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Review

Mitochondrial proteolysis: Its emerging roles in stress responses

Shiori Sekine, Hidenori Ichijo *

Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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ABSTRACT

Background: Mitochondria are multifunctional organelles that not only serve as cellular energy stores but are also actively involved in several cellular stress responses, including apoptosis. In addition, mitochondria themselves are also continuously challenged by stresses such as reactive oxygen species (ROS), an inevitable by-product of oxidative phosphorylation. To exert various functions against these stresses, mitochondria must be equipped with appropriate stress responses that monitor and maintain their quality.
Scope of review: Interestingly, increasing evidence indicates that mitochondrial proteolysis has important roles in mitochondrial and cellular stress responses. In this review, we summarize current advances in mitochondrial proteolysis-mediated stress responses.
Major conclusions: Mitochondrial proteases do not only function as surveillance systems of protein quality control by degrading unfolded proteins but also regulate mitochondrial stress responses by processing specific mitochondrial proteins.
General significance: Studies on the regulation of mitochondrial proteolysis-mediated stress responses will provide the novel mechanistic insights into the stress response research fields.

1. Introduction

Mitochondria not only serve as cellular energy stores by producing ATP through oxidative phosphorylation but also function as platforms for various cellular stress responses. Apoptosis regulation is one of the well-known functions of mitochondria [1]. In addition, mitochondria are also involved in innate immunity responses, such as antiviral responses through mitochondria-resident viral receptors, and inflammasome activation through the release of specific mitochondria-derived molecules [2].

However, mitochondria themselves are also continuously challenged by several stresses. For example, reactive oxygen species (ROS), which is an inevitable by-product of oxidative phosphorylation, induce protein modifications, lipid peroxidation and mitochondrial DNA (mtDNA) damage, which ultimately results in mitochondrial dysfunction. Because mtDNA encodes several subunits of OXPHOS genes, ROS-induced mutations in mtDNA promote the further production of ROS through the impairment of oxidative phosphorylation. For quality control of mitochondria and the maintenance of their activity against these stresses, mitochondria must be equipped with systems to monitor their health condition and evoke appropriate stress responses. Recent studies discovered that some mitochondrial stress responses, such as content mixing by fusion, the mitochondrial unfolded protein response (mtUPR) and mitophagy, play important roles in quality control of the mitochondria [3].

Various types of proteases reside in each compartment of mitochondria, including the matrix, inner mitochondrial membrane (IMM) and mitochondrial intramembrane space (IMS) [4]. Interestingly, these proteases do not only function as surveillance systems of protein quality control by degrading unfolded proteins but also regulate mitochondrial stress responses by processing specific mitochondrial proteins. In this review, we focus on the emerging roles of mitochondrial proteolysis in the mitochondrial and cellular stress responses.

2. Main text

2.1. Mitochondria-resident proteases

Mitochondria are composed of approximately 1500 proteins. The majority of these are nuclearly encoded and thus synthesized in the cytosol. Many of these proteins are synthesized as precursor forms that contain mitochondria-targeting signals; these signal peptides are cleaved by mitochondria-localized peptidases after the import into mitochondria. Mitochondria also contain elaborate proteolytic systems that conduct the quality control of mitochondrial proteins. Severely damaged unfolded proteins, such as oxidized proteins or unassembled OXPHOS subunits, are recognized and rapidly degraded by these proteases. Mitochondria house several types of proteases in each compartment, which include the matrix, IMM and IMS [4]. Interestingly, recent studies have suggested that mitochondrial proteolysis also has important roles in the mitochondrial and cellular stress responses in addition to their conventional protein quality surveillance functions (Fig. 1). In

* Corresponding author.
E-mail address: ichijo@mol.f.u-tokyo.ac.jp (H. Ichijo).

