Complex Formation and Turnover of Mitochondrial Transporters and Ion Channels

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**Abstract**

Mitochondria are responsible for many vital cellular functions in eukaryotic cells, such as ATP production, steroid synthesis and prosthetic group biogenesis. The vital functions of mitochondria are possible due to the compartmental nature of this organelle. Mitochondria form a dynamic network that can exist as a network throughout a cell or as distinct individual structures. Mitochondria are also composed of two membranes, an inner and outer membrane. The inner mitochondrial membrane (IMM) is significantly larger than the outer membrane and must fold upon itself to be contained within the outer mitochondrial membrane (OMM). These folds are known as cristae. Altogether these different membrane compartments specialize in different functions of the mitochondria. The OMM is responsible for passage of small metabolites into and out of the mitochondria while excluding macromolecules. The IMM is a highly selective barrier between the solutes of the cytosol and those within the mitochondrial matrix. Cristae specialize in oxidative phosphorylation. The functions of these membranes are afforded by membrane proteins that are able to transport specific solutes. The appropriate localization, assembly into multi-subunit protein complexes, and wild-type function of these membrane proteins therefore is vital for mitochondria to maintain appropriate function and support cellular survival. This review will address the composition and functions of mitochondrial membrane localized multi-subunit protein complexes along with how these proteins undergo degradation to maintain homeostatic functions of mitochondria in the context of mitochondria specific transporters and ion channels. Due to the large number of known mitochondrial membrane transporters and ion channels this review will focus on the topics presented at the Mitochondrial Ion Channels and Transporters Symposium hosted by the New York University College of Dentistry in September 2015 in honor of Casey Kinnally.

Keywords: mitochondrial membrane complex, degradation, mitochondrial transporter, mitochondrial ion channel

Abbreviations: CyP-D – cyclophilin-D, ETC – electron transport chain, IMM – inner mitochondrial membrane, IMS – inter-membrane space, MIM – mitochondrial insertion machinery, OMM – outer mitochondrial membrane, OXPHOS – oxidative phosphorylation, SAM – sorting and assembly machinery, TIM – translocase of the inner membrane, TOM – translocase of the outer membrane, UPS – ubiquitin-proteasome system, VDAC – voltage-dependent anion channel

**Mitochondrial membrane protein localization and topology**

The functions of mitochondria are ultimately dictated by the movement of solutes between the mitochondrial matrix and the mitochondrial inter-membrane space (IMS) and cytosol. The barriers between these compartments are the IMM and OMM. The IMM is a highly impermeable barrier to solutes and only those that are substrates of specific transporters and ion channels may pass through. This means that for each solute there is a specific transporter or ion channel resulting in a large variety of these in the IMM. Because of this controlled membrane permeability concentration and charge gradients of solutes, such as protons and metabolites, can generate a membrane potential across the IMM and this is the driving force behind many of the essential functions of mitochondria, such as ATP production (1,2). The gradients also control the passage of solutes through the IMM transporters and channels as these are sensitive to the membrane potential resulting in a very complex system of regulated solute passage. The OMM, on the other hand, allows for the passage of solutes using just a few types of more accommodating channels and transporters, such as the voltage-dependent anion channel (VDAC) family. This membrane is also subject to regulated channel opening by charge and molecular interactions. Therefore the organization and functions of these membrane resident channels and transporters are crucial for mitochondrial function.

Within these membranes mitochondrial transporter proteins have a similar topology and organization. Domains and loops are exposed to both sides of the membrane to allow for the passage of the solute from one side of the membrane to the other. These membrane proteins also have multiple transmembrane domains that pass through the membrane either as a single polypeptide or as multiple polypeptides with one or more transmembrane domains. This topology allows for whole concerted molecular conformational changes during solute transport (3). This arrangement also means that the single protein is exposed to three different environments, the hydrophobic membrane, and the aqueous environments either side of the membrane (Figure 1). In the context of this mini-review this means mitochondrial transporters are capable of interacting with proteins as well as being subject to turnover from three compartments.

