Complex Formation and Turnover of Mitochondrial Transporters and Ion Channels

Gavin P. McStay,

432 Theobald Science Center,

Department of Life Sciences,

New York Institute of Technology,

Old Westbury,

NY 11568.

E-mail: [gmcstay@nyit.edu](mailto:gmcstay@nyit.edu)

Telephone number: +1-516-686-1202

**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).

**Acknowledgments**

This work was supported by NYIT startup funds and an Institutional Support for Research & Creativity (ISRC) grant to GPM.

**Bibliography**

1. Szabo I, Zoratti M. Mitochondrial channels: ion fluxes and more. Physiol Rev. 2014 Apr;94(2):519–608.

2. Bioenergetics, 4th Edition | David Nicholls, Stuart Ferguson | ISBN 9780123884312.

3. Palmieri F. Mitochondrial carrier proteins. FEBS Lett. 1994 Jun 6;346(1):48–54.

4. Bohnert M, Pfanner N, van der Laan M. Mitochondrial machineries for insertion of membrane proteins. Curr Opin Struct Biol. 2015 Aug 18;33:92–102.

5. Stuart R. Insertion of proteins into the inner membrane of mitochondria: the role of the Oxa1 complex. Biochim Biophys Acta. 2002 Sep 2;1592(1):79–87.

6. Perocchi F, Jensen LJ, Gagneur J, Ahting U, von Mering C, Bork P, et al. Assessing systems properties of yeast mitochondria through an interaction map of the organelle. PLoS Genet. 2006 Oct 20;2(10):e170.

7. Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. Cell. 2009 Aug 21;138(4):628–644.

8. Becker T, Wenz LS, Thornton N, Stroud D, Meisinger C, Wiedemann N, et al. Biogenesis of mitochondria: dual role of Tom7 in modulating assembly of the preprotein translocase of the outer membrane. J Mol Biol. 2011 Jan 7;405(1):113–124.

9. Thornton N, Stroud DA, Milenkovic D, Guiard B, Pfanner N, Becker T. Two modular forms of the mitochondrial sorting and assembly machinery are involved in biogenesis of alpha-helical outer membrane proteins. J Mol Biol. 2010 Feb 26;396(3):540–549.

10. Schmidt O, Harbauer AB, Rao S, Eyrich B, Zahedi RP, Stojanovski D, et al. Regulation of mitochondrial protein import by cytosolic kinases. Cell. 2011 Jan 21;144(2):227–239.

11. Rao S, Schmidt O, Harbauer AB, Schönfisch B, Guiard B, Pfanner N, et al. Biogenesis of the preprotein translocase of the outer mitochondrial membrane: protein kinase A phosphorylates the precursor of Tom40 and impairs its import. Mol Biol Cell. 2012 May;23(9):1618–1627.

12. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science. 2003 Jul 25;301(5632):513–517.

13. Ren D, Kim H, Tu HC, Westergard TD, Fisher JK, Rubens JA, et al. The VDAC2-BAK rheostat controls thymocyte survival. Sci Signal. 2009 Aug 25;2(85):ra48.

14. Roy SS, Ehrlich AM, Craigen WJ, Hajnóczky G. VDAC2 is required for truncated BID-induced mitochondrial apoptosis by recruiting BAK to the mitochondria. EMBO Rep. 2009 Dec;10(12):1341–1347.

15. Naghdi S, Várnai P, Hajnóczky G. Motifs of VDAC2 required for mitochondrial Bak import and tBid-induced apoptosis. Proc Natl Acad Sci U S A. 2015 Oct 13;112(41):E5590–E5599.

16. Lazarou M, Stojanovski D, Frazier AE, Kotevski A, Dewson G, Craigen WJ, et al. Inhibition of Bak activation by VDAC2 is dependent on the Bak transmembrane anchor. J Biol Chem. 2010 Nov 19;285(47):36876–36883.

17. Rostovtseva TK, Sheldon KL, Hassanzadeh E, Monge C, Saks V, Bezrukov SM, et al. Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration. Proc Natl Acad Sci U S A. 2008 Dec 2;105(48):18746–18751.

18. Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, et al. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature. 1996 May 23;381(6580):335–341.

19. Renault TT, Chipuk JE. Death upon a kiss: mitochondrial outer membrane composition and organelle communication govern sensitivity to BAK/BAX-dependent apoptosis. Chem Biol. 2014 Jan 16;21(1):114–123.

20. Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends Cell Biol. 2008 Apr;18(4):157–164.

21. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell. 2002 Nov 1;111(3):331–342.

22. Dejean LM, Martinez-Caballero S, Guo L, Hughes C, Teijido O, Ducret T, et al. Oligomeric Bax is a component of the putative cytochrome c release channel MAC, mitochondrial apoptosis-induced channel. Mol Biol Cell. 2005 May;16(5):2424–2432.