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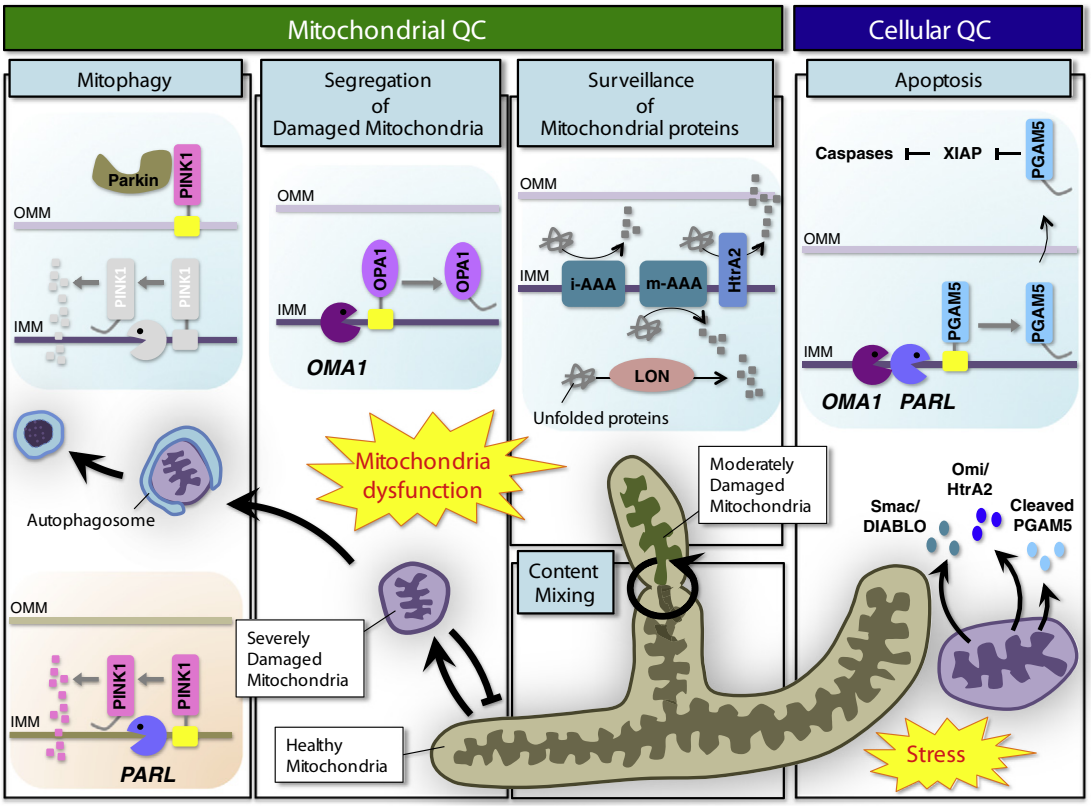

Fig. 1. The mitochondrial proteolysis-mediated stress responses. Mitochondrial proteolysis has important roles in both mitochondrial quality control (QC) and cellular QC. The best known function of mitochondrial proteases is the degradation of unfolded mitochondrial proteins. In addition, some proteases mediate the cleavage of specific substrates, which helps evoke the appropriate stress responses. For example, severely damaged mitochondria are segregated from healthy mitochondrial network through OMA1-mediated cleavage of mitochondrial fusion machinery OPA1. Although PINK1 is continuously cleaved and degraded by PARL in healthy mitochondria, PINK1 escapes from proteolytic degradation and is stabilized on damaged mitochondria, which promotes mitophagy, an autophagic degradation of dysfunctional mitochondria. PGAM5 is another example of a mitochondrial protein that is cleaved in response to the loss of mitochondrial membrane potential. PARL and OMA1 cooperatively regulate the cleavage of PGAM5. A recent study has suggested that cleaved PGAM5 is released into the cytosol and promotes apoptosis through XIAP inhibition.

the next section, we summarize the current understanding of the emerging roles of mitochondrial proteolysis.