OMM proteins are exposed to the cytosolic environment on their external face and can be directly involved in signal transduction pathways in the cytosol. The internal face of OMM proteins is exposed to the IMS and proteins that are specifically localized in this compartment. The topology of OMM proteins is facilitated by the protein complexes responsible for insertion. These include the TOM (translocase of the outer membrane) complex, and the SAM (sorting and assembly machinery) and MIM (mitochondrial insertion machinery) complexes that promote biogenesis of β-barrel and α-helical proteins respectively (4). IMM proteins are exposed to the IMS on the external face and the matrix on their internal face. In a similar fashion, IMM protein topology is facilitated by the protein complexes responsible for membrane insertion, TIM22 and TIM23 (translocase of the inner membrane) complexes for nuclear encoded polypeptides, and Oxa1 for mitochondrial encoded polypeptides (5). Therefore, proteins within the IMM are not directly accessible to the cytoplasm and are handled using different processes from those in the OMM.

Mitochondrial membranes are made up of high amounts of protein and the proteins present in these membranes do not exist in isolation. Many mitochondrial membrane proteins are part of larger multi-subunit protein complexes (2). The protein composition of these multi-subunit protein complexes can be of proteins involved in similar functions or of proteins with seemingly different functions. Assembly of the multi-subunit protein complexes can be accomplished through interactions of fully matured proteins after translation and full insertion into the membrane. However, some multi-subunit protein complexes are assembled through an ordered pathway that is regulated by the presence of certain assembly factors that determine if cellular conditions are appropriate. This latter type of pathway is associated with IMM complexes that are composed of proteins derived from the mitochondrial genome (Figure 2).

**Mitochondrial Membrane Protein Complexes**

Passage of ions, metabolites and proteins through mitochondrial membranes requires the function of specific channels or transporters. Many of these proteins exist as oligomeric protein complexes with either multiple copies of the same polypeptide (homo-oligomer) or two or more different polypeptides (hetero-oligomer). The oligomeric nature of these proteins allows for conformational flexibility required for solute transport and regulation. The composition of these oligomers can also be dynamic as alternative subunit isoforms can be substituted in protein complexes (3). Recent advances in protein identification technology has revealed unexpected associations between mitochondrial membrane proteins allowing for speculation about linked roles of proteins with seemingly unrelated functions (6).

An example of a large oligomeric complex of the OMM is the TOM complex. This is the first port of entry of nuclear-encoded mitochondrial targeted polypeptides that are destined for the four mitochondrial compartments and interacts with other protein complexes required for correct folding and targeting such as the SAM and the MIM complexes (4). The TOM complex is composed of 7 different proteins, one of which acts as the pore for protein translocation, Tom40, while the others, Tom20, Tom22 and Tom70 are receptors for different pre-proteins and Tom5, Tom6 and Tom7 act to assemble and stabilize the TOM complex (7). Experiments in the budding yeast *Saccharomyces cerevisiae* have provided many molecular details regarding the composition, assembly, function and regulation of this complex. The conservation of the composition of the TOM complex is maintained between budding yeast and mammalian cells indicating the ancestral nature of the import of proteins into mitochondria. Assembly of the TOM complex is regulated by the actions of the small Tom6 and Tom7 proteins; Tom6 acts as a promoter of TOM assembly, while Tom7 acts as an antagonist of the process (8,9). Regulation of the assembly of the TOM complex is achieved through phosphorylation of subunits that promote or inhibit assembly. Casein Kinase 2 (CK2) phosphorylates the Tom20 and Tom22 subunits promoting their import and assembly of the final complex. Alternatively, Protein Kinase A phosphorylates Tom40 and inhibits insertion into the OMM. These regulatory phosphorylation events control the formation of the TOM complex which ultimately dictates the expression of mitochondrial proteins in their final functional location. This is important for conditions where mitochondrial function may not be essential for cellular viability, such as fermentative metabolism (10,11).

