, B., Brennicke, A., and Wissinger, B. (1994). Differential RNA editing in closely related introns in *Oenothera* mitochondria. Molecular and General Genetics *243*, 39-46.

Llamas, E., Pulido, P., and Rodriguez-Concepcion, M. (2017). Interference with plastome gene expression and Clp protease activity in Arabidopsis triggers a chloroplast unfolded protein response to restore protein homeostasis. PLoS Genetics *13*, e1007022.

Logan, D.C. (2006). Plant mitochondrial dynamics. Biochimica et Biophysica Acta *1763*, 430-441.

Logan, D.C. (2012). Mitochondrial dynamics: when form meets function. Available at: http://6e.plantphys.net/essay12.03.html [Accessed 4th January 2023].

Lurin, C., Andres, C., Aubourg, S., Bellaoui, M., Bitton, F., Bruyere, C., Caboche, M., Debast, C., Gualberto, J., Hoffmann, B., *et al.* (2004). Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. The Plant Cell *16*, 2089-2103.

Maclean, A.E., Hertle, A.P., Ligas, J., Bock, R., Balk, J., and Meyer, E.H. (2018). Absence of complex I is associated with diminished respiratory chain function in European mistletoe. Current Biology *28*, 1614-1619 e1613.

Maldonado, M., Guo, F., and Letts, J.A. (2021). Atomic structures of respiratory complex III_2, complex IV, and supercomplex III_2-IV from vascular plants. Elife *10*, e62047.

Maldonado, M., Padavannil, A., Zhou, L., Guo, F., and Letts, J.A. (2020). Atomic structure of a mitochondrial complex I intermediate from vascular plants. Elife *9*, e56664.

Marechal-Drouard, L., Kumar, R., Remacle, C., and Small, I. (1996). RNA editing of larch mitochondrial $tRNA^{His}$ precursors is a prerequisite for processing. Nucleic Acids Research *24*, 3229-3234.

Marechal-Drouard, L., Ramamonjisoa, D., Cosset, A., Weil, J.H., and Dietrich, A. (1993). Editing corrects mispairing in the acceptor stem of bean and potato mitochondrial phenylalanine transfer RNAs. Nucleic Acids Research *21*, 4909-4914.

Martin, W.F., Garg, S., and Zimorski, V. (2015). Endosymbiotic theories for eukaryote origin. Philosophical transactions of the Royal Society of London Series B, Biological Sciences *370*, 20140330.

Masters, B.S., Stohl, L.L., and Clayton, D.A. (1987). Yeast mitochondrial RNA polymerase is homologous to those encoded by bacteriophages T3 and T7. Cell *51*, 89-99.

McDonald, A.E., Vanlerberghe, G.C., and Staples, J.F. (2009). Alternative oxidase in animals: unique characteristics and taxonomic distribution. Journal of Experimental Botany *212*, 2627-2634.

Michel, F., Umesono, K., and Ozeki, H. (1989). Comparative and functional anatomy of group II catalytic introns - a review. Gene *82*, 5-30.

Millar, A.H., Eubel, H., Jansch, L., Kruft, V., Heazlewood, J.L., and Braun, H.P. (2004). Mitochondrial cytochrome *c* oxidase and succinate dehydrogenase complexes contain plant specific subunits. Plant Molecular Biology *56*, 77-90.

Millar, A.H., Siedow, J.N., and Day, D. (2015). Respiration and photorespiration. In Biochemistry and Molecular Biology of Plants, 2nd edition, B.B. Buchanan et al., eds. (John Wiley and Sons), pp. 610-655.

Millen, R.S., Olmstead, R.G., Adams, K.L., Palmer, J.D., Lao, N.T., Heggie, L., Kavanagh, T.A., Hibberd, J.M., Gray, J.C., Morden, C.W., *et al.* (2001). Many parallel losses of infA from chloroplast DNA during angiosperm evolution with multiple independent transfers to the nucleus. The Plant Cell *13*, 645-658.