2.2. Examples of mitochondrial proteolysis that evokes stress responses

2.2.1. Evoking the mitochondrial stress responses

2.2.1.1. $\Delta\psi_A$ loss-dependent cleavage of OPA1

2.2.1.1.1. Fusion and fission of mitochondria. Mitochondria are dynamic organelles that continuously fuse and divide. These processes are regulated by dynamin-related GTPases, including OPA1 and Mitofusin1/2 for fusion and Drp1 for fission [5]. The knockout (KO) mice for each of these genes are embryonic lethal, indicating that mitochondrial fusion and fission have important roles in the development and maintenance of individuals [5]. The pathophysiological significance is also indicated [5]. For example, mutations in *OPA1* cause degeneration of retinal ganglion cells in autosomal dominant optic atrophy. Heterozygous mutations in *Mitofusin2* cause Charcot-Marie-Tooth type 2A (CMT2A), which is an autosomal dominant disease characterized by axonal peripheral neuropathy. Although how the defects in mitochondrial fusion cause these neuronal dysfunctions is not fully understood, recent studies have suggested that the mitochondria ensure the overall activities of them by exchanging their contents, including mtDNA, proteins, and lipids. This model is called "content mixing" [5]. When content mixing is prevented by the genetic ablation of fusion machineries such as OPA1 or both Mitofusin 1 and 2, the mitochondria contain only a third of the normal levels of mtDNA compared with wild-type mitochondria [6,7]. In addition to reduced overall mtDNA levels, some mitochondria lose mtDNA due to severe defects in the distribution of mtDNA nucleoids [6]. Mutations in mtDNA are generally complemented by content mixing, but the cells lacking mitochondria fusion machineries cannot tolerate mtDNA mutations, ultimately showing defective respiratory phenotypes [6]. The defects in respiratory functions are also seen in yeast that carry mitochondrial fusion defects [8–10].

2.2.1.1.2. The cleavage of mitochondrial fusion machinery gene OPA1. Mitochondrial fusion is highly dependent on the mitochondrial membrane potential ($\Delta\psi_A$). For one reason, $\Delta\psi_A$ loss-dependent cleavage of the mitochondrial fusion machinery gene *OPA1* contributes to this process [11,12]. The *OPA1* gene encodes eight distinct splice variants in mammals. In addition to the conventional cleavage of N-terminal mitochondrial targeting signals, mammalian OPA1 contains two cleavage sites, designated as the S1 site and the S2 site (Fig. 2). Cleavage at the S2 site always occurs under steady state [13]. However, cleavage at the S1 site is stress-dependent [11,12]. Individual long or short isoforms are insufficient for fusion, and both forms must be present at an appropriate balance for fusion [13]. Therefore, the complete $\Delta\psi_A$ loss-dependent conversion to the short form inhibits fusion.

2.2.1.1.3. Cleavage of OPA1 during stress responses. Thus, why mitochondrial fusion is inhibited under $\Delta\psi_A$ loss condition? As previously mentioned, the mitochondrial contents are exchanged after fusion. This system is good for compensating for moderate dysfunctional mitochondria, but it is dangerous to fuse healthy mitochondria with severely damaged mitochondria. Indeed, it has been reported that mitochondria that are losing $\Delta\psi_A$ are specifically segregated from the healthy mitochondrial network and subsequently degraded via autophagy [14]. Therefore, the $\Delta\psi_A$ loss-dependent cleavage of OPA1 is one mechanism that ensures the segregation of damaged mitochondria from the healthy mitochondrial network (Fig. 1).