Another highly abundant OMM protein is VDAC, also known as porin. This protein acts as a gateway between the cytosol and IMS allowing passage of crucial solutes required for metabolic pathways both inside and outside of the mitochondria. In addition to the function of solute passage through the OMM, VDAC has been implicated in cell death pathways by interacting with other proteins and protein complexes. A specific isoform of VDAC was found to interact with the pro-apoptotic protein BAK, a protein involved in the release of IMS proteins into the cytosol to cause apoptosis through activation of caspases (12). The interaction of BAK with VDAC2 is proposed to maintain BAK in an inactive state and upon pro-apoptotic signaling disruption of the interaction occurs and allows for oligomerization of BAK and cytochrome *c* release. The functional consequences of this interaction have been demonstrated to restrict death of murine thymocytes to various pro-apoptotic stimuli and result in lower numbers of thymocytes in mice (13). VDAC2 has also been proposed to act as a receptor for BAK insertion in the OMM (14). The molecular details of this interaction are thought to involve the hydrophobic C-terminal tail of BAK and a pocket on the surface of VDAC2 that could act to recruit BAK (15,16). VDAC is also involved in interactions with cytosolic proteins. Tubulin αβ dimers have been shown to directly bind to and regulate the gating function by inducing VDAC closure (17). This provides a putative mechanism allowing for communication between the cytoskeleton and mitochondrial metabolism.

BAK, and the related pro-apoptotic protein BAX, are responsible for the release of IMS proteins into the cytosol, such cytochrome c, SMAC/Diablo, that promote caspase activation and apoptosis. These proteins have a highly homologous structure to bacterial pore forming toxins (18). Therefore, the notion that BAK and BAX form pores to release IMS proteins has prevailed. The mechanism of pore formation by BAK and BAK is thought to be mediated by homo-oligomerization of the protein upon activation during apoptosis (19,20). The pore formed by BAK or BAX must be able to accommodate large molecular weight proteins, ranging from 12 – 50 kDa (the molecular weights of cytochrome *c* and SMAC/Diablo), but has been shown to accommodate the release of molecules up to 2 MDa (21) and is able to conduct current across mitochondrial membranes, identified as the MAC (mitochondria apoptotic channel) (22–24). Speculation also exists that BAK and BAX are able to rearrange mitochondrial OMM lipids to accommodate these large molecular weight solutes (19). Homo-oligomerization of BAX and BAK is stimulated by pro-apoptotic signaling and can be directly monitored using cross-linking (25,26) as well as size-exclusion chromatography approaches (27). Interestingly, BAX oligomerization is sensitive to detergent, as the apoptosis associated homo-oligomers are maintained in the presence of the detergent CHAPS, but can be triggered by various detergents in the absence of any pro-apoptotic stimulus (28) indicating very specific interactions between BAX and amphipathic detergent-like molecules. These interactions are thought to regulate pore formation and have been described for long chain monounsaturated sphingosine derivatives, that are breakdown products of sphingolipids (29). The exact molecular details of the BAX/BAK pore are not currently known and this is still a very active area of research.

