Mitochondrial quality control by the ubiquitin–proteasome system

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Abstract
Mitochondria perform multiple functions critical to the maintenance of cellular homeostasis and their dysfunction leads to disease. Several lines of evidence suggest the presence of a MAD (mitochondria-associated degradation) pathway that regulates mitochondrial protein quality control. Internal mitochondrial proteins may be retrotranslocated to the OMM (outer mitochondrial membrane), multiple E3 ubiquitin ligases reside at the OMM and inhibition of the proteasome causes accumulation of ubiquitinated proteins at the OMM. Reminiscent of ERAD [ER (endoplasmic reticulum)-associated degradation], Cdc48 (cell division cycle 42)/p97 is recruited to stressed mitochondria, extracts ubiquitinated proteins from the OMM and presents ubiquitinated proteins to the proteasome for degradation. Recent research has provided mechanistic insights into the interaction of the UPS (ubiquitin–proteasome system) with the OMM. In yeast, Vms1 [VCP (valosin-containing protein) (p97)/Cdc48-associated mitochondrial-stress-responsive 1] protein recruits Cdc48/p97 to the OMM. In mammalian systems, the E3 ubiquitin ligase parkin regulates the recruitment of Cdc48/p97 to mitochondria, subsequent mitochondrial protein degradation and mitochondrial autophagy. Disruption of the Vms1 or parkin systems results in the hyper-accumulation of ubiquitinated proteins at mitochondria and subsequent mitochondrial dysfunction. The emerging MAD pathway is important for the maintenance of cellular and therefore organismal viability.

Introduction
Mitochondria perform many critical functions in eukaryotes. Among these, mitochondria sustain cells with a continuous supply of ATP, integrate biosynthetic pathways, co-ordinate redox signalling and regulate apoptotic balance. Because of the multiple critical processes mitochondria facilitate, their dysfunction leads to the loss of cellular function and, in multicellular organisms, the onset of disease. In humans, mitochondrial dysfunction causes numerous pathologies including cancer [1,2], heart failure [3], neurological diseases [4], myopathies [5] and a multitude of other familial disorders.

Although the molecular bases for mitochondrial dysfunction are poorly understood and probably diverse, damage by ROS (reactive oxygen species) is thought to be a primary cause. Because mitochondria house the reactions of the electron transport chain and are a final destination for molecular oxygen, they are the major cellular source of ROS. During oxidative phosphorylation, electrons may escape the electron transport chain and react with molecular oxygen to form the highly reactive superoxide anion, which can form other highly reactive ROS and reactive nitrogen species. Although mitochondria are equipped with mechanisms to quench free radicals and minimize oxidative damage to component proteins and lipids, these systems are not sufficient for eliminating damage.

Mitochondria are further subjected to unique biochemical stresses because of their complex biogenesis. Mitochondrial biogenesis requires the co-ordinated assembly and sorting of both nuclear- and mitochondrially encoded proteins. The double membrane structure of mitochondria results in several distinct mitochondrial compartments, the matrix, the inner membrane, the intermembrane space and the outer membrane. Nuclear-encoded mitochondrial proteins must be translocated from the cytosol and sorted to one of these compartments. After sorting, they must often be co-ordinately assembled into multiprotein complexes with mitochondrially encoded proteins and cofactors. Although these processes are tightly regulated by dedicated translocases and chaperone proteins, import, sorting and assembly may fail.

Thus mitochondria are subjected to unique and sometimes overwhelming biochemical stresses, resulting from both their function as a containment vessel for oxidative phosphorylation and from their unique biogenic requirements. The acute failure of ROS detoxifying and protein sorting and assembly systems may initiate progressive mitochondrial failure. ROS generation increases with mitochondrial defects. Therefore mitochondrial dysfunction can spiral into a vicious cycle whereby damaged mitochondria produce more reactive ROS that further damage mitochondria and disrupt protein homeostasis, leading to more ROS and so on [6]. If mitochondria continuously sustain damage, how do they...
Mitochondrial quality control