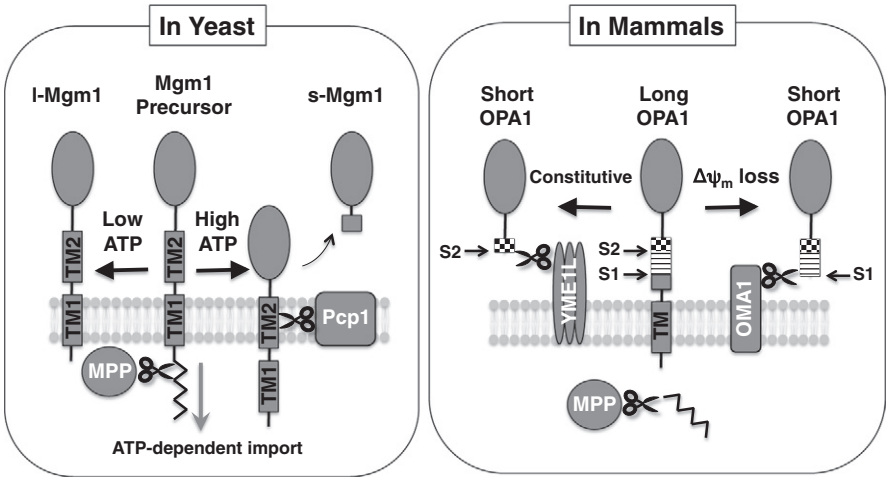

Fig. 2. Cleavage of OPA1 and its yeast ortholog Mgm-1. In yeast, Mgm-1 cleavage is mediated by Pcp1, a yeast ortholog of PARL. In yeast, Mgm-1 cleavage is dependent on the ATP levels. In the presence of high ATP, Pcp1 can access the cleavage site in the second transmembrane domain (TM2) and generate s-Mgm-1 by cleavage. However, in low ATP conditions, the import of Mgm-1 is stopped at the first transmembrane domain (TM1). Therefore, Pcp1 cannot cleave Mgm-1, which results in the generation of l-Mgm-1. In mammals, two inner mitochondrial membrane-localized proteases, YME1L and OMA1, are involved in the cleavage of OPA1. YME1L mediates the constitutive cleavage of OPA1 at S2 site, and OMA1 mediates mitochondrial membrane potential loss-dependent cleavage of OPA1 at S1 site.

More recently, as another function of OPA1 cleavage (or an appropriate balance of the OPA1 long and short isoforms), its relevance to lipid metabolism has also been reported [15]. Mice lacking OMA1, a mitochondria-resident protease that was recently identified as a protease that is required for $\Delta\psi_A$ the loss-dependent cleavage of OPA1 (see Section 2.3.1.2), exhibit obese phenotypes due to decreased energy expenditure [15]. Consistent with the well-known facts that obese/lean phenotypes are well correlated with cold resistance [16], OMA1 KO mice are vulnerable to cold-shock stress [15]. Enhanced lipid utilization for fulfilling the energy requirement is an important response against cold stress [17,18]. However, β oxidation is impaired in OMA1 KO mice-derived brown adipose tissue (BAT) (an important adipose tissue for heat production under cold stress). Therefore, this is one of the reasons that account for the cold-sensitive phenotypes of the OMA1 KO mice. Interestingly, after cold exposure, OPA1 cleavage is induced in BAT, and this cleavage is completely blocked in OMA1 KO mice-derived BAT [15]. Because UCP1, an inner mitochondrial membrane-resident uncoupler, is activated by catecholamines under the control of the central nervous system, which senses cold stress [16], cold stress-dependent cleavage of OPA1 in BAT may be induced downstream of UCP1 activation. In fact, the cleavage of OPA1 is also observed in catecholamine-stimulated primary brown adipocytes [19]. Interestingly, impaired β oxidation is also seen in OPA1-ablated primary brown adipocytes [15]. Moreover, OPA1 lacking the S1 cleavage site cannot rescue the defects in β oxidation [15], indicating that OPA1 cleavage influences lipid metabolism in some specific tissues, such as BAT.

2.2.1.2. $\Delta\psi_A$ loss-dependent down-regulation of PINK1 cleavage. OPA1 cleavage is induced by $\Delta\psi_A$ loss, but there is a protein whose cleavage is inhibited in $\Delta\psi_A$ loss-dependent manner, that is PINK1, which is a mitochondrial Ser/Thr protein kinase that has been identified as a gene product responsible for early-onset autosomal Parkinson disease (PD) [20]. Recently, dysfunctional mitochondria that lack $\Delta\psi_A$ were reported to be selectively eliminated by autophagy through the cooperatively functions of PINK1 and Parkin, an E3 ligase that has also been identified as a gene product responsible for PD [20, 21][21]. This process is termed "mitophagy" [20]. The most important points of mitophagy are that damaged mitochondria are specifically recognized and degraded by autophagy. For specific elimination of damaged mitochondria, the systems to distinguish between healthy mitochondria and damaged one are required. One of these systems is the segregation of damaged mitochondria through $\Delta\psi_A$ loss-dependent cleavage OPA1 as mentioned above [14], and the other is the stabilization of PINK1 on damaged mitochondria. In healthy mitochondria that maintain their $\Delta\psi_A$, PINK1 is continuously cleaved by the IMM-localized protease PARL and then degraded. By contrast, in damaged mitochondria that lack $\Delta\psi_A$, the transport of PINK1 across the OMM and IMM is stopped, and PINK1 stabilizes on the OMM [22–24]. Stabilized PINK1 acquires kinase activity through auto-phosphorylation and recruits Parkin in a kinase activity-dependent manner [25], which ultimately induces mitophagy.