Within the IMM there are also many multi-subunit protein complexes that are responsible for solute transport. In the context of oxidative phosphorylation the complexes of the ETC (electron transport chain) exist as multi-subunit complexes that act as proton pumps. The F1-F0 ATPase is also a multi-subunit complex that uses the proton gradient across the IMM to generate ATP. The majority of the oxidative phosphorylation (OXPHOS) complexes are encoded by genes derived from both the nuclear and mitochondrial genomes. Assembly of these multi-subunit complexes progresses through a modular assembly pathway and requires the action of complex specific assembly factors to ensure a balanced output of OXPHOS complexes as gene expression from the nucleus and mitochondria is not directly coordinated (30–34). Within the last two decades, the OXPHOS complexes have been suggested to exist in supercomplexes, which are higher order associations of one or more OXPHOS complexes. These supercomplexes have been proposed to allow the passage of electrons through the IMM during electron transport to minimize electron leakage from the ETC and avoid formation of damaging reactive oxygen or nitrogen species (35). The isolation and detection of these mitochondrial supercomplexes is also detergent-dependent, with digitonin, Triton X-100 and NP-40 maintaining supercomplexes while dodecyl maltoside does not (36). The formation of supercomplexes may depend on the pre-existence of assembled OXPHOS complexes (36), however, in yeast assembly intermediates of cytochrome oxidase associate with the cytochrome bc1 complex (32) indicating a putative coordinated assembly process of OXPHOS complexes in this organism. In mammalian cells, supercomplexes can be composed of a subset of OXPHOS complexes or all OXPHOS complexes, termed respirasomes, supramolecular complexes that ultimately transfer electrons from NADH to oxygen. In yeast, the main supercomplexes are formed between cytochrome bc1 complex and cytochrome oxidase (37). The stoichiometry of these supercomplexes can also include multiples of the same OXPHOS complex. The supercomplexes have been proposed to exist dynamically to allow for switching between efficient electron transfer or enhance electron release from the ETC to form reactive oxygen species.

Two other crucial proteins required for metabolic action within cells are the adenine nucleotide translocase (ANT) and the phosphate carrier (PiC). These channels bring in the substrates for the F1-F0 ATP synthase, ADP and inorganic phosphate. The ANT and PiC are canonical members of the mitochondrial channel family which are composed of 6 trans-membrane domains. These proteins were originally thought to function as dimers but recent studies suggest these proteins function as monomers (38,39).

A larger molecular organization of these transporters has been implicated in the formation of the mitochondrial permeability transition pore (MPTP), an IMM pore that forms under conditions of metabolic, oxidative, or Ca2+ stress. The molecular composition of the MPTP has been speculated since the 1970s when pore activity was first demonstrated in isolated mitochondria (40,41). The ANT was the first protein implicated as the pore forming component of the MPTP based on sensitivity to adenine nucleotides and the ANT inhibitors bongkrekic acid and carboxyatractyloside (42). More recently, other pore forming components have been proposed, including the PiC (43) as well as the F1-F0 ATP synthase in the form of a dimer (44) or the *c*-subunit ring (45). The only verified component of the MPTP is the mitochondrial matrix peptidyl-prolyl *cis-trans* isomerase cyclophilin-D (CyP-D). This was verified using specific inhibitors of the enzymatic activity of the enzyme (46) as well as mice lacking expression of CyP-D in models of tissue injury where opening of the MPTP is required for pathologies, e.g., ischemia-reperfusion in the heart (47). Similar genetic approaches have been attempted to verify the involvement of the ANT, PiC or F1-F0 ATP synthase. However, due to the essential functions of these proteins the generation of these knockout animals has resulted in skepticism of the results (48). Proteins of the OMM have also been implicated to be components of the MPTP, including VDAC and TSPO (translocator protein). These OMM proteins were identified using co-precipitation approaches using detergent lysed mitochondria and through effects of ligands on MPTP opening. However, genetic evidence in mice and cell lines lacking these proteins does not suggest they act as essential components of the MPTP (49,50) suggesting they may have a regulatory role. The exact molecular identity of the MPTP is still under intense investigation with the hopes to identify molecular targets to inhibit MPTP opening.

