

Chloroplasts and Mitochondria: Similarities and Differences

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Abstract— *Eukaryotic cells contain two organelles originally derived from endosymbiotic bacteria: mitochondria and plastids (only plants). In eukaryotes, (owner mitochondria and chloroplast) ATP synthase complex is located in the inner membrane of mitochondria, and thylakoids membrane of chloroplast. ATP synthesis utilization and provision of both ADP and Pi need to be fine – tuned for optimal ATP synthase activity. Mitochondria and chloroplast have their DNA. The vast majority of mitochondrial and plastid proteins are encoded in the nucleus, synthesized by cytosolic ribosomes and subsequently imported into the organelles via active protein transport systems.*

Keywords— *ATP synthesis, Chloroplast, Mitochondria, Protein targeting.*

I. INTRODUCTION

Several proposals have been made to explain the rise of multicellular life forms. An internal environment can be created and controlled, germ cells can be protected in novel structures, and increased organismal size allows a “division of labor” among cell types. These proposals describe advantages of multicellular versus unicellular organisms at levels of organization at or above the individual cell. It have been focused on a subsequent phase of evolution, when multicellular organisms initiated the process of development that later became the more complex embryonic development found in animals and plants. The advantage here is realized at the level of the mitochondria and chloroplast [20].

Eukaryotic cells have chloroplast and mitochondria that both are membrane bound organelles. Prokaryotic cells, for example, bacteria have not chloroplast and mitochondria. Mitochondria occur in the cells of animals and plants but chloroplast only occur in the photosynthesising tissues of plants. These two organelles are best known for their roles in energy metabolism, notably respiration and photosynthesis [85].

Respiration occurs in mitochondria. Mitochondria were originally identified as the site of oxidative energy metabolism [13]. Mitochondria are also the host for enzymes of the Krebs cycle and β – oxidation of fatty acids. In today’s world mitochondria are known not only as the “power station” of the cell, but also for playing a vital role in the transmission of extra – and intracellular signals that activate reaction cascades leading to cellular senescence and programmed cell death (PCD) [104]. The discovery of a number of human diseases associated with mitochondrial dysfunctions once again brought mitochondria into the spotlight of biological research.

Chloroplasts are members of a class of plant cell organelles known as plastids that all originate from protoplastids. During plant development the protoplastids differentiate to form three major groups of plastids, the green chloroplasts, the colored chromoplasts and the colorless leucoplasts. The most abundant and important plastids are the chloroplasts. Chloroplasts harvest energy from sunlight to split water and fix carbon dioxide to produce sugars. This process called photosynthesis also converts harvested solar energy into a conserved form of energy: ATP and NADPH through a complex set of processes.

II. SYNTHESIS OF ATP IN MITOCHONDRIA AND CHLOROPLAST

As it has been mentioned earlier mitochondria and chloroplasts are best known for their roles in energy metabolism, notably respiration and photosynthesis [85].

It is clear that ATP synthesis is the central bioenergetic engine of all organisms and represents the smallest molecular motor, which was optimized in the course of evolution [17].

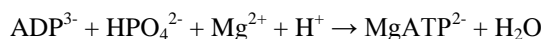
In eukaryotes, the ATP synthase complex is located in the inner membrane of mitochondria, with ATP synthesis reaction occurring on the membrane side toward matrix compartment. In plants, the enzyme is in addition localized in the thylakoid membrane of chloroplasts, with the ATP – forming – moiety facing the stroma. These topological differences between the mitochondrial and chloroplastic ATP synthases bring about two very distinct metabolic environments for ATP synthesis, where ATP utilization and provision of both ADP and Pi need to be fine–for optimal ATP synthase activity. In chloroplasts, ATP synthase receives protons from thylakoid lumen, which volume is small as compared to the mitochondrial