It is noteworthy that there are many causative gene mutations for Parkinson's disease that are not associated with defective mitochondrial proteolysis. Therefore, the defective PINK1/Parkin-dependent mitophagy is one of the causative factors in the pathophysiology of Parkinson's disease. However, the discovery of PINK1/Parkin-dependent mitophagy is important as an identification of sophisticated mechanisms that ensure the mitochondrial quality control. It can be regarded as an important example that $\Delta\psi_A$ loss-dependent blockade of protein import and subsequent protein stabilization by escaping proteolysis act as a trigger of the mitochondrial stress responses (Fig. 1).

2.2.2. Evoking the cellular stress responses

2.2.2.1. $\Delta\psi_A$-loss dependent cleavage of PGAM5 by PARL

2.2.2.1.1. The mitochondrial protein phosphatase PGAM5. As mentioned above, PARL is responsible for cleavage of the mitochondrial kinase PINK1 in healthy mitochondria. Interestingly, we identified that PARL also functions in damaged mitochondria and cleaves a different substrate, mitochondria-resident phosphatase PGAM5, in a $\Delta\psi_A$ loss-dependent manner [26].

We originally identified PGAM5 as a novel activator of ASK1, which is a stress-responsive MAP3K, the most upstream kinase of the stress-responsive MAP kinase pathway [27]. The stress-responsive MAP kinase pathway, which is an important cellular signaling pathway, is activated by various stresses and plays important roles in the cellular stress responses [28]. Therefore, PGAM5 might be a mitochondria-resident stress responsive gene.

PGAM5 belongs to the PGAM family, which is an evolutionarily conserved enzyme family, the prototype of which converts 3-phosphoglycerate to 2-phosphoglycerate during glycolysis [29]. Although all the amino acid residues composing the catalytic core of the other family members are conserved in PGAM5, we previously reported that PGAM5 lacks mutase activity and instead acts as a

Ser/Thr-specific protein phosphatase [27]. Because the phosphatase activity of PGAM5 is abolished when the putative catalytic center, His105, is mutated, PGAM5 is a histidine-based protein phosphatase. These results suggest that PGAM5 is a novel and unique protein phosphatase that is distinctly separate from the already known protein phosphatase family [30]. We also revealed that the protein phosphatase activity of PGAM5 is required for the activation of ASK1 and its downstream kinases p38 and JNK [27].

2.2.2.1.2. The cleavage of PGAM5. PGAM5 is localized to the mitochondria through its N-terminal transmembrane (TM) domain. Some reports suggest that PGAM5 is localized in the OMM [31], but our various biochemical assays using isolated mitochondria indicate that PGAM5 is mainly localized in the IMM [26]. When searching for the stresses that PGAM5 responds to, we found that PGAM5 is cleaved by PARL in response to $\Delta\psi_A$ loss [26]. PARL, an IMM-localized protease, belongs to a rhomboid protease family that is a member of the intramembrane cleaving proteases (I-Clips) [32]. I-Clips are an evolutionarily conserved group of multipass membrane proteins that catalyze the cleavage of TM domains within lipid bilayers. Consistently, the cleavage site sequence determined by EDMAN degradation analysis is located in the N-terminal TM domain of PGAM5 [26]. Interestingly, PGAM5 cleavage is inhibited by both DCI, a rhomboid protease inhibitor, and O-phenanthroline, a metalloprotease inhibitor [26], indicating that some other metalloprotease is also involved in the cleavage of PGAM5. Indeed, the $\Delta\psi_A$ loss-dependent cleavage of PGAM5 is also inhibited by knockdown of metallopeptidase OMA1, a recently identified protease that is responsible for $\Delta\psi_A$ loss-dependent OPA1 cleavage (see Section 2.3.1.2). Double knockdown of PARL and OMA1 completely blocks the cleavage of PGAM5 [26], indicating that these two proteases cooperatively regulate the cleavage of PGAM5 (Fig. 3). Although whether these proteases directly cleave PGAM5 is unknown, the cleavage site sequence in the TM domain of PGAM5 contains several rhomboid protease recognition sequences, including critical helix-breaking residues [33,34], suggesting that PARL might directly cleave PGAM5.