Insertion of IMM-destined nuclear encoded mitochondrial transporters and ion channels requires the action of the TOM complex which then passes proteins to the translocase of the inner membrane complexes TIM22 or TIM23. The TIM23 complex is responsible for the insertion of single trans-membrane span proteins with cleavable mitochondrial targeting motifs while the TIM22 complex is responsible for insertion of the mitochondrial carrier proteins and hydrophobic TIM proteins (51). The TIM22 complex inserts proteins using the help of the small TIM proteins Tim9 and Tim10 that are localized to the IMS and act as chaperones to carry these IMM destined proteins. The TIM22 complex is a 300 kDa complex composed of the proteins Tim22, Tim54 and Tim18 and the small TIM proteins Tim9, Tim10 and Tim12. The Tim18 protein has been identified as a distal homologue to a subunit of succinate dehydrogenase (SDH – complex II in the ETC) (52,53). More recently, Tim18 and SDH3, one of the essential subunits of SDH, were shown to interact in yeast mitochondria and SDH3 was found to be an accessory subunit to the TIM22 complex, demonstrating potential co-ordination between ETC function and mitochondrial protein import (54). Along the same lines, interactions between the TIM23 complex and the ANT in yeast have been reported, indicating a link between protein biogenesis and metabolite transport (55–58).

**Complexes between OMM and IMM**

Mitochondria require and produce many small molecules that need to pass from the matrix to the cytosol for metabolic reactions. A direct passage from the matrix to the cytosol has been proposed for certain metabolites in the form of contact sites. These supramolecular complexes are comprised of proteins from both the IMM and OMM. These structures have been observed using electron microscopy images and have been characterized biochemically using detergent extraction. The IMM component is proposed to be ANT while the OMM component is proposed to be VDAC. This would ensure directed passage of adenine nucleotides from the matrix to the cytosol. The formation of contact sites seems to be dictated by the “c” (cytosolic facing) conformation of ANT, when bound to the inhibitor carboxyatractyloside or ADP. These contact sites are proposed to channel ATP directly from mitochondria to the first step of glycolysis where glucose is phosphorylated by hexokinase or to phosphorylation of creatine by creatine kinase. In fact, these enzymes are dynamically associated with contact sites, hexokinase on the surface of the OMM by binding with VDAC and creatine kinase which interacts with ANT and VDAC in the IMS (59).

**Mitochondrial Protein Turnover**

Turnover completes the life-cycle of proteins and early indications pointed to the turnover of mitochondrial proteins being different between the inner and outer mitochondrial membranes. Protein components of the OMM have a shorter half-life than protein components of the IMM (4 days versus 12 days) as determined by metabolic radiolabeling (60). Recently it was determined the main mechanism for degradation of proteins localized to the OMM is via the major cytosolic protein degradation system, the ubiquitin-proteasome system (UPS). Cytosolic ubiquitin moieties are covalently attached to loops exposed to the cytosol through the action of E3 ubiquitin ligases. Ubiquitylated OMM proteins are then recognized by cytosolic adaptor proteins, such as p62 via the attached ubiquitin moiety, which then recruits AAA-ATPases, such as p97, to perform extraction of the protein from the OMM. The extracted protein is then a substrate of the proteasome for degradation (61) (Figure 3).

In the case of IMM proteins, turnover usually is mediated via proteases resident to the IMM. Proteins that are proteolytically processed by these proteases generally undergo complete degradation within the membrane without the need for extraction from the membrane. These proteases are generally in the class of ATP-independent metalloproteases (Oma1) or ATP-dependent proteases, such *m*-AAA proteases (AFG3L2, paraplegin) or *i*-AAA protease (YME1L) (Figure 4).

Interestingly a second mechanism for degradation of IMM proteins, that seems to go against topological restrictions, has been described where these proteins are degraded through the cytosolic UPS. This implies either IMM proteins can be marked for degradation within the IMM or are retrotranslocated into the OMM where ubiquitin attachment can occur. Whatever the order of the process, for this to happen proteins must be translocated from the IMM to the OMM, a seemingly energy requiring process potentially mediated via the mitochondrial import machinery acting in reverse (62–64).