intermembrane space (IMS) and which pH value can drop to the values below 5 [102], while in the mitochondrial IMS it drops only slightly below 7 [11 & 12). In mitochondria, adenylates are transported through the membrane, whereas their stromal pool is self – sufficient to support chloroplastic ATP synthase; the activity of adenylate transport between chloroplast and cytosol is very low, representing 1% of activity of the triose phosphate translocator [14]. While generation of proton electrochemical potential became the central theory in the chemiosmotic concept of ATP synthase operation [84], the optimal conditions of delivery of ADP and phosphate were analyzed in the concept of thermodynamic buffering [59 & 60], underlying the importance of auxiliary buffering enzymes such as adenylate kinase (AK) and creatine kinase in provision of the stable flux of ADP to ATP synthase. This theory was extended in relation to operation of AK in the IMS of mitochondria [15]. The energy balance of photosynthetic cells is provided by equilibration of adenylate levels by chloroplasts and mitochondria (and the cytosol) and the role of AK in this equilibration appears to be important.

III. MAGNESIUM AND THE ROLE OF ITS IN ATP SYNTHESIS

Before any more explanation and commentary about mitochondria and chloroplast and their differences and similarities also ATP synthase it is better to have a statement about the role of magnesium in ATP synthase by these two important and crucial organelle.

The role of magnesium in ATP synthesis is underlined not only by the fact that MgATP is the actual product of the reaction, but also, as we show below that Mg^{2+} acts as a separate substrate in the ATP synthase reaction under physiological conditions, ADP can exist both in a free and Mg - bound state, and this dual chemical capacity determines a way that magnesium becomes a part of “energy charge”. The Mg^{2+} pool is not less important than protons and it is generated (kept stable) by the AK reaction, which determines the equilibrium value of Mg^{2+} in cellular compartments [16]. This results in efficient regulation of Mg - dependent enzymes and, one such enzyme is ATP synthase. The rotation mechanism of ATP synthase was suggested by Boyer (1989) [86] and then it was demonstrated empirically [51]. The role of proton translocation consists in deforming an open catalytic site to increase the affinity for ADP and P_i , which then bind and pass through the transition state, yielding tightly bound ATP in one binding change. ADP binding appears to be a key parameter controlling rotation during synthesis, while MgADP is inhibiting. The essential role of Mg^{2+} in ATP synthase catalysis was recently established in works of scientists. Previously it was assumed that the substrate of ATP synthase was MgADP [87]. Later studies, however, have indicated that it is free ADP in the presence of magnesium which represents the real substrate. It was shown [105] that inhibition of catalysis by vanadate in the presence of MgADP could be substituted by the Mg - vanadate complex indicating that Mg^{2+} plays a pivotal role in transition state formation during ATP synthesis. This state involves the preferential coordination with P_i and the repositioning of the P – loop to bring the nonpolar alanine 158 into the catalytic pocket, which is achieved in the presence of Mg^{2+} [35]. According to these more recent data, it is correct to consider ADP_{free} rather than MgADP as a true substrate, rather than MgADP as a true substrate, while Mg^{2+} acts independently. Therefore, the substrates of ATP synthase are ADP_{free} , $P_{i,free}$, Mg^{2+}_{free} and H^+ , while the product is MgATP. The reaction can be presented by the following equation (one proton is the substrate, whereas other protons have catalytic function):



The difference in pH between matrix and IMS results in deprotonation of phosphate and of ADP in the matrix side, facilitating the Mg - dependent mechanism. Magnesium participating in ATP synthase catalysis exhibits a profound catalytic effect as shown by [10]. The activity with ^{25}Mg , which has magnetic isotopic nucleus, is two to three times higher than with ^{24}Mg or ^{26}Mg isotopes, having spinless non – magnetic isotopic nuclei. This suggests that the ATP synthesis is a spin – dependent ion – radical process. It implies a reversible electron transfer from the terminal phosphate of ADP^{3-} to Mg^{+2} generating ion – radical pair with singlet and triplet spin states. The yields of ATP along the singlet and triplet channels are controlled by hyperfine coupling of unpaired electron in $^{25}Mg^{+2}$ ion with magnetic nucleus. The magnesium bivalent cation transforms the protein molecule mechanics into a chemical reaction [10]. Although this mechanism was suggested for the mitochondrial ATP synthase, potentially it can be generalized for all ATP synthases including the chloroplast and even for other Mg - dependent enzymes. Mg^{2+} uptake by mitochondria and its efflux are mediated by a channel or transporter responding to changes in membrane potential, in particular in pH gradient [40 & 68]. The concentration of Mg^{2+} in the mitochondrial matrix depends on P_i which interacts strongly with Mg^{+2} to decrease its concentration and, in the absence of external Mg^{+2} promotes respiration – dependent Mg^{+2} efflux and its decrease in the matrix to very low levels [41]. The uptake of P_i by respiring mitochondria converts ΔpH to $\Delta\Psi$ and provides additional Mg - binding sites permitting its large accumulations. This means that P_i , in addition to AK, buffers Mg^{+2} concentration and this buffering is important in the matrix of plant mitochondria, where AK is absent. While Mg^{2+} is an important catalyst (and substrate) of ATP synthesis and