2.2.2.1.3. Stress-dependent substrate switching of PARL. PARL cleaves PINK1 in healthy mitochondria, but it turns to cleave PGAM5 in damaged mitochondria. Thus, how is this stress-dependent substrate switching of PARL regulated? Because PINK1 import is blocked in damaged mitochondria, IMM-resident PARL cannot access it. However, knockdown of PINK1 *per se* cannot induce the cleavage of PGAM5 (unpublished data), suggesting that PINK1 and PGAM5 do not compete for access to PARL. Because the interaction between PARL and PGAM5 is increased in response to $\Delta\psi_A$ loss [26], there is likely a mechanism that positively regulates the $\Delta\psi_A$ loss-dependent recognition of PGAM5 by PARL. Discovering the stress-dependent substrate recognition mechanisms of PARL is one of the next interesting topics of future study.

2.2.2.2. The cleavage of PGAM5 and apoptosis. What is a function of cleaved PGAM5? Recently, cleaved PGAM5 was reported to be released from the mitochondria to the cytosol and to exhibit apoptosis-promoting activity [35] (Fig. 1). Interestingly, the N-terminal sequence of cleaved PGAM5 resembles the IAP-binding motif that is commonly seen in the N-termini of IAP-binding proteins such as Smac/DIABLO and HtrA2/Omi (Fig. 4). IAP-binding proteins competitively prevent interactions between caspases and IAP family proteins by binding to the IAP family proteins through the IAP-binding motif. Then, caspases are released from the IAP family-mediated inhibition, which results in caspase activation and apoptosis promotion [36]. Cleaved PGAM5 also binds to XIAP, an IAP family protein, through its N-terminal cleavage site sequence, indicating that the apoptosis-promoting activity of cleaved PGAM5 is exerted by the inhibition of XIAP.

PARL KO mice die prematurely due to massive apoptosis [37,38]. PARL contributes to the formation of cristae junction by generating cleaved OPA1 and prevents the release of cytochrome C [39]. However, several lines of evidence suggest that PARL is only partly responsible for the cleavage of OPA1 [11,12]. Future studies are needed to determine which substrate contributes to the drastic phenotypes of PARL KO mice.

Uncovering the physiological meaning of PGAM5 cleavage *in vivo* is another important question to elucidate. Although it is not known whether PGAM5 cleavage is involved in the following phenomena or not, one recent report has suggested that aged PGAM5 KO mice show a Parkinson's-like movement disorder [40]. The further analysis of PGAM5 cleavage from both mechanistic and pathophysiological points of view is now greatly anticipated.

2.3. The mechanisms of OPA1 cleavage

2.3.1. Proteases that cleaves OPA1

2.3.1.1. In yeast. Mgm-1 is a Yeast ortholog of OPA1 [41]. Mgm-1 exists as two isoforms, l-Mgm-1 and s-Mgm-1. l-Mgm-1 is a long isoform that is anchored in the IMM. s-Mgm-1 is a short isoform that is only loosely associated the IMM or OMM. s-Mgm-1 is a cleaved product of l-Mgm-1, and the protease Pcp1 cleaves l-Mgm-1 [42–44]. Pcp1 is a yeast ortholog