Turnover of proteins can also be mediated through passive bulk clearance of macromolecules, through specific signal transduction pathways or via excessive protein damage. The general bulk clearance pathway of mitochondria and resident macromolecules is via mitochondria specific autophagy, known as mitophagy. During this process mitochondria are marked with ubiquitin that acts as a signal to recruit an emerging autophagosome that will engulf the mitochondria and cause complete degradation to component building blocks. This process is unselective and causes complete degradation of the mitochondria and contents. Mitophagy can be initiated by mitochondrial membrane potential loss, an indication of the loss of mitochondrial function, through the activation of the ubiquitin E3 ligase Parkin resulting in OMM protein ubiquitination. Pathways that also result in excessive ubiquitylation of OMM proteins can lead to mitophagy rather than individual protein degradation (65). An emerging process of selective degradation of mitochondrial proteins involving mitochondrial derived vesicles has been recently described. In this process, proteins are selectively incorporated into vesicles that emerge from mitochondria and can be targeted to the lysosome for degradation (66). This process involves the proteins PINK1 (PTEN-induced kinase) and Parkin, two genes associated with susceptibility to Parkinson’s disease (67). In addition to the PINK1-Parkin pathway of mitophagy several other E3 ligases are thought to be responsible for induction of mitophagy, including Gp78 (68). Mitophagy usually requires fragmentation of the mitochondrial network and this is also regulated by OMM protein ubiquitylation, one such example is the activity of the OMM localized E3 ligase MARCH5 that ubiquitylates MiD49, a receptor for the mitochondrial fission inducing protein DRP1, resulting in proteasomal degradation (69)

**Turnover of OMM proteins**

The main types of transporters in the OMM are the resident protein Porin/VDAC, the TOM complex and the apoptosis-activated pore-forming proteins BAX (BCL-2 Associated X protein) and BAK (BCL-2 homologous Antagonist/Killer) that form the MAC.

VDAC is a channel that allows the passage of small molecules and metabolites into and out of mitochondria to ensure metabolic pathways are not compartmentally restricted. Therefore, it can be seen that expression of VDAC is essential for mitochondrial function and cellular viability. Therefore, constitutive presence and functionality of VDAC are required for mitochondrial function. The VDAC isoform, VDAC2, has been identified as a ubiquitylated protein in large proteomic screens (70,71), however, whether this modification is involved in UPS-dependent proteolysis is not known.

The TOM complex is responsible for the transport of nuclear derived polypeptides into mitochondrial compartments and again is essential for the proper function of mitochondria and cells. Multiple subunits of the TOM complex have been identified as being ubiquitylated, also in large proteomic screens, such as Tom5, Tom7, Tom22 and Tom70 (70,72). Later studies confirmed that Tom20, Tom40 and Tom70 were ubiquitylated after depolarization of mitochondria due to the action of the E3 ligase Parkin. Upon ubiquitylation these proteins were degraded by the UPS (73).

The pro-apoptotic proteins BAX and BAK allow for release of proteins from the IMS to the cytosol through the formation of a proteolipid pore. BAX translocates from the cytosol to the mitochondria upon pro-apoptotic signaling to facilitate apoptosis. At the mitochondria BAX can be ubiquitylated and undergo degradation by the UPS (74). This post-translational modification is a potential inhibitory mechanism of apoptosis and could contribute to survival of cancer cells (75). The process of BAX extraction from the OMM has not been described, however, BAX can retrotranslocate from the OMM due to the action of the anti-apoptotic protein BCL-xL (76), however, whether this is process involves ubiquitin or is the first step of BAX degradation is unknown. On the other hand, ubiquitylation of BAK is not as frequently described. The Human Papillomavirus (HPV) derived E6 proteins have been reported to be responsible for BAK degradation in epithelial cells in a UPS-dependent manner through the action of the ubiquitin E3 ligases UBE3A/E6AP (E6 associated protein), and HERC1, perhaps contributing as a risk factor to non-melanoma skin cancer in solar ultraviolet exposed tissue (77–79).