many other processes, the changes of its content result in significant shifts in bioenergetic state of the cell. These Mg^{2+} dependent shifts strongly affect Ca^{2+} concentration in the IMS [89 & 4].

Ca^{2+} uptake by mitochondria is inhibited by Mg^{2+} via a mixed type inhibition in the process of multistate catalytic binding and interconversion, in which phosphate is also involved as a regulator [90]. A frequently observed increase in $[Ca^{2+}]$ under stress conditions is, therefore, mediated by fluctuations in Mg^{2+} and results in activation of Ca^{2+} dependent stress induced enzymes. Thus the signaling and metabolic roles of Ca^{2+} are under control of magnesium, phosphate and adenylate energy charge that establishes equilibrium Mg^{2+} concentration

IV. DNA IN MICHONDRIA AND CHLOROPLAST

The DNA in both mitochondria and chloroplasts can be extremely unstable, as illustrated by the following examples.

- (i) The half – life of rat mitochondrial DNA (mtDNA), in days, is 6.7 for heart, 9.4 for liver, 10.4 for kidney, and 31 for brain [73],
- (ii) In the single – celled alga *Euglena*, the half – lives for chloroplast DNA (cpDNA) and mtDNA are 1.6 and 1.8 cell doublings, respectively, but nuclear DNA is so stable that turnover could not be detected (56 & 80).
- (iii) Two days after sowing mung bean seeds, the mtDNA in dark – grown seedlings turns over entirely in 24 hours [50].
- (iv) The half – life of mtDNA in yeast is ~ 4 hours (for a mutant defective in the mtDNA polymerase gamma) [106]
- (v) Light triggers the degradation of DNA in maize chloroplasts [10]. Four hours after exposing 10 – day – old dark grown seedlings to light, the leaf begins to green, and the average DNA content per chloroplast decreases to 54% by hour 6 and 9% by hour 24 .
- (vi) During 6 stages of development of maize leaf tissue, the size and structural integrity of cpDNA decreases progressively from branched molecules of multigenomic size in the basal meristem of seedlings to fragments of subgenomic size in adult plants, as observed in moving pictures of individual ethidium – stained DNA molecules [36].

A similar degradative progression of individual cpDNA molecules is observed during leaf development for tobacco and the legume *Medicago truncatula* [57] and *Arabidopsis* [18]. (vii) In fully expanded leaves of adult plants of *Arabidopsis* [18 & 19] and maize, [36] more than half the chloroplasts contain no detectable DNA. How can we explain this remarkable instability of organellar DNA? It is suggested that the ROS generated during electron transport that accompanies oxidative phosphorylation and photosynthesis leads to oxidative stress and extensive damage to the DNA [20]. For *Euglena*, repair of the mtDNA and cpDNA is the only option because it is a unicellular organism. For dark – grown mung bean seedlings, repair again is the only option for mtDNA since respiration must provide the energy for this aerobic organism. The mtDNA is so extensively damaged that it turns over completely in one day. For a light – grown plant, however, there is another option. If some of the organellar DNA can be sequestered in quiescent germ line cells, the highly damaged organellar DNA in somatic cells can be left unrepaired; it is eventually degraded and its nucleotides are recycled for their nutritive value [23]. Similarly, oxidatively damaged mtDNA in active somatic cells can either be repaired or abandoned, as long as undamaged mtDNA is retained in quiet germ line cells. For the mesozoan *Dicyema japonicum*, mtDNA is retained in "stem" mitochondria of germ cells, but mtDNA is undetectable in most somatic cells of mature larvae and adults, a result of either dilution without replication [49] or, it is suggested that, abandonment and degradation of mtDNA [20].