Millerd, A., Bonner, J., Axelrod, B., and Bandurski, R. (1951). Oxidative and phosphorylative activity of plant mitochondria. Proceedings of the National Academy of Sciences of the United States of America *37*, 855-862.

Møller, I.M. (2001). Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual Review of Plant Physiology and Plant Molecular Biology *52*, 561-591.

Møller, I.M. (2016). What is hot in plant mitochondria? Physiologia Plantarum *157*, 256-263.

Møller, I.M., Rasmusson, A.G., and Van Aken, O. (2021). Plant mitochondria - past, present and future. The Plant Journal *108*, 912-959.

Møller, I.M., and Sweetlove, L.J. (2010). ROS signalling – specificity is required. Trends in Plant Science *15*, 370-374.

Montoya, J., Ojala, D., and Attardi, G. (1981). Distinctive features of the 5'-terminal sequences of the human mitochondrial mRNAs. Nature *290*, 465-470.

Moullan, N., Mouchiroud, L., Wang, X., Ryu, D., Williams, E.G., Mottis, A., Jovaisaite, V., Frochaux, M.V., Quiros, P.M., Deplancke, B., *et al.* (2015). Tetracyclines disturb mitochondrial function across eukaryotic models: a call for caution in biomedical research. Cell Reports *10*, 1681-1691.

Moutinho, A., Hussey, P.J., Trewavas, A.J., and Malho, R. (2001). cAMP acts as a second messenger in pollen tube growth and reorientation. Proceedings of the National Academy of Sciences of the United States of America *98*, 10481-10486.

Mower, J.P. (2020). Variation in protein gene and intron content among land plant mitogenomes. Mitochondrion *53*, 203-213.

Murphy, M.P. (2009). How mitochondria produce reactive oxygen species. The Biochemical Journal *417*, 1-13.

Myouga, F., Hosoda, C., Umezawa, T., Iizumi, H., Kuromori, T., Motohashi, R., Shono, Y., Nagata, N., Ikeuchi, M., and Shinozaki, K. (2008). A heterocomplex of iron superoxide dismutases defends chloroplast nucleoids against oxidative stress and is essential for chloroplast development in Arabidopsis. The Plant Cell *20*, 3148-3162.

Negruk, V.I., Eisner, G.I., Redichkina, T.D., Dumanskaya, N.N., Cherny, D.I., Alexandrov, A.A., Shemyakin, M.F., and Butenko, R.G. (1986). Diversity of *Vicia faba* circular mtDNA in whole plants and suspension cultures. Theoretical and Applied Genetics *72*, 541-547.

Ng, S., De Clercq, I., Van Aken, O., Law, S.R., Ivanova, A., Willems, P., Giraud, E., Van Breusegem, F., and Whelan, J. (2014). Anterograde and retrograde regulation of nuclear genes encoding mitochondrial proteins during growth, development, and stress. Molecular Plant *7*, 1075-1093.

Ng, S., Ivanova, A., Duncan, O., Law, S.R., Van Aken, O., De Clercq, I., Wang, Y., Carrie, C., Xu, L., Kmiec, B., *et al.* (2013). A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in Arabidopsis. The Plant Cell *25*, 3450-3471.

Nguyen, T.T., Planchard, N., Dahan, J., Arnal, N., Balzergue, S., Benamar, A., Bertin, P., Brunaud, V., Dargel-Graffin, C., Macherel, D., *et al.* (2021). A case of gene fragmentation in plant mitochondria fixed by the selection of a compensatory restorer of fertility-like PPR gene. Molecular Biology and Evolution *38*, 3445-3458.

Noeske, J., Wasserman, M.R., Terry, D.S., Altman, R.B., Blanchard, S.C., and Cate, J.H. (2015). High-resolution structure of the *Escherichia coli* ribosome. Nature Structural & Molecular Biology *22*, 336-341.

O'Leary, B.M., and Plaxton, W.C. (2016). Plant Respiration. In eLS, pp. 1-11.