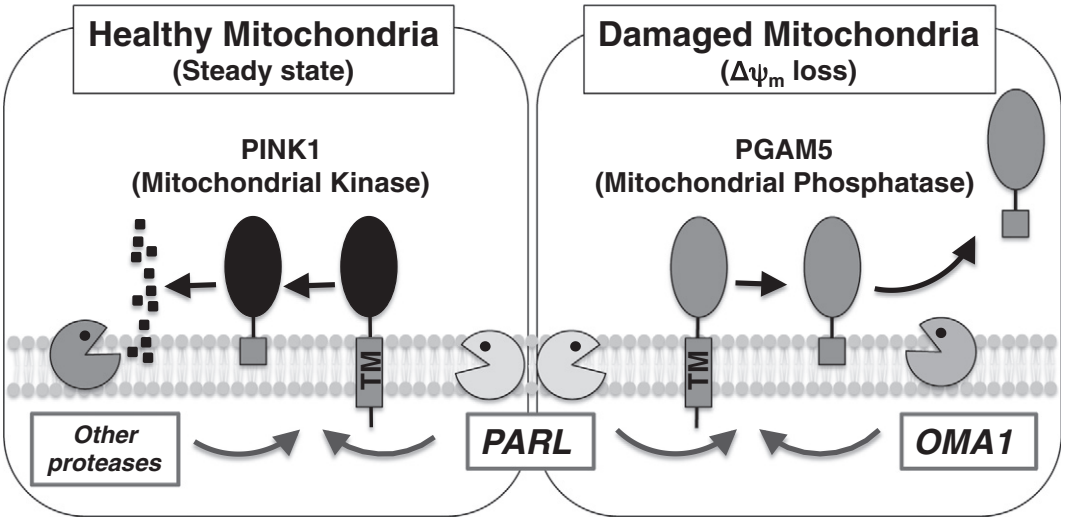

Fig. 3. Stress-dependent substrate switching of PARL. In healthy mitochondria that maintain their membrane potential, PARL cleaves mitochondrial kinase PINK1. By contrast, in damaged mitochondria that lose mitochondrial membrane potential, PARL instead cleaves mitochondrial phosphatase PGAM5. In addition to PARL, OMA1 is also involved in the mitochondrial membrane potential loss-induced cleavage of PGAM5.

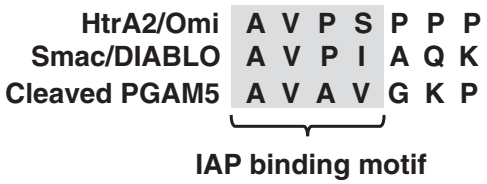

Fig. 4. N-terminal amino acids sequence of cleaved PGAM5 resembles to IAP binding motif.

of PARL. Mgm-1 contains two TM domains, TM1 and TM2, in its N-terminal region after the mitochondrial targeting sequence. Pcp1 cleaves Mgm-1 within the TM2 domain [42–44]. The Pcp1-mediated cleavage of Mgm-1 is influenced by the ATP level [45]. According to this study, under low ATP conditions, Mgm-1 import is stopped at TM1; thus, Pcp1 cannot access the TM2 cleavage site. By contrast, under high ATP conditions, the import of Mgm-1 is promoted; thus, Pcp1 can recognize and access TM2 domain (Fig. 2). Additionally, both forms of Mgm-1 are required for mitochondrial fusion in yeast [41]. Therefore, when l-Mgm-1 is present in excess after ATP depletion, the mitochondrial network structure is rapidly disrupted [45]. The import machineries-mediated correct positioning of the TM domains of substrates within the lipid bilayers appears to be commonly used to regulate substrate recognition by Pcp1. For example, m-AAA-driven import is also important for the cleavage of the other Pcp1 substrate, Ccp1 [46].

2.3.1.2. In mammals. It is widely accepted that the cleaving protease of Mgm-1 is Pcp1 in yeast. By contrast, a number of proteases have been reported as proteases for OPA1 in mammals; these proteases include PARL and the m- and i-AAA proteases, HtrA2 and OMA1 [47]. However, several lines of evidence support a role for two proteases as cleaving proteases of OPA1. One is i-AAA protease, YME1L [13], and the other is metallopeptidase OMA1 [48,49], both of which are membrane-embedded metal-dependent proteases. They are targeted to the IMM, and their catalytic domains face the IMS. YME1L is responsible for constitutive S2 site cleavage, while OMA1 mediates stress-dependent S1 site cleavage (Fig. 2). The cleavage of OPA1 was recently confirmed to completely be blocked in YME1L/OMA1 double KO mice-derived MEFs [50].

Because OMA1-mediated S1 cleavage is induced in response to $\Delta\psi_A$ loss, it has been expected to reveal the precise mechanisms through which $\Delta\psi_A$ loss is sensed and subsequently linked to the activation of proteases. Recently, it has been reported that OMA1 is activated and auto-catalytically degraded in response to $\Delta\psi_A$ loss [51,52]. Thomas Langer's group proposed a model in which the N-terminal hydrophobic region and the adjacent cluster of positively charged amino acids of OMA1 may function as a $\Delta\psi_A$ sensor [51]. When these amino acids are mutated, the $\Delta\psi_A$ loss-dependent auto-catalytic degradation of OMA1 is inhibited. Because the co-expression of the mutant with the deleted cluster of positively charged amino acids and protease-inactive mutant restores the cleaving activity for OPA1, N-terminal positively charged amino acids are not required for protease activity *per se* but are important for $\Delta\psi_A$ loss-dependent activation. Future studies are expected to determine what types of $\Delta\psi_A$ loss-dependent changes these amino acids are sensing. It is unknown whether $\Delta\psi_A$ responsiveness is explained by the following model, but the lipid microdomain model has been proposed as one of the regulatory mechanisms of proteolysis in the IMM (see the next section).