4.1 DNA damage and repair in mitochondria and chloroplasts

From an evolutionary perspective, the only objective for an organism is to replicate its DNA and pass it on to the next generation. Unintended alterations in chromosomal DNA molecules can arise in various ways, including DNA polymerase errors and changes to the DNA template from internal (ROS, for example) and external (radiation, for example) sources. Only internal sources because these can be modulated during development. Changes in DNA can be perceived and acted upon as needed during development. Changes in DNA can occur as nucleotide alterations, insertions/deletions, inversions, and DNA strand breaks. Those lesions recognized as "damage" can be either repaired or removed by degrading the DNA [42].

Most information on the repair of mtDNA comes from yeast and somatic cells of mammals [57; 72; 63; 96; 54], whereas very little is known about mtDNA repair in plants or about cpDNA repair [24; 82; 25; 5]. A detected change in mtDNA is the result of both the rate of damage and the efficiency of correcting the damage. The power of genetics can sometimes be used to study each of these parameters separately in yeast. Overall, two conclusions seem generally supported. First, most DNA damage in mitochondria is due to oxidative damage, as may be expected for the site of respiration, and base excision repair

(BER) is the main way to rectify oxidative damage [72; 71; 61]. If BER fails, human mtDNA molecules containing the damage are usually degraded and base substitution (point mutation) is thus avoided [28]. Degradation of damaged DNA molecules to avoid mutation is feasible for the high – genome – copy cytoplasmic organelles, but not for the diploid nucleus. Such degradation would mask a higher rate of damage in the organelles than in the nucleus. The second conclusion is that the capacity to repair stress – induced DNA damage is lower in mitochondria than the nucleus, because mitochondria are the principal site of ROS production, employ fewer repair processes than do nuclei, or lack protective histones on their mtDNA molecules [75; 72; 61; 45; 53; 83]. Damage to organellar DNA is indicated by a rapidly increasing mutation rate (point mutations per kb of mtDNA) as mouse tissues age [71], an accumulation of mtDNA deletions with age in humans, monkeys, and rodents [75 & 62], and a decline in structural integrity of cpDNA molecules as leaves develop. Thus, it would be advantageous to shelter organellar DNA before tissues mature in the adult.

V. GENES IN MITOCHONDRIA AND CHLOROPLAST (INHERITANCE: LAWS AND MECHANISMS)

The literature on the inheritance of genes in mitochondria and chloroplasts (hereafter, organelle genes) has changed, grown, and advanced tremendously [30].

Some of the most exciting advances since the previous reviews have been made in understanding the molecular and cellular mechanisms of organelle division and distribution between daughter cells (partitioning) in yeast, animals, and plants; genetic studies of segregation and within generation selection of mitochondrial genes in mammals and *Drosophila*; and the controversial subject of mitochondrial bottlenecks in mammals. Other exciting discoveries dealt with the mechanisms of uniparental inheritance in *Chlamydomonas* and mammals, and a controversy over whether there is a low level of biparental inheritance and recombination in humans. The growing excitement about mitochondrial genetics in humans and mammals has been driven in large part by their application to human diseases caused by mitochondrial mutations, and by the widespread use of mitochondrial genes to study the population genetics and evolution of humans and other animals. These subjects have also been reviewed in the past three years, but mainly as separate subjects and not in the context of organelle heredity in general.

5.1 *Chlamydomonas* Chloroplasts

5.1.1 Vegetative Segregation is rapid in *Chlamydomonas* chloroplasts

Chlamydomonas reinhardtii has been used extensively for chloroplast genetics since the pioneering studies of Sager [93 & 94] and Gillham [77 & 78]. In contrast to the plants discussed above, *Chlamydomonas* cells have one chloroplast, which divides into two equal parts just before the cell divides; consequently, vegetative segregation cannot be explained by the partitioning of chloroplasts. Most data come from crosses of antibiotic – resistant by sensitive clones. Vegetative segregation can be studied in vegetative zygotes, which divide by mitosis instead of meiosis, or in the meiotic and early mitotic divisions of the small percentage of zygospores that show biparental inheritance. In either case, segregation is complete within a few cell generations. This is much too fast to be accounted for by random partitioning of the approximately 50 – 100 genomes.