Oda, K., Yamato, K., Ohta, E., Nakamura, Y., Takemura, M., Nozato, N., Akashi, K., Kanegae, T., Ogura, Y., Kohchi, T., *et al.* (1992). Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA: A primitive form of plant mitochondrial genome. Journal of Molecular Biology *223*, 1-7.

Oldenburg, D.J., and Bendich, A.J. (1996). Size and structure of replicating mitochondrial DNA in cultured tobacco cells. The Plant Cell *8*, 447-461.

Oldenkott, B., Yamaguchi, K., Tsuji-Tsukinoki, S., Knie, N., and Knoop, V. (2014). Chloroplast RNA editing going extreme: more than 3400 events of C-to-U editing in the chloroplast transcriptome of the lycophyte *Selaginella uncinata*. RNA *20*, 1499-1506.

Olyslaegers, G., and Verbelen, J.-P. (1998). Improved staining of F-actin and co-localization of mitochondria in plant cells. Journal of Microscopy *192*, 73-77.

Palade, G.E. (1952). The fine structure of mitochondria. The Anatomical Record *114*, 427-451.

Palmfeldt, J., and Bross, P. (2017). Proteomics of human mitochondria. Mitochondrion *33*, 2-14.

Parsons, D.F., Bonner Jr, W.D., and Verboon, J.G. (1965). Electron microscopy of isolated plant mitochondria and plastids using both the thin-section and negative-staining techniques. Canadian Journal of Botany *43*, 647-655.

Prokisch, H., Scharfe, C., Camp, D.G., 2nd, Xiao, W., David, L., Andreoli, C., Monroe, M.E., Moore, R.J., Gritsenko, M.A., Kozany, C., *et al.* (2004). Integrative analysis of the mitochondrial proteome in yeast. PLoS Biology *2*, e160.

Pulido, P., Llamas, E., Llorente, B., Ventura, S., Wright, L.P., and Rodriguez-Concepcion, M. (2016). Specific Hsp100 chaperones determine the fate of the first enzyme of the plastidial isoprenoid pathway for either refolding or degradation by the stromal Clp protease in Arabidopsis. PLoS Genetics *12*, e1005824.

Ramrath, D.J.F., Niemann, M., Leibundgut, M., Bieri, P., Prange, C., Horn, E.K., Leitner, A., Boehringer, D., Schneider, A., and Ban, N. (2018). Evolutionary shift toward protein-based architecture in trypanosomal mitochondrial ribosomes. Science *362*, 6413.

Rao, R.S., Salvato, F., Thal, B., Eubel, H., Thelen, J.J., and Møller, I.M. (2017). The proteome of higher plant mitochondria. Mitochondrion *33*, 22-37.

Rasmusson, A.G., Soole, K.L., and Elthon, T.E. (2004). Alternative NAD(P)H dehydrogenases of plant mitochondria. Annual Review of Plant Biology *55*, 23-39.

Roger, A.J., Munoz-Gomez, S.A., and Kamikawa, R. (2017). The origin and diversification of mitochondria. Current Biology *27*, R1177-R1192.

Rolland, S.G., Schneid, S., Schwarz, M., Rackles, E., Fischer, C., Haeussler, S., Regmi, S.G., Yeroslaviz, A., Habermann, B., Mokranjac, D., *et al.* (2019). Compromised mitochondrial protein import acts as a signal for UPR^{mt}. Cell Reports *28*, 1659-1669 e1655.

Rugen, N., Straube, H., Franken, L.E., Braun, H.P., and Eubel, H. (2019). Complexome profiling reveals association of PPR proteins with ribosomes in the mitochondria of plants. Molecular & Cellular Proteomics *18*, 1345-1362.

Ruzvidzo, O., Dikobe, B.T., Kawadza, D.T., Mabadahanye, G.H., Chatukuta, P., and Kwezi, L. (2013). Recombinant expression and functional testing of candidate adenylate cyclase domains. Methods in Molecular Biology *1016*, 13-25.