**Turnover of IMM proteins**

The mitochondrial carrier family contains the largest number of proteins to mediate solute transport across the IMM. They are composed of 6 transmembrane domains with short loops exposed to the IMS and longer loops exposed to the matrix. This family of proteins is imported into mitochondria through the TOM complex of the OMM and then the TIM22 or TIM23 protein complex assists in insertion into the IMM (80). Polypeptides derived from mitochondrial DNA and translated by mitochondrial matrix ribosomes are inserted via Oxa1 (5). These regulated pathways of insertion ensure IMM proteins are correctly folded and are therefore functional. Proteins that are incorrectly inserted are recognized by IMM chaperones that then instruct proteases to degrade the non-functional protein. Proteins that become damaged through reactive chemical species are also targeted for degradation.

F1-F0 ATP synthase uses the electrochemical gradient across the IMM to generate the majority of ATP for the entire cell. This multi-protein complex is derived from both nuclear and mitochondrial gene products and follows a modular assembly pathway and requires stoichiometric incorporation of the polypeptides to form the active assembled enzyme (31). Ineffective assembly of F1-F0 ATP synthase leads to a dysfunctional enzyme that is unable to support the ATP requirements of a cell, potentially resulting in disease. To ensure appropriate enzyme assembly a number of check points need to be passed prior to assembly of the enzyme. Mitochondria need to identify and handle these accumulating assembly intermediates to prevent potentially damaging effects of these non-functional protein complexes (81). Specifically, in the absence of the β-subunit of F1-F0 ATP synthase the stability of other subunits is reduced by proteolytic degradation. The partner subunit of the β-subunit is the α-subunit that together form the F1 complex of F1-F0 ATP synthase. In the absence of the β-subunit, the α-subunit is proteolytically degraded almost immediately after translation and never accumulates as a stable polypeptide (82). The identity of the protease responsible for degradation of the α-subunit is currently unknown, but is likely to reside in either the matrix or as a matrix facing IMM protease. F1-F0 ATP synthase subunits have also been observed to undergo degradation by AFG3L2 in mammalian cells. Decreased expression of AFG3L2 by short interfering RNA caused an accumulation of newly translated ATP6 indicating a constitutive turnover of the newly translated polypeptide (83).

The IMM metalloprotease Oma1 has been described to degrade a temperature sensitive mutant of the IMM insertase Oxa1. Wild-type Oxa1 is responsible for the insertion of IMM proteins derived from the mitochondrial genome and is vital for OXPHOS complex production with lack of function of this protein resulting in respiratory deficiency in yeast (84). Oma1 is a quality control protein as it is involved in degrading misfolded IMM proteins or stalled assembly intermediates of OXPHOS complexes, such as cytochrome oxidase subunit 1 in yeast (85). In humans, Oma1 is responsible for the degradation of Opa1, an IMM protein involved in mitochondrial fusion (86,87) as well as of newly translated respiratory subunits Co1, CytB, and Nd1 (83). How Oma1 recognizes its targets is not known and is currently under investigation in our laboratory.

A very intriguing mechanism of rapid turnover of mitochondrial IMM proteins has been described in the past 10 years for members of the uncoupling family of proteins. These proteins are able to mediate proton transport across the IMM, dissipating the mitochondrial membrane potential and generally decreasing efficiency of ATP production by the introduction of a leak across the IMM. Two members of this family, UCP2 and UCP3, have been described to have short half-lives of approximately 0.5-4 hours (63,88). The close homolog, UCP1 has a longer half-life ranging from 20-70 hours (89). *In vitro*, UCP3 turnover is accelerated by the presence of cytosolic proteasomes and is dependent on ubiquitin indicating that the UPS was degrading UCP3. The dependence of UCP2 degradation on the UPS has not been shown, but is anticipated to be through similar mechanisms. The molecular mechanism by which UCP proteins that are localized in the IMM can become accessible to the UPS is purely speculative, ranging from intra-mitochondrial ubiquitylation of UCP3 or retrotranslocation of the protein to the OMM where it can be recognized by the UPS. Intriguingly this mechanism appears to rely on a high mitochondrial membrane potential (greater than 120mV) and occurs when mitochondria are in a healthy state. Ubiquitylation of IMM proteins is not isolated to UCP proteins, the IMM chaperone prohibitin is ubiquitylated during spermatogenesis and potentially plays a part in destruction of sperm post-fertilization (90).