5.1.2 Genome partitioning is probably stochastic but not strictly random

One possible explanation for rapid segregation is that when the two gamete chloroplasts fuse in the zygote, the plastid genomes from the parents tend to remain in different parts of the chloroplast and consequently tend to segregate together rather than strictly randomly [67]. The chloroplast genomes are grouped in about 5 – 15 nucleoids, and it is possible that the 10 or more genomes in each nucleoid tend to be replication products of one genome. In other words, genome partitioning is stochastic but not strictly random; like molecules tend to segregate together because they are joined in nucleoids and/or the nucleoids from the gametes are not completely mixed in the zygote.

5.1.3 Genome replication is stochastic

Different *Chlamydomonas* zygotes from the same mating give rise to clones with very different frequencies of alleles from the two parents. Some zygote clones are uniparental, with organelle genomes from only one parent or the other. Frequency distributions of gene frequencies in a large number of zygote clones bear a striking resemblance to the gene frequency distributions of Mendelian populations undergoing random genetic drift [33]. When the mitotic division of vegetative zygotes [66], or the meiotic divisions of zygospores [22], was delayed for a time by starvation, the variance in gene frequencies increased and more uniparental zygote clones were produced. These data suggested that plastid genomes continue to replicate during starvation and that replication is stochastic, with some genomes replicating more often than others by chance. The result is that gene frequencies within cells undergo stochastic changes, which is called intracellular

random drift by analogy to random drift of nuclear gene frequencies in populations of organisms [30]. Stochastic replication by itself will not completely eliminate an allele from a cell or clone, but may reduce it to a frequency too low to detect. Alternatively, there may be some degradation of organelle DNA molecules, which will then be replaced by additional replications of other molecules (turnover). Note that the stochastic replication of genomes, and the stochastic partitioning of genomes into daughter organelles when an organelle divides, can also explain how a mutant genome becomes homoplasmic in plant plastids.

5.2 Yeast Mitochondria

Much has been learned about organelle heredity from the study of another model genetic system, mitochondrial genes in budding or baker's yeast (*Saccharomyces cerevisiae*). The best markers are mutant genes conferring antibiotic resistance; respiration – deficient mutants (petites) are also used but their inheritance is strongly affected by selection. When heteroplasmic zygotes are produced by mating yeast strains that differ in one or more mitochondrial alleles, the majority of diploid progeny are homoplasmic after no more than 20 cell generations. Strictly random partitioning could only explain this rate of segregation if there were no more than 2 to 5 segregating units [30]. This is much smaller than the number of mtDNA molecules in diploid cells [approximately 100] and slightly smaller than the number of nucleoids. Mitochondria from the two parents cannot be the segregating units because they fuse in the zygote. Consequently, vegetative segregation in yeast must be explained by some combinations of the same factors that were invoked above for chloroplast genes in *Chlamydomonas*: (a) partitioning of genes that is stochastic but not strictly random, with similar molecules tending to remain together; (b) stochastic replication; or (c) turnover. There is experimental evidence only for the first two processes, but it is likely that all three are involved.

5.3 Mitochondrial fusion and fission

A yeast cell may contain a single large mitochondrial network, or a network plus a few small separate mitochondria, or many small discrete mitochondria, depending on its physiological state. Yeast mitochondrial genomes undergo multiple pairings with recombination in zygotes, showing that genomes from the two parents can interact extensively. Considerable progress has been made in understanding mitochondrial fusion and fission in yeast. Fission is accomplished by the dynamin system in yeast and animals [74]. The dynamin Dnm1p localizes to mitochondria at division sites and tips and is required for normal mitochondrial morphology. Mitochondrial fusion requires the *fzo1* (fuzzy onion) gene, a homologue of the fuzzy onion gene that is required for mitochondrial fusion in *Drosophila*. In yeast, normal mitochondrial morphology requires a balance between the activities of Dnm1p and Fzo1 [52].