Sabetta, W., Vannini, C., Sgobba, A., Marsoni, M., Paradiso, A., Ortolani, F., Bracale, M., Viggiano, L., Blanco, E., and de Pinto, M.C. (2016). Cyclic AMP deficiency negatively affects cell growth and enhances stress-related responses in tobacco Bright Yellow-2 cells. Plant Molecular Biology *90*, 467-483.

Sagan, L. (1967). On the origin of mitosing cells. Journal of Theoretical Biology *14*, 255-274.

Schleiff, E., and Becker, T. (2011). Common ground for protein translocation: access control for mitochondria and chloroplasts. Nature Reviews Molecular Cell Biology *12*, 48-59.

Schmidt, O., Pfanner, N., and Meisinger, C. (2010). Mitochondrial protein import: from proteomics to functional mechanisms. Nature Reviews Molecular Cell Biology *11*, 655-667.

Schmitz-Linneweber, C., and Small, I. (2008). Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends in Plant Science *13*, 663-670.

Schuster, W., Ternes, R., Knoop, V., Hiesel, R., Wissinger, B., and Brennicke, A. (1991). Distribution of RNA editing sites in *Oenothera* mitochondrial mRNAs and rRNAs. Current Genetics *20*, 397-404.

Senkler, J., Rugen, N., Eubel, H., Hegermann, J., and Braun, H.P. (2018). Absence of complex I implicates rearrangement of the respiratory chain in European mistletoe. Current Biology *28*, 1606-1613 e1604.

Senkler, J., Senkler, M., and Braun, H.P. (2017a). Structure and function of complex I in animals and plants - a comparative view. Physiologia Plantarum *161*, 6-15.

Senkler, J., Senkler, M., Eubel, H., Hildebrandt, T., Lengwenus, C., Schertl, P., Schwarzlander, M., Wagner, S., Wittig, I., and Braun, H.P. (2017b). The mitochondrial complexome of *Arabidopsis thaliana*. The Plant Journal *89*, 1079-1092.

Shen, C., Zhang, D., Guan, Z., Liu, Y., Yang, Z., Yang, Y., Wang, X., Wang, Q., Zhang, Q., Fan, S., *et al.* (2016). Structural basis for specific single-stranded RNA recognition by designer pentatricopeptide repeat proteins. Nature Communications *7*, 11285.

Shi, X., Castandet, B., Germain, A., Hanson, M.R., and Bentolila, S. (2017). ORRM5, an RNA recognition motif-containing protein, has a unique effect on mitochondrial RNA editing. Journal of Experimental Botany *68*, 2833-2847.

Shi, X., Germain, A., Hanson, M.R., and Bentolila, S. (2016). RNA Recognition Motif-Containing Protein ORRM4 broadly affects mitochondrial RNA editing and impacts plant development and flowering. Plant Physiology *170*, 294-309.

Shine, J., and Dalgarno, L. (1974). The 3'-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proceedings of the National Academy of Sciences of the United States of America *71*, 1342-1346.

Sloan, D.B. (2013). One ring to rule them all? Genome sequencing provides new insights into the "master circle" model of plant mitochondrial DNA structure. The New Phytologist *200*, 978-985.

Sloan, D.B., Alverson, A.J., Chuckalovcak, J.P., Wu, M., McCauley, D.E., Palmer, J.D., and Taylor, D.R. (2012). Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates. PLoS Biology *10*, e1001241.

Small, I.D., and Peeters, N. (2000). The PPR motif - a TPR-related motif prevalent in plant organellar proteins. Trends in Biochemical Sciences *25*, 46-47.

Small, I.D., Schallenberg-Rudinger, M., Takenaka, M., Mireau, H., and Ostersetzer-Biran, O. (2020). Plant organellar RNA editing: what 30 years of research has revealed. The Plant Journal *101*, 1040-1056.

Spang, A., Saw, J.H., Jorgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., van Eijk, R., Schleper, C., Guy, L., and Ettema, T.J.G. (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature *521*, 173-179.

Sutton, C.A., Conklin, P.L., Pruitt, K.D., and Hanson, M.R. (1991). Editing of pre-mRNAs can occur before cis- and trans-splicing in Petunia mitochondria. Molecular Cell Biology *11*, 4274-4277.

