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Delivery of Drugs and Macromolecules to Mitochondria

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Abstract

Mitochondria is where the bulk of the cell's ATP is produced. Mutations occur to genes coding for members of the complexes involved in energy production. Some are a result of damages to nuclear coded genes and others to mitochondrial coded genes. This review describes approaches to bring small molecules, proteins and RNA/DNA into mitochondria. The purpose is to repair damaged genes as well as to interrupt mitochondrial function including energy production, oxygen radical formation and the apoptotic pathway.

Keywords

Mitochondrial DNA; Mitochondrial disease; Translocators; Protein and RNA import; Membrane insertion; Lipophilic cations

1. Introduction

Before embarking on a discussion of macromolecule or drug delivery to mitochondria the uniqueness of the organelle must be considered. Mitochondria are different from other subcellular organelles with respect to their complex two membrane structure. It is hypothesized that mitochondria were originated by endosymbiosis. Research on mitochondrial DNA supports that mitochondria have similarity with α -proteobacteria [1]. The double membrane structure most likely evolved to help preserve some of the essential functions of the organelle. Perhaps the most important function of mitochondria is the syntheses of ATP from ADP and phosphate where the free energy required to perform the reaction ($\Delta G_0 = + 7.3$ Kcal/mole) is derived from energy released during the transfer of electrons through the electron transport system to oxygen. For ATP synthesis to occur, it is necessary that the mitochondria maintain an acidic inner space and an electrochemical potential across the inner membrane. Thus, it is essential that the organelle have the ability to selectively control the permeation of chemicals entering into it. If chemicals could enter freely, then these two features could be destroyed.

During the past few years a number of outstanding review articles have been published that discussed various ways to bring drugs or chemicals into mitochondria [2–6]. This review article will summarize various unique features of mitochondria and then review how others suggested that those might be exploited to permit compounds to enter it. This article will focus primarily on ways of getting protein and DNA into mitochondria since small molecule entry has been well reviewed by others.

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2. Structural features of mitochondria

2.1. Double membrane and matrix space

Mitochondria are composed of a double membrane. Like all membranes, these two are primarily composed of phospholipid bilayers with proteins embedded in them. Since there are two membranes, two aqueous spaces are defined. The inner most is the matrix space while the one between the membranes is simply called the intermembrane space. The outer membrane, like the plasma membrane, has a lipid to protein ratio of 1:1. The outer membrane does not offer a barrier to small molecules. These can simply diffuse through pores in the membrane formed by a membrane spanning protein called porin. Large molecules such as proteins cross the outer membrane by using a unique protein import apparatus. ATP synthesis occurs in the matrix space so it is the inner membrane that provides the bulk of the protection for what enters the organelle. To allow specific compounds to reach the matrix space a number of proteins in the inner membrane function as transporters with each having a specific ligand to move across the membrane. For example, one of these (ATP/ADP carrier) allows ADP to cross the inner membrane while simultaneously transferring out an ATP from the matrix space. Many other such transporter have been described. The composition of inner membrane is different from outer membrane in that it is more proteinaceous and it contains an unusual phospholipid, cardiolipin. Other than the metabolite transporters, the inner membrane also has three functionally different proteins; proteins involved in the respiratory chain complex, ATP synthase and protein import machinery.

Within the human mitochondrial matrix space is a small genome of 16569 bp that codes for 13 hydrophobic proteins all of which are involved in electron transfer, along with 22 transfer RNAs and two ribosomal RNAs [7,8]. The matrix space is also the site for major metabolic pathways including the citric acid cycle, urea cycle and fatty acid oxidation. Citric acid cycle is necessary to generate NADH that is the source of electrons for the electron transport system. The terminal acceptor for these electrons is oxygen. During the past decades a number of chemicals have been found that inhibit the transfer of electrons and from these we have learned something about the ability of molecules to enter the matrix space. Cyanide and azide ion both inhibit cytochrome oxidase, thereby blocking the flow of electrons through complex IV and enhance reactive oxygen species (ROS) generation at complex III [9,10]. Carbon monoxide has strong affinity for heme and it binds the heme containing cytochrome oxidase [11]. In contrast, a simple ion such as chloride or sodium can not diffuse into the matrix space, and like phosphate ion, requires a specific translocator on the inner membrane to allow it to get from the cytosol to the matrix space. Calcium ions enter the matrix space but it too requires the action an energy dependant transporter.