**Summary**

The life-cycle of mitochondrial membrane transporters and ion channels proceeds through translation, incorporation into membrane, interactions with other proteins, performance of function and then removal. In this review, we addressed the interactions with other proteins and the turnover of these proteins. In the high protein concentrations found in mitochondrial membranes proteins do not exist in isolation and interact with other proteins. The consequences of these interactions can connect seemingly separate pathways to coordinate signaling in cells. These interactions are dynamic and can alter depending on cellular signal transduction pathways. The complexes involving transporters and ion channels can bring about changes in electron transfer efficiency and reactive oxygen species production, can couple metabolite transport to cell death processes and co-ordinate mitochondrial protein import with ETC function. These complexes also may be the mechanism for communication from the cytosol to the matrix of mitochondria.

The mechanism of degradation of a transporter or ion channel depends on the membrane where the protein resides. Proteins of the OMM are post-translationally modified by ubiquitin which renders them susceptible to the cytosolic UPS system. These proteins are extracted from the membrane and degraded by the proteasome. Bulk clearance of mitochondria and mitochondrial proteins can be achieved by mitochondria specific autophagy after high levels of OMM protein ubiquitylation. Proteins located in the IMM are subject to the action of proteases localized to the IMM and are degraded *in situ*. Unique examples of IMM proteins being subject to degradation by the cytosolic UPS exist. However, the exact molecular details of this process are far from understood.

Further investigation into the dynamic nature of mitochondrial membrane complex formation and regulation will allow for identification of as of yet unknown mitochondrial membrane complexes and how these complexes are incorporated into signal transduction pathways initiated by cell cycle progression, cell death and metabolic switches. There are still many unanswered questions regarding the turnover of mitochondrial membrane proteins including selective degradation of substrates, how protease activity is regulated and the nature of interactions between proteases and their substrates.

**Figure legends**

**Figure 1** Protein complexes found in mitochondrial membranes. Organization of different types of mitochondrial membrane complexes including soluble proteins found in the matrix, IMS or cytoplasm. Examples of each type of complex are provided. The ERMES (endoplasmic reticulum-mitochondria encounter structure) does not possess channel or transporter function but is thought to be involved in lipid transfer between mitochondria and the endoplasmic reticulum (91). It is indicated here as an example of a complex composed of proteins from all compartments considered in this review but will not be discussed further.

**Figure 2** Generation of mitochondrial membrane complexes. Polypeptides synthesized on cytoplasmic ribosomes are inserted into the OMM or IMM. Polypeptides synthesized in the mitochondrial matrix and inserted into the IMM. Peptides synthesized and folded in the cytoplasm respond to stimuli that cause translocation to the OMM. In the OMM or IMM proteins interact with other proteins to elicit a wide variety of functions.

**Figure 3** Mechanisms of OMM protein degradation. Proteins in the OMM undergo ubiquitylation by ubiquitin E3 ligases. These proteins are then retrotranslocated and degraded by the cytosolic UPS.

**Figure 4** Mechanisms of IMM protein degradation. Proteins in the IMM can be degraded by IMM localized proteases (top left) or matrix proteases (bottom left). Some proteins (UCP2 and UCP3) undergo ubiquitin dependent degradation by the cytosolic UPS, either by ubiquitylation first followed by retrotranslocation (top right) or retrotranslocation followed by ubiquitylation (bottom right).

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