Swiezawska, B., Jaworski, K., Pawelek, A., Grzegorzewska, W., Szewczuk, P., and Szmidt-Jaworska, A. (2014). Molecular cloning and characterization of a novel adenylyl cyclase gene, HpAC1, involved in stress signaling in *Hippeastrum x hybridum*. Plant Physiology and Biochemistry/Societe Francaise de Physiologie Vegetale *80*, 41-52.

Takenaka, M., Zehrmann, A., Verbitskiy, D., Kugelmann, M., Hartel, B., and Brennicke, A. (2012). Multiple Organellar RNA Editing Factor (MORF) family proteins are required for RNA editing in mitochondria and plastids of plants. Proceedings of the National Academy of Sciences of the United States of America *109*, 5104-5109.

Tiranti, V., Savoia, A., Forti, F., D'Apolito, M.F., Centra, M., Rocchi, M., and Zeviani, M. (1997). Identification of the gene encoding the human mitochondrial RNA polymerase (h-mtRPOL) by cyberscreening of the Expressed Sequence Tags database. Human Molecular Genetics *6*, 615-625.

Tran, H.C., and Van Aken, O. (2020). Mitochondrial unfolded protein-related responses across kingdoms: similar problems, different regulators. Mitochondrion *53*, 166-177.

Trotta, A., Bajwa, A.A., Mancini, I., Paakkarinen, V., Pribil, M., and Aro, E.M. (2019). The role of phosphorylation dynamics of CURVATURE THYLAKOID 1B in plant thylakoid membranes. Plant Physiology *181*, 1615-1631.

Unseld, M., Marienfeld, J.R., Brandt, P., and Brennicke, A. (1997). The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides. Nature Genetics *15*, 57-61.

Uyttewaal, M., Mireau, H., Rurek, M., Hammani, K., Arnal, N., Quadrado, M., and Giege, P. (2008). PPR336 is associated with polysomes in plant mitochondria. Journal of Molecular Biology *375*, 626-636.

Van Aken, O., Ford, E., Lister, R., Huang, S., and Millar, A.H. (2016). Retrograde signalling caused by heritable mitochondrial dysfunction is partially mediated by ANAC017 and improves plant performance. The Plant Journal *88*, 542-558.

Van Aken, O., Pecenkova, T., van de Cotte, B., De Rycke, R., Eeckhout, D., Fromm, H., De Jaeger, G., Witters, E., Beemster, G.T., Inze, D., *et al.* (2007). Mitochondrial type-I prohibitins of *Arabidopsis thaliana* are required for supporting proficient meristem development. The Plant Journal *52*, 850-864.

Van Aken, O., and Rasmusson, A.G. (2022). Plant Mitochondria: Methods and Protocols. In New York: Humana Press.

Van Aken, O., Zhang, B., Law, S., Narsai, R., and Whelan, J. (2013). AtWRKY40 and AtWRKY63 modulate the expression of stress-responsive nuclear genes encoding mitochondrial and chloroplast proteins. Plant Physiology *162*, 254-271.

Van Gestel, K., Kohler, R.H., and Verbelen, J.P. (2002). Plant mitochondria move on F-actin, but their positioning in the cortical cytoplasm depends on both F-actin and microtubules. Journal of Experimental Botany *53*, 659-667.

Vanderauwera, S., Vandenbroucke, K., Inze, A., van de Cotte, B., Muhlenbock, P., De Rycke, R., Naouar, N., Van Gaever, T., Van Montagu, M.C., and Van Breusegem, F. (2012). AtWRKY15 perturbation abolishes the mitochondrial stress response that steers osmotic stress tolerance in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America *109*, 20113-20118.

Vanlerberghe, G.C., and McIntosh, L. (1996). Signals regulating the expression of the nuclear gene encoding Alternative Oxidase of plant mitochondria. Plant Physiology *111*, 589-595.