Rotenone, a very hydrophobic compound that is isolated from a plant root simply diffuses across the membrane and binds to a protein in complex 1 and inhibits the transfer of electron from NADH to coenzyme Q [12]. Some antibiotic compounds cross the inner membrane and bind to components of the electron transfer system [13,14]. Other hydrophobic molecules, even those carrying a positive charge can enter mitochondria by simply diffusing across the membranes. Rhodamine and JC1 has been used to stain mitochondria. Triphenylphosphonium (TPP) ion is another example of a cationic hydrophobic molecule that can cross the mitochondrial membrane. Investigators have shown that drugs attached to TPP can diffuse into mitochondria [15–17]. Thus, hydrophobic molecules have the ability to simply diffuse across the membrane. Ions with delocalized charge can also diffuse across the membrane while ions with a localized charge (i.e. Na^+ or Ca^{2+}) require translocators.

2.2. Translocators and transporters

Binding of a drug to virtually any one of the transporters or even porin, the outer membrane protein, could disrupt mitochondria function by preventing an essential compound from entering or leaving. For example, atractyloside binds to the ATP/ADP carrier and affects mitochondria by uncoupling oxidative phosphorylation [14]. To date, not many such drugs have been described.

The vast majorities of mitochondrial proteins are coded by nuclear genes and after synthesis on cytosolic ribosomes are translocated to mitochondria. A receptor-translocator complex exists in mitochondrial membrane that allows these proteins to enter mitochondria [18–20]. Since proteins that are destined for the matrix space have to cross two membranes, there are actually two separate translocators. One is found on each membrane with the one on the outer membrane abbreviated TOM (translocator outer membrane) with the other being TIM (translocator innner membrane). The nuclear encoded proteins that are recognized by the TOM and TIM complexes possess an N-terminal leader sequence [18–22]. Attaching the leader to essentially any protein will enable the protein to be translocated into mitochondria. In fact, fusing the leader to a segment of DNA or RNA will allow those entities to be taken up by mitochondria [23]. More details about using the TOM and TIM complex will be discussed in a subsequent section of the review.

3. Specific diseases that are of mitochondrial origin

Mitochondria harbors roughly 1000 proteins of which only 13 are mitochondria genome coded. Thus, mitochondrial diseases can arise from defects in both nuclear and mitochondrial genomes [24–28] (Table 1). Mitochondria diseases that arise from the defective nuclear encoded proteins have been recently reviewed by Mackenzie et al [29]. The review discusses pyruvate dehydrogenase deficiency, primary hyperoxaluria type 1, severe alcoholic liver disease and human deafness dystonia syndrome. In pyruvate dehydrogenase deficiency, a point mutation was observed that caused the enzyme-complex to have lower activity in the mitochondrial matrix space when compared to healthy individuals. Primary hyperoxaluria type 1 is the result of two point mutations in the alanine/glyoxylate aminotransferase 1 protein. These mutants are targeted to mitochondria instead of peroxisomes. Human deafness dystonia syndrome is caused by a mutation to the inter membrane space (IMS) protein deafness dystonia peptide 1. Several other mitochondrial diseases occur due to mutation in the nuclear encoded mitochondrial proteins such as those involved in mtDNA maintenance/replications and mtDNA polymerase [30,31].

The vast majority of diseases of mitochondrial origin are due to defects mainly in the electron transport complexes [32–34]. These are the result of damaged or mutated proteins being incorporated into the complexes. The damaged proteins can be any one of the 13 that are coded by the mitochondrial genome or ones being coded by a nuclear gene since the complexes are composed of subunits of each. A list of the diseases associated with the mitochondrial genome is presented in Figure 1.

4. Why should drugs be targeted to mitochondria?

In addition to providing energy for the cell through oxidative phosphorylation, mitochondria also serve as a source of carbon compounds for some important pathways. For example, acetyl CoA is produced in mitochondria by the action of the pyruvate dehydrogenase complex. Acetyl CoA is needed for the synthesis of citrate in the matrix space but is also needed to make malonyl CoA in the cytosol to be used in fatty acid biosynthesis. Acetyl CoA can not pass through the membrane but citrate can. Thus, in theory, a drug that interfered with the translocation of citrate that produces acetyl CoA might be useful for weight control.