Elaborate quality control mechanisms have evolved for protecting mitochondria from ROS and repairing or eliminating damaged mitochondria. These mechanisms include autophagic consumption of dysfunctional mitochondria [7], constant fusion and fission to partition and dissipate damaged mitochondria and mitochondrial proteolytic systems to degrade damaged mitochondrial proteins. Proteases for mitochondrial protein quality control are found within each mitochondrial compartment. Two AAA+ proteases (AAA is ATPase associated with various cellular activities), Lon and ClpXP, reside in the matrix and degrade oxidatively damaged proteins in the matrix. A pair of AAA metalloproteases patrol opposite faces of the mitochondrial inner membrane. The m-AAA (matrix AAA) protease faces and performs protein quality control functions on the matrix side, while the i-AAA (intermembrane AAA) protease does the same at the intermembrane space side [8,9]. These proteases are important for clearing dysfunctional proteins from the inner membrane, which may accumulate due to failures in respiratory chain complex assembly or oxidative damage. Although the mitochondrial proteases collectively are critical for the prevention of mitochondrial dysfunction, their study has failed to explain aspects of mitochondrial quality control, particularly the degradation of outer membrane proteins.

The UPS (ubiquitin–proteasome system) and ERAD [ER (endoplasmic reticulum)-associated degradation]

The UPS is a eukaryotic proteolytic system that operates at the cytosol and the ER membrane. After being marked for degradation by ubiquitination, these proteins must be fully extracted from the ER membrane. The mechanism for this extraction is conserved throughout eukaryota. During ER stress, the AAA ATPase Cdc48 (cell division cycle 42)/p97, in complex with its adaptor proteins Npl4 and Ufd1, is recruited to the ER, extracts ubiquitinated proteins and presents them to the proteasome, which degrades them [13]. Thus the UPS regulates protein quality control at the ER, an organelle that excludes it.

MAD (mitochondria-associated degradation)

Because the UPS is similarly excluded from mitochondria, an ERAD-like system, MAD, has been proposed to operate at mitochondria [10–12,14,15]. In this system, matrix or inner membrane proteins are retrotranslocated to the outer membrane for ubiquitination and targeting to the proteasome. Several observations support the existence of such a system. Proteins associated with various mitochondrial compartments are ubiquitinated and subjected to proteasomal degradation, but without a mechanistic explanation this could be discounted as being pre-import regulation. If an ERAD-like system operates at the OMM, then by analogy, the OMM, like the ER membrane, should also harbour E3 ubiquitin ligases. Indeed, several E3 ligases reside at the OMM. Current data suggest that they may perform both regulatory and protein quality control functions.

Mitol/March5 ubiquitinates Drp1 and Fis1, two proteins regulating mitochondrial fission [16]. Depletion of Mitol/March5 results in excess fission, leading to mitochondrial fragmentation. In a non-regulatory role, Mitol/March5 also ubiquitinates the mSOD1 (mutant SOD1 [superoxide dismutase 1]) that causes amyotrophic lateral sclerosis, and which accumulates in mitochondria [17]. Mitol/March5 depletion causes increased mitochondrial mSOD1 accumulation, suggesting that ubiquitination of mSOD1 at mitochondria is important for its degradation, thereby implicating the UPS at mitochondria. Additional mitochondrial E3 ligases include Mulan, Mdm30, Mfb1 and Rsp5, which all induce changes in mitochondrial morphology on knockdown or deletion [11]. Mdm30 ubiquitinates Fzo1, which is important for mitochondrial fusion in budding yeast [18], but targets for the other ligases have not been discovered. A recent proteomics study identified over 100 ubiquitinated proteins in mitochondrial extracts purified from mouse heart, providing numerous putative targets for known and yet-to-be-discovered mitochondrial E3 ligases [19].