23. Pavlov EV, Priault M, Pietkiewicz D, Cheng EH, Antonsson B, Manon S, et al. A novel, high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast. J Cell Biol. 2001 Nov 26;155(5):725–731.

24. Martinez-Caballero S, Dejean LM, Kinnally MS, Oh KJ, Mannella CA, Kinnally KW. Assembly of the mitochondrial apoptosis-induced channel, MAC. J Biol Chem. 2009 May 1;284(18):12235–12245.

25. Eskes R, Desagher S, Antonsson B, Martinou JC. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol Cell Biol. 2000 Feb;20(3):929–935.

26. Gross A, Jockel J, Wei MC, Korsmeyer SJ. Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. EMBO J. 1998 Jul 15;17(14):3878–3885.

27. Antonsson B, Montessuit S, Sanchez B, Martinou JC. Bax is present as a high molecular weight oligomer/complex in the mitochondrial membrane of apoptotic cells. J Biol Chem. 2001 Apr 13;276(15):11615–11623.

28. Antonsson B, Montessuit S, Lauper S, Eskes R, Martinou JC. Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome c release from mitochondria. Biochem J. 2000 Jan 15;345 Pt 2:271–278.

29. Chipuk JE, McStay GP, Bharti A, Kuwana T, Clarke CJ, Siskind LJ, et al. Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. Cell. 2012 Mar 2;148(5):988–1000.

30. Mick DU, Fox TD, Rehling P. Inventory control: cytochrome c oxidase assembly regulates mitochondrial translation. Nat Rev Mol Cell Biol. 2011 Jan;12(1):14–20.

31. Rak M, Gokova S, Tzagoloff A. Modular assembly of yeast mitochondrial ATP synthase. EMBO J. 2011 Mar 2;30(5):920–930.

32. McStay GP, Su CH, Tzagoloff A. Modular assembly of yeast cytochrome oxidase. Mol Biol Cell. 2013 Feb;24(4):440–452.

33. Mimaki M, Wang X, McKenzie M, Thorburn DR, Ryan MT. Understanding mitochondrial complex I assembly in health and disease. Biochim Biophys Acta. 2012 Jun 1;1817(6):851–862.

34. Smith PM, Fox JL, Winge DR. Biogenesis of the cytochrome bc(1) complex and role of assembly factors. Biochim Biophys Acta. 2012 Feb 1;1817(2):276–286.

35. Acin-Perez R, Enriquez JA. The Function of the Respiratory Supercomplexes: The plasticity model. Biochim Biophys Acta. 2013 Dec 21;

36. Acín-Pérez R, Fernández-Silva P, Peleato ML, Pérez-Martos A, Enriquez JA. Respiratory active mitochondrial supercomplexes. Mol Cell. 2008 Nov 21;32(4):529–539.

37. Schägger H, Pfeiffer K. Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J. 2000 Apr 17;19(8):1777–1783.

38. Crichton PG, Harding M, Ruprecht JJ, Lee Y, Kunji ERS. Lipid, detergent, and Coomassie Blue G-250 affect the migration of small membrane proteins in blue native gels: mitochondrial carriers migrate as monomers not dimers. J Biol Chem. 2013 Jul 26;288(30):22163–22173.

39. Kunji ERS, Crichton PG. Mitochondrial carriers function as monomers. Biochim Biophys Acta. 2010 Jul 1;1797(6-7):817–831.

40. Haworth RA, Hunter DR. The Ca2+-induced membrane transition in mitochondria. Arch Biochem Biophys. 1979 Jul;195(2):460–467.

41. Hunter DR, Haworth RA. The Ca2+-induced membrane transition in mitochondria. Arch Biochem Biophys. 1979 Jul;195(2):453–459.

42. Halestrap AP, Woodfield KY, Connern CP. Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. J Biol Chem. 1997 Feb 7;272(6):3346–3354.

43. Leung AWC, Varanyuwatana P, Halestrap AP. The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition. J Biol Chem. 2008 Sep 26;283(39):26312–26323.

44. Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, et al. Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci U S A. 2013 Apr 9;110(15):5887–5892.

45. Alavian KN, Beutner G, Lazrove E, Sacchetti S, Park H-A, Licznerski P, et al. An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. Proc Natl Acad Sci U S A. 2014 Jun 16;

46. Crompton M, Ellinger H, Costi A. Inhibition by cyclosporin A of a Ca2+-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. Biochem J. 1988 Oct 1;255(1):357–360.

47. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature. 2005 Mar 31;434(7033):658–662.

48. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, et al. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature. 2004 Jan 29;427(6973):461–465.