A recent discovery showed that mitochondria is involved in apoptosis (programmed cell death). It appears that compounds that increase the permeability of the outer membrane can cause cytochrome c to be released; this event is one of the early triggers of the apoptotic pathway. Naturally occurring compounds that are involved are actually proteins of the Bcl-2 family abbreviated as Bax and Bid. These proteins form pores that permits cytochrome c to exit from IMS. On the other hand, Bcl-2 and Bcl-X_L inhibit apoptosis [35–38]. Despite intensive investigations, how Bax and Bak form pores in the outer membrane is still not known. The widely accepted theory is that during apoptosis, Bax and Bak change their conformations, oligomerize and form pore in the outer membrane that facilitate the release of cytochrome c. Bcl-2 can inhibit Bax and Bak oligomerization by binding the preformed oligomer [39]. The pro and antiapoptotic proteins, Bax, Bak, Bcl-2 and Bcl-X_L all have the ability to form pores in the outer membrane though the shape and ion selectivity of the pores are different [40–43]. The structures of six Bcl-2 family member proteins have been determined [44–46]. The structures of these proteins possess remarkable similarity although their amino acid sequences differ. The structure of Bcl-X_L shows that it forms a hydrophobic groove that makes a binding site for proapoptotic protein such as BAD suggesting that the interaction is mainly hydrophobic [47]. This study led the prediction that small molecules that could be used as inhibitors of these proteins. ABT-737 is a potent inhibitor that binds the same hydrophobic region of Bcl-X_L and has been used in clinical trials [48].

Voltage dependent anion channel (VDAC) is a highly conserved protein with homology to bacterial porin and it forms an outer membrane pore [49]. VDAC plays a major role in mitochondria associated apoptosis. There are many conflicting theories about its role in apoptosis. It was found that proapoptotic protein Bax was co-immunoprecipitated with VDAC suggesting an interaction between them [50]. Other investigators showed that Bax can favor the high conductance state of VDAC that allowed cytochrome c to be released from the intermembrane space [51]. Rostovtseva et al found the proapoptotic protein tBid, not Bax, binds VDAC and prevents the exchange of ATP/ADP between cytosol and mitochondria leading to mitochondrial swelling resulting in rupture of the mitochondrial membrane. Subsequently, cytochrome c is released into cytoplasm leading to cell death [52]. Studies showed that mitochondria bound hexokinase II also has role in apoptosis [53]. Hexokinase II binds VDAC inhibiting the binding of Bax. Since the binding of Bax to VDAC promotes apoptosis, hexokinase II prevents the cytochrome c release into cytosol and hence prevents apoptosis. Drugs that induce apoptosis would be useful as therapeutic agents.

During the passage of electrons through the electron transport system, oxygen radicals are formed (ROS) which leads to a concept termed oxidative stress [54–56]. Mitochondria destroy the free radicals using vitamin E, ascorbate, coenzyme Q 10, cytochrome c and glutathione or enzymatically with superoxide dismutase, glutathione peroxidase and catalase. If this defense system is compromised free radicals could accumulate in mitochondria. These radicals could cause mtDNA mutation [57], lipid peroxidation [58] and protein oxidation [59]. mtDNA mutations are associated with many physiological complications including Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis [26,60]. Antioxidant supplements as well as drugs have been used to help scavenge the free radicals produced in mitochondria [61].

Drugs are necessary for both inhibiting mitochondria in order to kill cancer cells as well as to protect the cells from oxidative damage and to repair defects. Because of the complex nature of the organelle it will be necessary to use many different strategies to get a drug or macromolecule into mitochondrial once it is taken up by the plasma membrane.

5. Strategies used to bring compounds into mitochondria that disrupts its function

5.1. Lipophilic cations

Many of the chemicals that disrupt mitochondrial function take advantage of the hydrophobic nature of the membrane. For example CCCP is a compound used to disrupt the electrochemical potential of the organelle and it simply diffuses across the membrane. The concept of hydrophobic molecules as carriers of drugs into mitochondria was turned into reality when triphenyl phosphonium ion was shown to cross into the matrix space [61,62]. Functional groups could be attached to the phosphorous atom that will be carried into the matrix space and released there. Alternatively, it might be possible to modify the phenyl rings so that drugs could be attached to them. These could then be cleaved from the carrier by enzymes in the matrix space. TPP or a methyl derivative, TPMP have the property of being relatively lipid-soluble, despite their net positive charge. The hydrophobic nature as well as the delocalized positive charge allows for the efficient penetration into mitochondria without requiring a receptor [61].

Oxidative damage to mitochondria is very common in most age-related human diseases including neurodegenerative disorders. This is due to the formation of reactive oxygen species (ROS), produced by the mitochondria [54]. The selective delivery of antioxidants to damaged mitochondria is therefore an effective therapeutic strategy in such human disorders. For example, Murphy's lab employed VitE and coenzyme Q to treat the cells from patients having Friedreich Ataxia (FA) [61]. The mitochondrial protein frataxin is defective in the FA disease and it contributes to ROS formation in mitochondria. It was observed that VitE and coenzyme Q both were able to rescue the cells from the damage caused by ROS. However, these antioxidants do not have the ability to concentrate in mitochondria. Thus, the amounts needed to afford protection were very high. To overcome the problem, antioxidants were covalently coupled to a triphenylphosphonium cation (Fig. 2), and these compounds were selectively taken up by mitochondria [3,61]. The lipophilic cations easily permeate through the lipid bilayers and subsequently accumulate several hundred-fold within mitochondria because of a large mitochondrial membrane potential (150–170 mV; negative inside). Thus, TPP can be used as a carrier to target antioxidants to mitochondria to rescue cells from oxidative damage.