5.4 Bud position effects: non random partitioning

Early models of mitochondrial gene inheritance in yeast assumed that fusion was so frequent that a cell is effectively a single population of freely interacting genomes. That this could not be strictly true was demonstrated by pedigree studies of zygotes [30; 34; 91], which showed that (a) when the first bud comes from one end of the zygote, the majority of its mitochondrial genes come from the parent which formed that end of the zygote; and (b) buds that arise from the neck of the zygote receive markers from both parents, as well as a higher frequency of recombinant genotypes. This indicates that the mixing of mitochondrial genomes from the two parents is incomplete when the first bud is formed; later buds usually include markers from both parents, indicating more complete mixing. This interpretation was verified by showing that labeled mtDNA from one parent failed to enter the opposite side of the zygote until sometime after the first bud was formed, although it did enter first center buds [58]. The mitochondrial membranes from the two parents fused quickly, so delayed mixing of mtDNA was not due to delayed mitochondrial fusion; evidently, the movement of mtDNA across the zygote involves a different mechanism from the movement of mitochondria. Mitochondrial proteins also move more quickly through the mitochondrial network than does mtDNA [88; 58; 69].

5.5 Mitochondrial movement from mother to bud

Because *Saccharomyces* cells bud rather than undergoing binary fission, a mechanism is required to move mitochondria and their genes from the mother into the growing bud. The experimental studies of this process have been reviewed [74]. Mitochondria are actively transported from the mother cell into the bud, where they are immobilized at the tip of the bud until cytokinesis is complete. Mitochondria probably move along actin filaments by a motor that depends on actin polymerization [103], and movement also requires intermediate filaments encoded by the MDM gene [99]. It is not surprising that a mechanism evolved which ensures that buds receive at least some mitochondria, which are required for survival, and mitochondrial genomes, which are required for respiratory competence.

5.6 Stochastic replication

As was the case for *Chlamydomonas* chloroplast genes, yeast cells can become homoplasmic for mitochondrial genes without dividing, owing to random genetic drift of gene frequencies within the cell [31]. This was demonstrated using delayed division experiments with both budding and fission yeast [64], analogous to those in *Chlamydomonas*. Birky and colleagues [32] reported that many first central buds are uniparental, producing clones with mitochondrial genes from only one parent; however, when wild – type cells were mated with $\frac{1}{2}$ mutants that have mitochondria but no mtDNA, all first central buds receive mtDNA. They suggested that all first central buds probably receive mtDNA from both parents but that stochastic replication (possibly combined with turnover) eliminates genes from one parent or the other. Stochastic replication is almost certainly a major contributor to the production of homoplasmic cells during asexual reproduction in yeast, i.e., to vegetative segregation.

5.6.1 Nucleoid structure affects mitochondrial gene inheritance

It was suggested that the segregating units in yeast mitochondria might be nucleoids [30], and recent studies suggest that nucleoid structure does affect the inheritance of mitochondrial genes. The mtDNA molecules in a nucleoid appear to be held together by Holliday structures [100; 37; 38; 81], perhaps because mtDNA replication is initiated by recombination (7; 99; 44) as it is in T – even phage [46]. Mutations that affect the resolution of the Holliday structures also modify the inheritance of neutral ρ^- genomes in $\rho^- \times \rho^+$ crosses [38, 97].

VI. PROTEIN TARGETING TO MITOCHONDRIA AND CHLOROPLASTS

One of the most interesting subjects about mitochondria and chloroplasts, which should be considered earlier, is the origin of these organelles. As all of us know these organelles originally derive from endosymbiotic bacteria: The closest bacterial organisms to the endosymbiotic ancestors of these organelles have nearly a thousand genes (*Rickettsia* [98]) or several thousands (cyanobacteria [95]).