Until recently, there was no mechanistic evidence for MAD. However, data from Margineantu et al. [20] demonstrate that proteins are ubiquitinated at mitochondria, and some proteins may be retrotranslocated to the OMM to be accessed by the proteasome. They found that the OSCP (oligomycin-sensitivity-conferring protein) subunit of the.
F$_{1}$F$_{0}$-ATPase, which resides at the IMM (inner mitochondrial membrane), is retrotranslocated to the OMM where it is ubiquitinated and degraded by the proteasome. More broadly, treatment with proteasome inhibitors increased the accumulation of many mitochondrial proteins, including the mitochondrially encoded COX1 (cyclo-oxygenase 1), which because of its synthesis in the matrix would require retrotranslocation for access by the proteasome. Treatment with lysosomal and macroautophagy inhibitors has no such effect, suggesting that the increase in mitochondrial proteins did not result from a regulatory interaction between proteasomal inhibition and mitophagy. Consistent with this proposed phenomenon, Azzu et al. [21,22] found that UCP2 (uncoupling protein 2) and UCP3, which also reside at the IMM, are degraded by the proteasome. They constructed reconstituted systems in vitro, where UCP2 and UCP3 in intact, energized mitochondria are degraded by the proteasome, implying retrotranslocation. However, despite evidence for retrotranslocation to the OMM, a mechanism for the interaction between the UPS and the OMM remained undefined.

**Vms1 regulates mitochondrial protein degradation**

We recently identified a mechanism in yeast whereby a novel protein, which we have named Vms1 [VCP (valosin-containing protein) (p97)/Cdc48-associated mitochondrial-stress-responsive 1], recruits Cdc48/p97 and Npl4 to stressed mitochondria [15]. Under normal growth conditions the Vms1 complex resides in the cytosol. However, on treatment with mitochondrial toxins including uncouplers, respiratory chain poisons and oxidants, Vms1 recruits Cdc48/p97 and Npl4 to mitochondria [15]. Efforts are under way to identify additional substrates of the Vms1/MAD system. Tran et al. [23] recently found Vms1 to generally regulate ubiquitin unloading from ubiquitinylated mitochondrial proteins and further experiments demonstrated that VMS1 deletion decreased the rate of Fzo1 degradation, a known substrate of the mitochondrial E3 ligase Mdm30 [15]. Efforts are under way to identify additional substrates of the Vms1/MAD system. Tran et al. [23] recently found Vms1 to generally regulate ubiquitin unloading from Cdc48/p97, a function that would be important during MAD, but may also implicate Vms1 in some aspects of ERAD. Biochemically, the Vms1 system appears to be an arm of the UPS distinct from ERAD. By co-immunoprecipitation experiments we found that the association of Vms1 and Ufd1 with Cdc48/p97 is mutually exclusive, suggesting that the Ufd1–Npl4–Cdc48/p97 complex is devoted to ERAD, while the Vms1–Npl4–Cdc48/p97 complex is devoted to MAD. Yet, Cdc48 translocation to mitochondria is not absolutely dependent on Vms1. We observed translocation of a reduced fraction of Cdc48 to mitochondria in the absence of Vms1, indicating that there is at least one other mode for targeting Cdc48 to mitochondria. However, under conditions of mitochondrial stress that cause translocation of the Vms1 complex to mitochondria, the absence of Vms1 causes mitochondrial accumulation of polyubiquitinated proteins, biochemical defects and respiratory failure. Thus, in yeast, Vms1-dependent MAD is critical for cell viability during mitochondrial stress.

The Vms1 gene is highly conserved from yeast to humans and initial data suggest that the Vms1 system may be conserved throughout eukaryota. We found that Vms1 knockdown in Caenorhabditis elegans resulted in hypersensitivity to H$_2$O$_2$, which caused Vms1 to translocate to mitochondria [15]. Thus, as in yeast, Vms1 in C. elegans is critical, at mitochondria, for cellular and organismal viability under conditions of mitochondrial stress. Initial data suggest that the conservation of the Vms1 system also extends to mammals. Human Vms1 partially rescues the yeast VMS1 deletion phenotype and Vms1 in mammalian cells also tandem-affinity purifies with Cdc48/p97. Thus Vms1 functions as a Cdc48/p97 adaptor protein in mammalian systems. While the cellular function of Vms1 in mammals remains to be elucidated, two recent studies have implicated Cdc48/p97, and by analogy to yeast, Vms1, in OMM protein degradation in mammalian systems, thereby providing mechanistic insights into mammalian MAD.