Dequalinium is another delocalized cationic lipid and has the potential to target DNA to mitochondria [5,6]. It binds DNA tightly and can fold into a compact structure. This structure protects the bound DNA from nuclease digestion. Due to its delocalized positive charge it can pass through the plasma membrane by endocytosis. Unlike a nuclear targeting lipid vector, the DNA is not released from the liposome upon contact with the inner side of plasma membrane. Instead, DNA is released only when this liposome interacts with the mitochondrial membrane. The specific components present in the mitochondrial membrane interact with the positively charged liposome and DNA is released at the mitochondrial surface. If DNA is fused to a mitochondrial leader peptide, it will be imported into the matrix space using the mitochondrial import machinery (TOM/TIM). It was shown that dequalinium-DNA can be imported into mitochondrial matrix space when it was incubated with BT20 cells [63].

5.2. Protein-nucleic acid

Shortly after the discovery of the leader sequence, Schatz's laboratory showed that mitochondria can import single or double stranded DNA whose 5' end was covalently linked to the C-terminus of a mitochondria precursor protein [64]. The precursor initially employed was the leader sequence from COX IV fused to dihydrofolate reductase. The cysteine residue at the C-terminus of the preprotein was coupled to the 5' end of 24 nucleotide ssDNA using a bifunctional crosslinker. The protein-DNA adduct was imported into yeast mitochondria. The authors also showed that dsDNA can be imported into isolated mitochondria.

5.3. Peptide-nucleic acid

Peptide nucleic acids (PNAs) are synthetic DNA-like molecules in which the chains of pyrimidine and purine bases are linked by an aminoethyl (pseudopeptide) backbone (Fig. 3) [65,66]. This structure specifically hybridizes to complementary DNA and RNA and is resistance to nuclease and protease attack. As a result, PNAs have the potential to bind damaged mitochondria genome. However, poor permeation through the cell membrane has made it less attractive as a therapeutic agent although some cells like muscle cells are particularly capable of DNA or modified DNA uptake by an unknown mechanism [67].

Investigators reported that biotinylated PNA was easily incorporated into cells. The mitochondrial leader sequence from COX VIII was fused to the biotinylated PNA, and it was able to localize in mitochondria both *in vitro* and *in vivo* [67]. Although the mitochondrial leader peptide was able to carry PNA to mitochondria, the cost of synthesizing the adduct and its low permeability through the plasma membrane has made it unattractive to use. Investigators, instead, used TPP to bring PNA into mitochondria [68]. The investigators applied the PNA-adduct to treat MERRF disease, a disease caused by a point mutation, A8344G tRNA^{Lys}. The strategy was to study a DNA replication run off assay using two different templates, one from wild type and the other from mutant mtDNA. A PNA 11-mer was conjugated to TPP and was used in the *in vitro* replication assay. PNA inhibited up to 75% of the mutant DNA replication while no inhibition was found using the wild type DNA template. This PNA-adduct was also employed with cells from MERRF patients that contained both the wild type and mutant mtDNA. The PNA-TPP adduct was found in the mitochondria but the ratio of mutant and wild type DNA was not changed showing that the PNA could not inhibit the replication in intact cells. Though the experiment did not produce the expected results it showed that the PNA-TPP adduct has the potential to enter into mitochondrial matrix when added to a cell line. More work will be needed to make them a useful therapeutic delivery system.

5.4. Proteins and RNA

The leader sequence necessary for protein targeting is removed by a mitochondrial protease after it enters the matrix space. What is of potential interest is that the sequence and size of the leaders vary among the 1000 or so nuclear encoded proteins that enter mitochondria [18]. Further, it has been shown that the leader can bring in virtually any protein as well as segments of RNA or DNA. This means that the leader sequences can be used to bring native or foreign macromolecules into mitochondria.

It has been shown that the leader sequence of the Toho-1, a bacterial signal peptide, can bring GFP to mitochondria [69]. Other laboratories have also shown that bacteria signal peptides can function as a mitochondrial leader [70]. This implies that it could be possible by using a bacterial as well as a mammalian leader peptide to bring any protein into mitochondria. For example, one could use this approach to bring into mitochondria a correct version of a protein coded by damaged gene of either nuclear or mitochondrial origin. Obviously, it would be necessary to find a way to get the correct DNA into the cell, a topic beyond the scope of this review. However, there are well established methods to incorporate a foreign DNA into cells.