49. Baines C, Kaiser R, Sheiko T, Craigen W, Molkentin J. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat Cell Biol. 2007 May 1;9(5):550–555.

50. Sileikyte J, Blachly-Dyson E, Sewell R, Carpi A, Menabo R, Di Lisa F, et al. Regulation of the Mitochondrial Permeability Transition Pore by the Outer Membrane does not Involve the Peripheral Benzodiazepine Receptor (TSPO). J Biol Chem. 2014 Apr 1;

51. Neupert W, Herrmann JM. Translocation of proteins into mitochondria. Annu Rev Biochem. 2007 Jan;76:723–749.

52. Koehler CM, Murphy MP, Bally NA, Leuenberger D, Oppliger W, Dolfini L, et al. Tim18p, a new subunit of the TIM22 complex that mediates insertion of imported proteins into the yeast mitochondrial inner membrane. Mol Cell Biol. 2000 Feb;20(4):1187–1193.

53. Kerscher O, Sepuri NB, Jensen RE. Tim18p is a new component of the Tim54p-Tim22p translocon in the mitochondrial inner membrane. Mol Biol Cell. 2000 Jan;11(1):103–116.

54. Gebert N, Gebert M, Oeljeklaus S, von der Malsburg K, Stroud DA, Kulawiak B, et al. Dual function of Sdh3 in the respiratory chain and TIM22 protein translocase of the mitochondrial inner membrane. Mol Cell. 2011 Dec 9;44(5):811–818.

55. Mehnert CS, Rampelt H, Gebert M, Oeljeklaus S, Schrempp SG, Kochbeck L, et al. The mitochondrial ADP/ATP carrier associates with the inner membrane presequence translocase in a stoichiometric manner. J Biol Chem. 2014 Sep 26;289(39):27352–27362.

56. Stuart RA. Chapter 11 Supercomplex Organization of the Yeast Respiratory Chain Complexes and the ADP/ATP Carrier Proteins. Mitochondrial Function, Part A: Mitochondrial Electron Transport Complexes and Reactive Oxygen Species. Elsevier; 2009. p. 191–208.

57. Dienhart MK, Stuart RA. The yeast Aac2 protein exists in physical association with the cytochrome bc1-COX supercomplex and the TIM23 machinery. Mol Biol Cell. 2008 Sep;19(9):3934–3943.

58. Geissler A, Chacinska A, Truscott KN, Wiedemann N, Brandner K, Sickmann A, et al. The Mitochondrial Presequence Translocase. Cell. 2002 Nov;111(4):507–518.

59. Brdiczka DG, Zorov DB, Sheu S-S. Mitochondrial contact sites: their role in energy metabolism and apoptosis. Biochim Biophys Acta. 2006 Feb 1;1762(2):148–163.

60. Brunner G, Neupert W. Turnover of outer and inner membrane proteins of rat liver mitochondria. FEBS Lett. 1968 Aug;1(3):153–155.

61. Karbowski M, Youle RJ. Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. Curr Opin Cell Biol. 2011 Aug;23(4):476–482.

62. Mookerjee SA, Brand MD. Characteristics of the turnover of uncoupling protein 3 by the ubiquitin proteasome system in isolated mitochondria. Biochim Biophys Acta. 2011 Nov;1807(11):1474–1481.

63. Azzu V, Mookerjee SA, Brand MD. Rapid turnover of mitochondrial uncoupling protein 3. Biochem J. 2010 Feb 15;426(1):13–17.

64. Azzu V, Jastroch M, Divakaruni AS, Brand MD. The regulation and turnover of mitochondrial uncoupling proteins. Biochim Biophys Acta. 2010 Jul;1797(6-7):785–791.

65. Liu L, Sakakibara K, Chen Q, Okamoto K. Receptor-mediated mitophagy in yeast and mammalian systems. Cell Res. 2014 Jul;24(7):787–795.

66. Sugiura A, McLelland GL, Fon EA, McBride HM. A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. EMBO J. 2014 Oct 1;33(19):2142–2156.

67. Narendra D, Walker JE, Youle R. Mitochondrial quality control mediated by PINK1 and Parkin: links to parkinsonism. Cold Spring Harb Perspect Biol. 2012 Nov 1;4(11).

68. Fu M, St-Pierre P, Shankar J, Wang PTC, Joshi B, Nabi IR. Regulation of mitophagy by the Gp78 E3 ubiquitin ligase. Mol Biol Cell. 2013 Apr;24(8):1153–1162.

69. Xu S, Cherok E, Das S, Li S, Roelofs BA, Ge SX, et al. Mitochondrial E3 ubiquitin ligase MARCH5 controls mitochondrial fission and cell sensitivity to stress-induced apoptosis through regulation of MiD49 protein. Mol Biol Cell. 2015 Nov 12;

70. Matsumoto M, Hatakeyama S, Oyamada K, Oda Y, Nishimura T, Nakayama KI. Large-scale analysis of the human ubiquitin-related proteome. Proteomics. 2005 Nov;5(16):4145–4151.