**p97 and parkin are recruited to mitochondria**

Xu et al. [24] found that the degradation of OMM proteins Mfn1 and Mcl1 in mammalian cells is proteasome- and p97-dependent. They also found a partial association of p97 with mitochondria, indicating that a fraction of p97 associates with mitochondria to regulate the turnover of OMM proteins in mammalian systems. These findings were extended by Tanaka et al. [25], who also reported that p97 localizes to mitochondria, but with the additional finding that chemically uncoupling mitochondria causes large-scale translocation of p97 to mitochondria. This result is consistent with our studies on Vms1 in yeast, where uncoupling causes the recruitment of Cdc48/p97 to mitochondria by Vms1. They also found that uncoupling-stimulated p97 translocation to the OMM is dependent on the E3 ubiquitin ligase activity of the protein parkin, which has no orthologues in yeast. This finding provides a mechanistic link between Parkinson’s disease and mitochondrial dysfunction.

Mutations in parkin are a major cause of familial Parkinson’s disease, accounting for over 50% of all juvenile-onset cases [26], and Parkinson’s disease has been increasingly associated with mitochondrial dysfunction. Parkin is selectively recruited to depolarized mitochondria and marks them for mitophagy [27], a topic only superficially addressed in this review. Tanaka et al. [25] showed that uncoupling-stimulated, parkin-dependent, p97 OMM localization facilitates the proteasome-dependent degradation of Mfn1 and Mfn2,
which prevents re-fusion of depolarized mitochondria. Thus parkin acts upstream of recruitment of p97, and potentially Vms1, to the OMM to enable the selective mitophagy of depolarized mitochondria. Indeed, constitutive overexpression of parkin in heteroplasmic cybrid cell lines increases the ratio of wild-type to mutant mtDNA (mitochondrial DNA), probably by increasing the mitophagy of the defective mitochondria harbouring mutant mtDNA [28]. In addition to its role in mitophagy, the parkin–p97 pathway exerts a general protein quality control function at the OMM. Both Chan et al. [29] and Yoshii et al. [30] recently demonstrated that mitochondrial uncoupling causes the parkin-dependent degradation of multiple OMM proteins independent of mitophagy. This is an important distinction because it shows that the UPS broadly regulates OMM protein degradation, a feature required of a bona fide ERAD-like MAD system and provides a partial explanation of the mechanism, further elucidating the MAD pathway.

Summary and perspectives

Mitochondria are subject to unique stresses because they house oxidative phosphorylation, originate from two genomes and have two membranes. To maintain mitochondrial quality, a MAD pathway has been proposed to link mitochondrial protein quality control with the UPS. Findings from multiple groups have contributed to the delineation of a MAD pathway that targets proteins at the OMM or those retrotranslocated to it. Damaged proteins at the OMM are ubiquitinated by resident or selectively recruited E3 ligases, such as parkin in mammalian systems. Once ubiquitinated, they are extracted by Cdc48/p97, presented to the proteasome, and degraded. However, despite recent advances, some aspects of MAD remain superficially defined and trigger many interesting questions.

Although several studies have demonstrated that OMM proteins are ubiquitinated and degraded by the UPS (Figure 1), direct evidence for retrotranslocation is limited. To confirm retrotranslocation and to show that the UPS regulates protein quality for mitochondrial compartments other than the OMM in a true ERAD-like manner, a mechanism for retrotranslocation must be defined. Mechanistic details are also lacking for the mode by which the UPS is recruited to the OMM. In yeast, Vms1 recruits Cdc48/p97 to stressed mitochondria, although a fraction of Cdc48/p97 is recruited in a parkin-dependent manner. In mammalian systems, parkin is selectively recruited to depolarized mitochondria and ubiquitates proteins at the OMM, and Cdc48/p97 is recruited in a parkin-dependent manner. In yeast, Vms1 recruits Cdc48/p97 to stressed mitochondria, although a fraction of Cdc48/p97 is recruited by an unidentified Vms1-independent mechanism. The Vms1 gene is highly conserved in mammalian systems, where its role in mitochondrial protein degradation remains to be determined.

References

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