Vestergaard, C.L., Flyvbjerg, H., and Møller, I.M. (2012). Intracellular signaling by diffusion: can waves of hydrogen peroxide transmit intracellular information in plant cells? Frontiers in Plant Science *3*, 295.

von Heijne, G. (1986). Why mitochondria need a genome. FEBS letters *198*, 1-4.

Waltz, F., Corre, N., Hashem, Y., and Giege, P. (2020a). Specificities of the plant mitochondrial translation apparatus. Mitochondrion *53*, 30-37.

Waltz, F., and Giege, P. (2020). Striking diversity of mitochondria-specific translation processes across eukaryotes. Trends in Biochemical Sciences *45*, 149-162.

Waltz, F., Nguyen, T.T., Arrive, M., Bochler, A., Chicher, J., Hammann, P., Kuhn, L., Quadrado, M., Mireau, H., Hashem, Y., *et al.* (2019). Small is big in Arabidopsis mitochondrial ribosome. Nature Plants *5*, 106-117.

Waltz, F., Soufari, H., Bochler, A., Giege, P., and Hashem, Y. (2020b). Cryo-EM structure of the RNA-rich plant mitochondrial ribosome. Nature Plants *6*, 377-383.

Wang, X., and Auwerx, J. (2017). Systems phytohormone responses to mitochondrial proteotoxic stress. Molecular Cell *68*, 540-551 e545.

Wang, Z., and Wu, M. (2014). Phylogenomic reconstruction indicates mitochondrial ancestor was an energy parasite. PloS One *9*, e110685.

Wang, Z., and Wu, M. (2015). An integrated phylogenomic approach toward pinpointing the origin of mitochondria. Scientific Reports *5*, 7949.

Watson, Z.L., Ward, F.R., Meheust, R., Ad, O., Schepartz, A., Banfield, J.F., and Cate, J.H. (2020). Structure of the bacterial ribosome at 2 Å resolution. Elife *9*, e60482.

Weihe, A. (2004). The transcription of plant organelle genomes. In Molecular Biology and Biotechnology of Plant Organelles, H. Daniell, and C. Chase, eds. (Springer, Dordrecht), pp. 213-237.

Weihe, A., Hedtke, B., and Borner, T. (1997). Cloning and characterization of a cDNA encoding a bacteriophage-type RNA polymerase from the higher plant *Chenopodium album*. Nucleic Acids Research *25*, 2319-2325.

Whelan, J., and Murcha, M.W. (2015). Methods in Molecular Biology: Plant mitochondria. In New York: Springer.

Wirth, C., Brandt, U., Hunte, C., and Zickermann, V. (2016). Structure and function of mitochondrial complex I. Biochimica et Biophysica Acta *1857*, 902-914.

Wissinger, B., Schuster, W., and Brennicke, A. (1991). Trans splicing in *Oenothera* mitochondria: *nad1* mRNAs are edited in exon and trans-splicing group II intron sequences. Cell *65*, 473-482.

Wolfe, K.H., Li, W.H., and Sharp, P.M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proceedings of the National Academy of Sciences *84*, 9054-9058.

Yagi, Y., Hayashi, S., Kobayashi, K., Hirayama, T., and Nakamura, T. (2013). Elucidation of the RNA recognition code for pentatricopeptide repeat proteins involved in organelle RNA editing in plants. PloS One *8*, e57286.

Yan, J., Yao, Y., Hong, S., Yang, Y., Shen, C., Zhang, Q., Zhang, D., Zou, T., and Yin, P. (2019). Delineation of pentatricopeptide repeat codes for target RNA prediction. Nucleic Acids Research *47*, 3728-3738.

Yassin, A.S., Haque, M.E., Datta, P.P., Elmore, K., Banavali, N.K., Spremulli, L.L., and Agrawal, R.K. (2011). Insertion domain within mammalian mitochondrial translation initiation factor 2 serves the role of eubacterial initiation factor 1. Proceedings of the National Academy of Sciences of the United States of America *108*, 3918-3923.