Since the endosymbiosis, many of the genes of the endosymbiotic bacteria have been lost, leaving the organelle genomes with less than a hundred proteins – coding genes each [101; 70]. The vast majority of mitochondrial and plastid proteins are encoded in the nucleus, synthesized by cytosolic ribosomes and subsequently imported into the organelles via active protein transport systems. The total number of proteins present in mitochondria and chloroplasts is thought to be about 2000 – 3000 for each of them [3]. Mitochondria originated much earlier than plastids and thus the first plastids arose in cells that already contained an efficient system for targeting cytosolically synthesized proteins to mitochondria. One might have expected evolution to have seized this opportunity to reuse the same machinery for targeting proteins to plastids, but in fact this seems not to be the case; the two protein import systems have clearly been derived independently and do not share homology. In this situation, it is thus easy to understand why protein targeting is usually highly specific. Nevertheless, it is becoming increasingly clear that despite the profound differences in the two import machineries, a certain number of proteins are efficiently recognized by both systems and are imported into both organelles [85].

6.1 Targeting protein to mitochondria

Mitochondria have two complexes of proteins called TOM proteins and TIM proteins, respectively located in the outer membrane and the inner membrane, which together form the protein import channel. The proteins that will be imported generally have a mitochondrial targeting sequence located at the N – terminus, although there are proteins that have internal or even C – terminal targeting signals. This latter case has been found only once, for a yeast mitochondrial helicase [27]. The N – terminal presequence cannot be described as a consensus sequence but contains conserved features that can be identified with more or less confidence. In plants, mitochondrial targeting sequences are generally longer than in other organisms (40 amino acids on average) [9], they have a net positive charge (rich in arginine and poor in acidic amino acids) and contain many aliphatic residues (mainly leucine and alanine). The structure adopted by the presequence is generally an amphiphilic K helix [47].

It can be noted also that plant mitochondrial targeting sequences are particularly rich in serine residues. How the translated protein is actually targeted to the mitochondria is not well understood yet. In the case of a protein targeted to the matrix of mitochondria and possessing an N – terminal presequence, cytosolic protein factors interact with the presequence. These factors are generally chaperones and can require ATP. The presequence is then transferred to the mitochondrial TOM complex proteins. These proteins, namely TOM70, TOM20 and TOM22, are generally negatively charged and can thus form

electrostatic interactions with the presequence. Once the presequence is engaged in the outer membrane channel, negative charges present on the inner membrane protein TIM23, along with the electrochemical gradient across the inner membrane (gradient created by the electron transport along the mitochondrial respiratory chain), allow the presequence to tow the protein through both the outer and the inner membrane. The last steps of protein import are carried out by mitochondrial chaperones, which literally pull the protein inside the matrix. The imported protein can then be cleaved from its import signal by specific proteases, and be refolded to carry out its function inside the mitochondria.

6.2 Targeting protein to chloroplast

Chloroplasts also possess an outer envelope protein complex called TOC, and an inner envelope protein complex, TIC, which differ in many ways from the equivalent mitochondrial complexes. Chloroplast proteins can be located in even more compartments than mitochondrial proteins. In addition to the envelope membranes and the inter membrane space and the stroma, many important chloroplast proteins (photosynthesis – related proteins) are located in the membrane and the lumen of the thylakoids. In this study will be focused only on the presequence needed for the protein to cross the double membrane envelope of the chloroplast. These targeting sequences are different from their mitochondrial counterparts but do present some similarities. They are about 50 amino acids long; rich in the hydroxylated residue serine and unlike mitochondrial presequences they do not contain many positively charged residues, especially in the first ten amino acids, and do not contain many leucine residues. However, like mitochondrial targeting sequences, they contain very few acidic amino. The structure of the presequence is somewhat less well defined than for mitochondria [48].

Proteins targeted to the chloroplast are probably also recognized in the cytosol by chaperone proteins [48], before interacting with the components of the import machinery. The major difference with protein import into mitochondria is that there is no comparable electrical gradient in chloroplasts. None of the proteins from the TOC and TIC complexes have homologues in the TOM or TIM machinery [2]. Protein import into chloroplasts largely depends on the subsequent action of different protein chaperones, the process requiring GTP and ATP. A large GTPase protein, TOC160, is one of the most cytosolic – accessible TOC proteins, and is involved in recognition of the presequence. The TOC and TIC protein complexes are in close contact with each other. TIC22 is the first protein from the inner membrane complex to interact with the presequence [9]. TIC110 is believed to form the canal through which the proteins are eventually imported into the stroma. It seems that TIC110 is also in close interaction with stromal chaperones, which could be the final motor for the import of the chloroplast – targeted protein. As in mitochondria, specific proteases can remove the presequence from the mature protein.