5.4.1. Factors to be considered when trying to replace a damaged protein

Coding: One problem when trying to express a mitochondria coded protein in cytosol is that the genetic code for a few amino acids is different in mitochondria than in the nuclear coded genes. ATA codes for methionine in mitochondria but codes for isoleucine in cytosol. TGA codes for tryptophan in mitochondria but it is a stop codon in cytosol. Thus, it will be necessary to change the codons according to the universal nuclear code. Simple PCR reactions can be used to accomplish these changes.

Solubility: All thirteen proteins expressed in mitochondria are very hydrophobic. There is a possibility that if these proteins were expressed in cytosol they might aggregate and precipitate. It is also possible that these very hydrophobic proteins might just bind to any membrane. When these proteins are expressed in mitochondria they are targeted to the inner membrane by a co-translational import mechanism, that is, the protein starts to be incorporated into the inner membrane while it is coming off mitochondrial ribosome [71]. It has been shown that a co-transport/translocation mechanism for protein import from nuclear coded genes also exists [72]. Thus, the hydrophobic nature of allotropically expressed proteins might not be a serious problem if the nuclear coded protein could follow a co-translational import model [73].

Insertion into inner membrane and assembly: Finally, after targeting to the inner mitochondria membrane, it would be necessary to replace a protein that is embedded in a membrane-bound complex. It is not known precisely how each component assembles into a complex. Most likely the proteins are not free to diffuse in and out of the complex. Thus, it may not be possible for a newly imported protein simply to displace the damaged protein from an existing complex. Rather, it might be possible to imagine that the new protein can be inserted into the complex during mitochondrial biogenesis.

Several mechanisms exist to insert nuclear coded proteins into the inner membrane (Fig. 4). The carrier proteins such as ATP/ADP carrier protein does not contain any N-terminal-leader sequence but possesses several internal signals necessary for import. The carrier proteins are first recognized by Tom70, an outer membrane protein. In the IMS, they interact with small TIM proteins so that they are not precipitated in the hydrophilic environment of the IMS. Finally, they interact with the TIM22 complex and subsequently are released to inner membrane [74].

Some inner membrane proteins contain a leader peptide that is followed by a hydrophobic sequence. The leader peptide first interacts with the TOM complex and then is transferred to the TIM23 complex. The leader peptide crosses the inner membrane and is cleaved by the protease in the matrix space while the hydrophobic signal keeps the protein in the inner membrane. Oxa1p from yeast follows this mechanism [75].

A few preproteins are integrated into the inner membrane by 'the conservative pathway'. The preproteins contain typical mitochondrial leader sequences that are recognized by TOM and then by TIM23 complex. The proteins are then completely released into matrix space where the leader sequence is removed. The proteins are finally inserted to inner membrane by an export-like system. The export is carried out primarily by Oxa1 complex in the inner membrane. The subunit 9 of F₁-F₀ ATPase of *N. crassa* and Oxa1 are examples of proteins using this mechanism [76].

The proteins that are expressed in the mitochondria (cytochrome b of the bc₁ complex; Cox1, Cox2, and Cox3 of the cytochrome oxidase; and Atp6, Atp8, and Atp9 of the F₀F₁-ATPase) are very hydrophobic and are inserted into inner membrane by a co-translational path way. The co-translational import is facilitated by the C-terminus of Oxa1 complex which is extended into matrix that binds the nascent polypeptide chain [18,71].

The above mentioned mechanisms illustrate various ways of how a protein can be imported into the inner membrane. One or more of these approaches might have to be employed to replace defective proteins in the electron transport complex. Most probably the method which uses the internal signal could not be used since putting an internal signal into a mitochondrial coded protein may disrupt the protein's function. The other pathways can be used to insert allotropically expressed mitochondria-coded proteins into inner membrane by fusing a signal peptide to the N-terminus to replace a damaged protein.

Mitochondrial membrane insertion of allotopically expressed proteins have been reported. ATPase6 and ATPase8 were expressed in cytosol and were imported into mitochondria with the use of a mitochondrial leader peptide. Nagley et al showed that a construct containing a leader peptide from ATPase9 fused to ATPase6 subunit from F₁-F₀ ATPase synthase expressed in cytosol were targeted to mitochondria [77]. This chimeric protein eventually replaced the defective ATPase6 component in the complex. Later Manfredi et al and Zullo et al separately showed that ATPase6 subunit can be expressed in cytosol and be brought to mitochondria [78,79]. More work will have to be done to determine how general were these findings.