Yin, P., Li, Q., Yan, C., Liu, Y., Liu, J., Yu, F., Wang, Z., Long, J., He, J., Wang, H.W., *et al.* (2013). Structural basis for the modular recognition of single-stranded RNA by PPR proteins. Nature *504*, 168-171.

Zalman, L.S., Nikaido, H., and Kagawa, Y. (1980). Mitochondrial outer membrane contains a protein producing nonspecific diffusion channels. The Journal of Biological Chemistry *255*, 1771-1774.

Zanlungo, S., Quinones, V., Moenne, A., Holuigue, L., and Jordana, X. (1995). Splicing and editing of *rps10* transcripts in potato mitochondria. Current Genetics *27*, 565-571.

Zaremba-Niedzwiedzka, K., Caceres, E.F., Saw, J.H., Backstrom, D., Juzokaite, L., Vancaester, E., Seitz, K.W., Anantharaman, K., Starnawski, P., Kjeldsen, K.U., *et al.* (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature *541*, 353-358.

Zhang, B., Carrie, C., Ivanova, A., Narsai, R., Murcha, M.W., Duncan, O., Wang, Y., Law, S.R., Albrecht, V., Pogson, B., *et al.* (2012). LETM proteins play a role in the accumulation of mitochondrially encoded proteins in *Arabidopsis thaliana* and AtLETM2 displays parent of origin effects. The Journal of Biological Chemistry *287*, 41757-41773.

Zhang, X., Ivanova, A., Vandepoele, K., Radomiljac, J., Van de Velde, J., Berkowitz, O., Willems, P., Xu, Y., Ng, S., Van Aken, O., *et al.* (2017a). The transcription factor MYB29 is a regulator of *ALTERNATIVE OXIDASE 1A*. Plant Physiology *173*, 1824-1843.

Zhang, Y., Beard, K.F.M., Swart, C., Bergmann, S., Krahnert, I., Nikoloski, Z., Graf, A., Ratcliffe, R.G., Sweetlove, L.J., Fernie, A.R., *et al.* (2017b). Protein-protein interactions and metabolite channelling in the plant tricarboxylic acid cycle. Nature Communications *8*, 15212.

Zhao, Q., Wang, J., Levichkin, I.V., Stasinopoulos, S., Ryan, M.T., and Hoogenraad, N.J. (2002). A mitochondrial specific stress response in mammalian cells. The EMBO Journal *21*, 4411-4419.

Zimmerly, S., Hausner, G., and Wu, X. (2001). Phylogenetic relationships among group II intron ORFs. Nucleic Acids Research *29*, 1238-1250.

Zubo, Y.O., Potapova, T.V., Tarasenko, V.I., Borner, T., and Konstantinov, Y.M. (2014). The rate of transcription in Arabidopsis chloroplasts depends on activity of alternative electron transfer pathway in mitochondria. Doklady Biochemistry and Biophysics *455*, 76-79.

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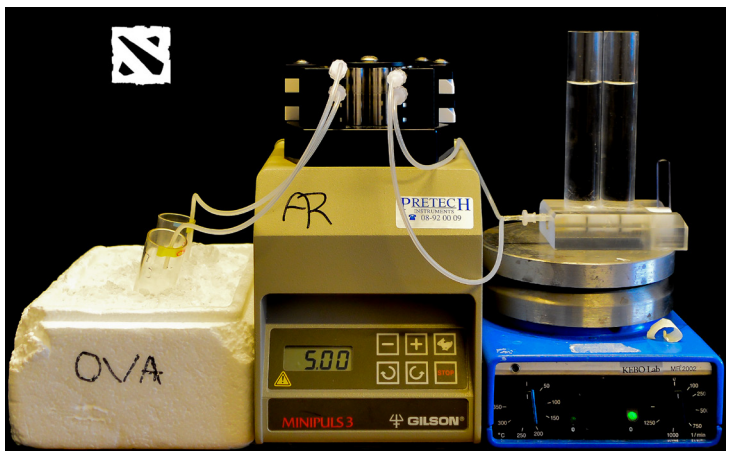

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