6.3 Dual targeting proteins to chloroplast and mitochondria

There are approximately 50 proteins in different species reported up to date to be encoded by a single gene, synthesized as one gene product, but imported into both mitochondria and chloroplasts using an ambiguous dual targeting peptide (dTP) [29; 6; 69]. The first protein shown to be dually targeted to mitochondria and chloroplasts was glutathione reductase (GR) from *Pisum sativum*. Since then, other proteins involved in many essential organellar functions, such as DNA and RNA synthesis and processing, protein folding and fate, energy metabolism, and stress response, have been shown to be dually targeted. Eighteen of the dually targeted proteins are aminoacyl – tRNA synthetases identified in *A. thaliana* [6 & 76]. The overall properties of dTPs resemble standard characteristics of mTPs and cTPs, but there are quantitative and distributional differences. Continuous identification of new dually targeted proteins gives opportunity for reinvestigating the overall properties. Determinants for dual targeting are not fully understood. It has been proposed that the information for organellar targeting can be organized in domains, as, for example, in dTPs of GR [29], RNA polymerase RpoT:2 [21], and Presequence Protease, or spread all through the targeting peptide [79] or associated with the occurrence of arginine and the properties of the second amino acid of the N – terminal sequence [28].

Moreover, expression of the 5' untranslated region (UTR) upstream of the ATG start codon has also been shown to be involved in generating a dTP [1]. Here, we have analyzed the amino acid content and distribution of 43 dual targeted proteins in *A. thaliana* using SequenceLogos and statistical methods. We have investigated targeting determinants of the dual targeting peptide of Thr – tRNA synthetase (ThrRS – dTP) studying organellar import of N and C – terminal deletion constructs coupled to GFP. Furthermore, it have been produced chemical quantities of the shortest peptide of ThrRS – dTP that was capable of conferring dual targeting capacity, ThrRS – dTP (2 – 60), and it has been studied its biochemical and biophysical properties.

VII. CONCLUSION

Chloroplasts and mitochondria are both membrane bound organelles of eukaryotic cells. They do not occur in prokaryotic cells, for example, bacteria. Mitochondria occur in the cells of animals and plants but chloroplasts only occur in the photosynthesising tissues of plants.

Chloroplasts are concerned with the process of photosynthesis whereas mitochondria are concerned with aerobic respiration. It is clear that one of the most important products of these two pivotal processes is ATP. ATP synthesis is the central bioenergetic engine of all organisms and represents the smallest molecular motor, which was optimized in the course of evolution. In eukaryotes, the ATP synthase complex is located in the inner membrane of mitochondria, with ATP synthesis reaction occurring on the membrane side toward matrix compartment.

In plants, the enzyme is in addition localized in the thylakoid membrane of chloroplasts, with the ATP – forming – moiety facing the stroma. So we observe that there are topological differences between the mitochondrial and chloroplastic ATP synthases. Also magnesium is an important element in ATP synthesis. The role of its, is to form of Mg^{2+} which acts as separate substrate in the ATP synthase. It has also been shown that DNA in mitochondria chloroplast has its own and that the DNA in both mitochondria and chloroplasts can be extremely unstable. Most information on the repair of mtDNA comes from yeast and somatic cells of mammals, whereas very little is known about mtDNA repair in plants or about cpDNA repair.

An ambiguous dual targeting peptide is a tool for importing one gene product into both mitochondria and chloroplast. The first protein shown to be dually targeted to mitochondria and chloroplasts was glutathione reductase (GR) from *Pisum sativum*.

It has been proposed that the information for organellar targeting can be organized in domains.

A protein domain is a conserved part of a given protein sequence and (tertiary) structure that can evolve, function, and exist independently of the rest of the protein chain.

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