71. Maor R, Jones A, Nühse TS, Studholme DJ, Peck SC, Shirasu K. Multidimensional protein identification technology (MudPIT) analysis of ubiquitinated proteins in plants. Mol Cell Proteomics. 2007 Apr;6(4):601–610.

72. Peng J, Schwartz D, Elias JE, Thoreen CC, Cheng D, Marsischky G, et al. A proteomics approach to understanding protein ubiquitination. Nat Biotechnol. 2003 Aug;21(8):921–926.

73. Yoshii SR, Kishi C, Ishihara N, Mizushima N. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. J Biol Chem. 2011 Jun 3;286(22):19630–19640.

74. Liu FT, Agrawal SG, Gribben JG, Ye H, Du MQ, Newland AC, et al. Bortezomib blocks Bax degradation in malignant B cells during treatment with TRAIL. Blood. 2008 Mar 1;111(5):2797–2805.

75. Li B, Dou QP. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. Proc Natl Acad Sci U S A. 2000 Apr 11;97(8):3850–3855.

76. Edlich F, Banerjee S, Suzuki M, Cleland MM, Arnoult D, Wang C, et al. Bcl-x(L) retrotranslocates Bax from the mitochondria into the cytosol. Cell. 2011 Apr 1;145(1):104–116.

77. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev. 2000 Dec 1;14(23):3065–3073.

78. Thomas M, Banks L. Inhibition of Bak-induced apoptosis by HPV-18 E6. Oncogene [Internet]. 1998; Available from: http://europepmc.org/abstract/med/9881696

79. Holloway A, Simmonds M, Azad A, Fox JL, Storey A. Resistance to UV-induced apoptosis by β-HPV5 E6 involves targeting of activated BAK for proteolysis by recruitment of the HERC1 ubiquitin ligase. Int J Cancer. 2015 Jun 15;136(12):2831–2843.

80. Ferramosca A, Zara V. Biogenesis of mitochondrial carrier proteins: molecular mechanisms of import into mitochondria. Biochim Biophys Acta. 2013 Mar;1833(3):494–502.

81. Rak M, Tzagoloff A. F1-dependent translation of mitochondrially encoded Atp6p and Atp8p subunits of yeast ATP synthase. Proc Natl Acad Sci U S A. 2009 Nov 3;106(44):18509–18514.

82. Rak M, McStay GP, Fujikawa M, Yoshida M, Manfredi G, Tzagoloff A. Turnover of ATP synthase subunits in F1-depleted HeLa and yeast cells. FEBS Lett. 2011 Aug 19;585(16):2582–2586.

83. Richter U, Lahtinen T, Marttinen P, Suomi F, Battersby BJ. Quality control of mitochondrial protein synthesis is required for membrane integrity and cell fitness. J Cell Biol. 2015 Oct 26;211(2):373–389.

84. Kaser M, Kambacheld M, Kisters-Woike B, Langer T. Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease. J Biol Chem. 2003 Nov 21;278(47):46414–46423.

85. Khalimonchuk O, Jeong M-Y, Watts T, Ferris E, Winge DR. Selective Oma1 protease-mediated proteolysis of Cox1 subunit of cytochrome oxidase in assembly mutants. J Biol Chem. 2012 Mar 2;287(10):7289–7300.

86. Ehses S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D, et al. Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol. 2009 Dec 28;187(7):1023–1036.

87. Head B, Griparic L, Amiri M, Gandre-Babbe S, van der Bliek AM. Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. J Cell Biol. 2009 Dec 28;187(7):959–966.

88. Rousset S, Mozo J, Dujardin G, Emre Y, Masscheleyn S, Ricquier D, et al. UCP2 is a mitochondrial transporter with an unusual very short half-life. FEBS Lett. 2007 Feb 6;581(3):479–482.

89. Puigserver P, Herron D, Gianotti M, Palou A, Cannon B, Nedergaard J. Induction and degradation of the uncoupling protein thermogenin in brown adipocytes in vitro and in vivo. Evidence for a rapidly degradable pool. Biochem J. 1992 Jun 1;284 ( Pt 2):393–398.

90. Thompson WE, Ramalho-Santos J, Sutovsky P. Ubiquitination of prohibitin in mammalian sperm mitochondria: possible roles in the regulation of mitochondrial inheritance and sperm quality control. Biol Reprod. 2003 Jul;69(1):254–260.

91. Lang A, John Peter AT, Kornmann B. ER-mitochondria contact sites in yeast: beyond the myths of ERMES. Curr Opin Cell Biol. 2015 Aug;35:7–12.