5.4.2. Import of restriction enzymes to be used for mitochondrial gene therapy—mtDNA mutations could produce a new site for a restriction enzyme. The strategy to destroy the mtDNA was to import a unique restriction endonuclease by fusing a leader peptide to it. NARP disease results from the point mutation in mtDNA (T8399G) which results in impair electron transport. The point mutation creates a new *SmaI-XmaI* site which is not present in wild type mtDNA. A mitochondrial leader sequence was fused to the *SmaI* endonuclease and was imported into mitochondria [80]. The protein selectively degraded the mutant mtDNA but not the wt mtDNA suggesting its potential therapeutic use.

5.4.3. Import of tRNA into mitochondria—Human mitochondria code all the tRNAs inside the matrix and hence have no tRNA import machinery. Plants, protozoa and fungi, however, have well defined tRNA import machinery in their mitochondrial membrane [81–84]. It has been shown that human cytoplasmic tRNA^{Lys}(UUU) is imported into the mitochondria of *T. brucei* [85], *L. tropica*. [86] and isolated *Leishmania* mitochondria [86]. The imported tRNA was able to function properly. This shows that human tRNA can utilize the fungal tRNA import machinery to be imported into mitochondria. Kolesnikova et al also showed that yeast tRNA^{Lys} could be imported into human mitochondria, although human mitochondria do not possess tRNA import machinery and was able to rescue mitochondrial function suffering from MERRF disease [87]. Very recently, investigators showed that human mitochondria could import tRNA after introducing *Leishmania* RNA import complex (RIC) into the mitochondria of human cells [89]. More importantly, imported tRNA^{Lys} into human mitochondria rescued the function of mitochondria possessing defective tRNA^{Lys}. This study shows that a defective tRNA in human mitochondria can be replaced by a cytosolic tRNA using fungal RIC [88].

5.4.4. Leaders as inhibitors of mitochondrial protein import—Peptides corresponding to a leader sequence typically were found not be good inhibitors of protein import. This was an unexpected finding since it is the leader alone that interacts with TOM 20, a major component of the import apparatus [18]. It is possible that during import of a precursor protein, a heat shock-like protein is needed to anchor the precursor protein to the receptor complex [89]. Should that be the case, then one would need more than just a small peptide to act as an inhibitor.

Finding an inhibitor, be it peptide or an organic compound, for protein import could lead to cell death if newly synthesized proteins could not be brought into mitochondria. An alternative use could be to prevent proteins such as hexokinase II from binding to porin, an event found in cancer cells [90]. In some advanced cancers an isozyme of hexokinase associates with the outer membrane by binding to porin. Thus, when ATP exits mitochondria it becomes the co-substrate for the kinase and allows the cancerous cell to utilize glucose more efficiently than does a non-cancerous cell. To date, few, if any, peptides or compounds have been developed to either prevent import from occurring or to inhibit the binding of proteins to mitochondria.

6. Future

Diseases related to mitochondrial function are often the result of mutations that lead to changes in the proteins involved in the electron transport system. To correct these, some form of gene therapy will have to be employed. Different problems will exist depending upon whether the mutation was to a nuclear or mitochondria gene. We understand how to bring a nuclear coded gene product to the mitochondria. In contrast, there are only a few examples of correctly inserting a mitochondria gene product after the protein is allotropically expressed. More work will have to be done in this area before it can be determined whether or not it will be feasible to replace all or most of the damaged mitochondrial coded gene products.

Small molecules have been shown to affect mitochondria function. Use of triphenyl phosphonium ions has made it possible to bring many different molecules into mitochondria. The range of compounds should be expanded to include ones that can induce apoptosis in cancer cells as well as to inhibit (or activate) damaged proteins that were a result of mutated genes. More compounds are needed to protect the cell from the damaged caused by oxidative stress. Various techniques are available to allow one to bring virtually any type compound, be it a macromolecule or a small organic molecule, into the organelle. It will be necessary to show that the compound is truly functional once it is imported. Unfortunately, due to the complex nature of mitochondria, no one technique will enable all molecules to be imported.

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Abbreviations

mtDNA	mitochondrial DNA
PNA	peptide nucleic acid
TOM	translocase outer membrane
TIM	translocase inner membrane
TPP	triphenyl phosphonium ion
IMS	inter membrane space
VDAC	voltage dependent anionic channel
MERRF	Myoclonic epilepsy and ragged-red fibres
NARP	Neurogenic weakness, ataxia and retinitis pigmentosa
MELAS	

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes

KSS

Kearns–Sayre syndrome

PEO

Progressive external ophthalmoplegia

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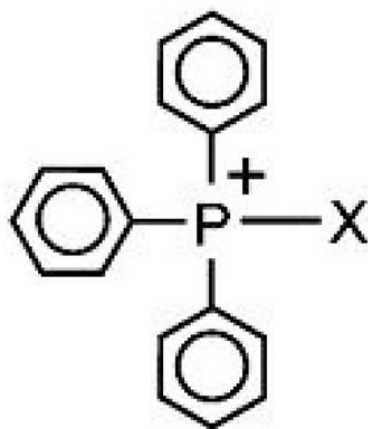
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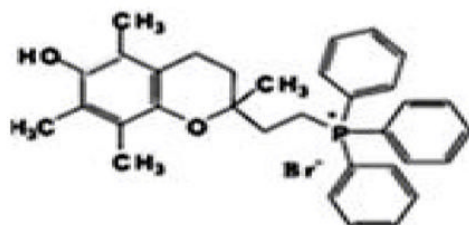
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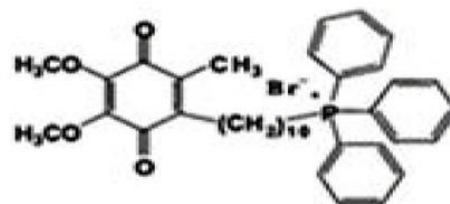
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A



B



C

Figure 2. Triphenyl phosphonium ions that can enter mitochondria because of their lipophilic properties. A. Triphenyl phosphonium ion (TPP). Antioxidant moieties (α -tocopherol and ubiquinol) fused to TPP. B. Mito-E₂ C. Mito-Q₁₀.

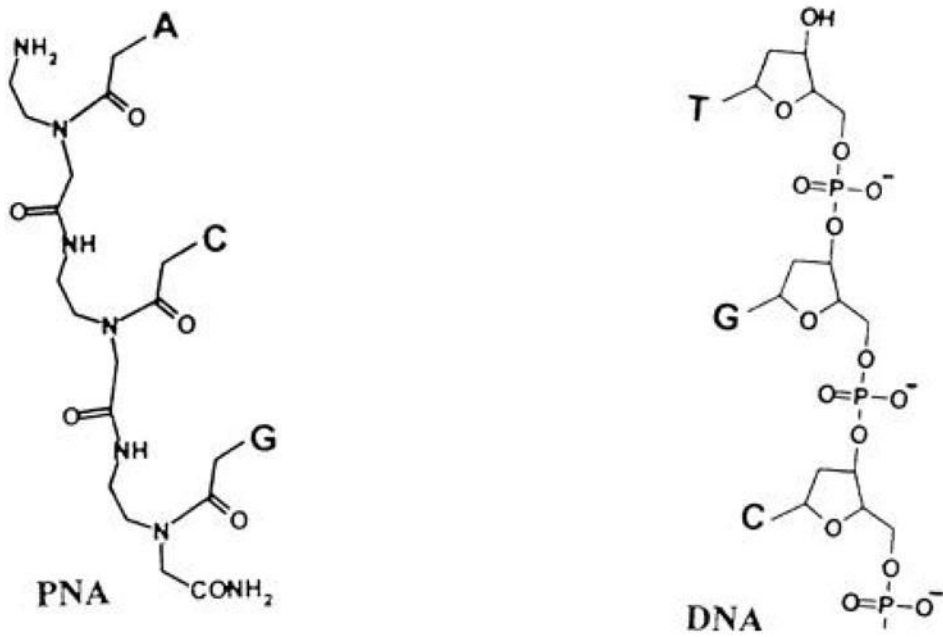


Figure 3.
Peptide nucleic acid (PNA) used to bring a DNA-like structure into mitochondria.

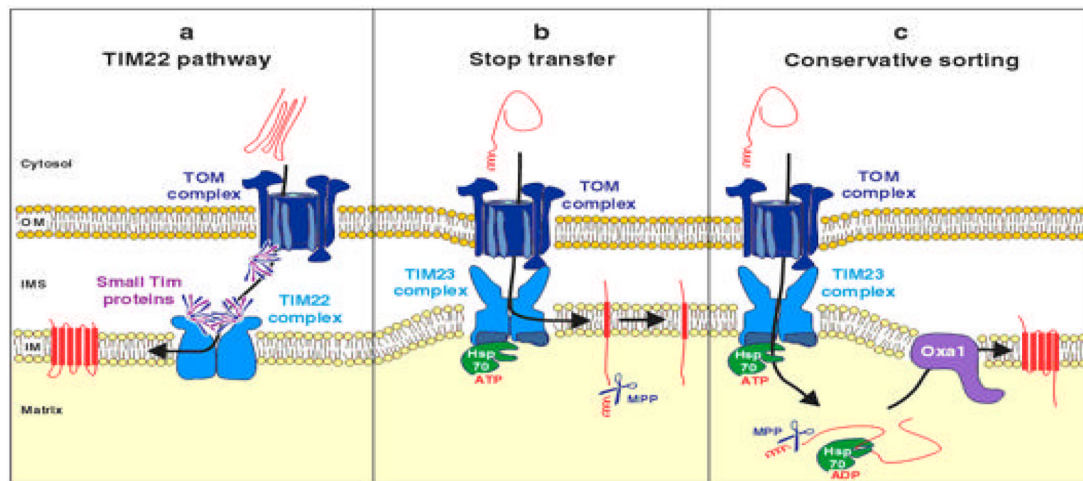


Figure 4. Inner membrane import pathways. The figure shows how the inner membrane proteins are imported and inserted into the inner membrane. The figure was taken from the reference 18.

Table 1

Diseases resulting from mutations of genes coding for tRNAs and proteins

mtDNA mutations in the tRNA ^{Leu} gene		
Mutation	Phenotype	
A3243G	MEALS MEALS/MERRF PEO KSS Myopathy	
A3243T	Encephalomyopathy	
T3250C	Myopathy with respiratory Failure	
A3251G	PEO, myopathy	
A3252G	Dementia, diabetes	
C3254G	Myopathy, cardiomyopathy	
C3256T	PEO, deafness	
A3260G	Myopathy, cardiomyopathy	
T3271C	MEALS	
A3288G	Myopathy	
T3291C	MEALS	
A3302G	Myopathy with respiratory failure	
C3303T	Cardiomyopathy	
mtDNA mutation in the tRNA ^{Lys} gene		
<u>Mutation</u>		
A8344G	<u>Phenotype</u> MERRF MELAS/MERRF PEO Myopathy Leigh's syndrome	
G8313A	Gastrointestinal dysfunction, dementia, ataxia, deafness, axonal neuropathy	
G8328A	Encephalopathy	
G8342A	PEO	
T8356C	MERRF	
G8363A	MERRF	
A8296G	Cardiomyopathy, deafness, ataxia	
Hypertrophic cardiomyopathy		
mtDNA mutations in other tRNA genes		
<u>Gene</u>	<u>Mutation</u>	<u>Phenotype</u>
tRNA ^{Phe}	A583G	MELAS
tRNA ^{Val}	G1606A	Ataxia, dementia, deafness
	G1642A	MELAS
tRNA ^{Ile}	A4269G	Encephalopathy
	T4274C	PEO
	T4285C	PEO
	A4295G	Cardiomyopathy
tRNA ^{Trp}	5537T insert	Ataxia, Leigh's syndrome
	G5540A	Ataxia, deafness
tRNA ^{Asp}	A5692G	Ataxia
	G5703A	PEO
tRNA ^{Cys}	A5814G	MELAS
tRNA ^{Ser}	7472C insert	Deafness, ataxia
	T7512C	MERRF/MELAS
tRNA ^{Asp}	G7543A	Infantile encephalopathy
tRNA ^{Glu}	T14709C	Myopathy and diabetes
Mitochondrial oxidative phosphorylation diseases resulting from nuclear gene mutations		
<u>Gene encoding protein respiratory components</u>		<u>Phenotype</u>
Complex I NDUFS1		Leigh syndrome
Complex I NDUFS2		Cardiomyopathy- Encephalomyopathy
Complex I NDUFS4		Leigh syndrome
Complex I NDUFS7		Leigh syndrome
Complex I NDUFS8		Leigh syndrome
Complex I NDUFV1		Leigh syndrome
Complex II SDHA		Leigh syndrome
Complex IV SURF1		Leigh syndrome
Nuclear genetic disorders of the mitochondrial respiratory chain due to mutations in translation factors:		
<u>Gene</u>		<u>Phenotype/Disease</u>
<i>EFG1</i>		Leigh syndrome
<i>MRPS16</i>		Lactic acidosis, dysmorphism
<i>EFTu</i>		Leukodystrophy and polymicrogyria
<i>PUS1</i>		Myopathy and sideroblastic anemia
Nuclear genetic disorders associated with multiple mtDNA deletions or mtDNA depletion:		
<u>Gene</u>		<u>Phenotype/Disease</u>
<i>POLG</i> , <i>POLG2</i> , <i>PEO1</i> and <i>SLC25A4</i>		Autosomal progressive external ophthalmoplegia
<i>TP</i>		Mitochondrial neurogastrointestinal encephalomyopathy (thymidine phosphorylase deficiency)

mtDNA mutations in the tRNA^{Leu} gene Mutation	Phenotype
<i>POLG</i> and <i>MPV</i>	Alpers–Huttenlocher syndrome
<i>DGUOK</i>	Encephalomyopathy and liver failure