2.3.2. Prohibitins-mediated lipid compartmentalization as a regulator of OPA1 cleavage?

2.3.2.1. Prohibitins. Prohibitins exist as two closely related proteins, PHB1 and PHB2 [53]. Prohibitins localize to the nucleus or the plasma membrane, but they are also targeted into the IMM through the N-terminal TM domains. They hetero-oligomerize through their C-terminal coiled-coil domains, which face the IMS, and present as a high molecular-weight (HMW) complex (~1.2 MDa) that is called the PHB complex. The analysis of purified yeast PHB complexes by single-particle electron microscopy revealed a ring-like structure with an outer diameter of approximately 200–250 Å [54].

2.3.2.2. Implication of prohibitins as a regulator of mitochondrial proteolysis. It has been suggested that prohibitins may function as regulators of mitochondrial proteolysis.

For example, following the ablation of prohibitins in yeast, the m-AAA protease-mediated degradation of non-assembled OXPHOS proteins is promoted [55]. m-AAA also forms a HMW complex (approximately 2 MDa). This complex contains prohibitins, which can interact with m-AAA but not with i-AAA. Because the lack of prohibitins disrupts the HMW complex of m-AAA, m-AAA may acquire its proteolytic activity after being released from the PHB complex.

Moreover, in mammals, prohibitins may also regulate OPA1 cleavage [56]. In MEF cells that lack of PHB2 (the expression level of PHB1 is also reduced), OPA1 cleavage is accelerated under steady state even if the $\Delta\psi_A$ is not lost.

The above results indicate that prohibitins may directly or indirectly inhibit mitochondrial proteolysis in the IMM.

2.3.2.3. The lipid compartmentalization model. How do prohibitins regulate mitochondrial proteolysis? An exciting model has just been proposed: prohibitins might act as membrane scaffolds in the IMM and organize the functional lipid microdomain that regulates protease activity and/or the interaction between proteases and substrates [53,57]. Currently, the following two lines of evidence support this model.

Prohibitins contain a PHB domain between the N-terminal TM domain and the C-terminal coiled-coil domain. This domain is commonly found in the so-called SPFH family [58]. The several members in this family also form a HMW complex and exist as ring assemblies with a diameter of approximately 200–250 Å. The fact that many SPFH proteins co-fractionate with detergent-resistant membranes raises the possibility that SPFH family proteins organize membrane microdomains with distinct lipid composition [58].

The other evidence is that a yeast genetic screen for factors that are essential for the survival of prohibitin-deficient yeast cells identified several lipid metabolism-related genes [59,60]. Moreover, the ablation of these genes affects the cleavage of Mgm-1. For example, in the absence of Psd1, the enzyme that mediates the terminal steps in phosphatidylethanolamine (PE) biosynthesis, Mgm-1 cleavage is inhibited [60,61]. The ablation of Ups1 or Gep1 reduces the PE and cardiolipin (CL) levels in the cells, and Mgm-1 cleavage is inhibited at the same time [60,62]. As mentioned before, in Yeast, Mgm-1 is cleaved by rhomboid protease Pcp1. It is noteworthy that the proteolytic activity of some types of bacterial rhomboid protease is regulated by lipids [63].

Based on the above observations, prohibitins may assist in the formation of membrane domains that are specifically enriched in CL and PE, which then regulate mitochondrial proteolysis, especially in the IMM [53,57].

It will be interesting whether the precise molecular mechanisms of $\Delta\psi_A$ loss-dependent OPA1 cleavage can be explained by the above-mentioned lipid compartmentalization model. Now, several questions are raised. Can $\Delta\psi_A$ loss-dependent disruption of the PHB complex and the subsequent remodeling lipid microdomain can be observed? Can $\Delta\psi_A$ loss-dependent activation of OMA1 be influenced by the PHB complex or lipids such as PE and CL? Numerous studies over the past several years have just begun to bear fruit now.

3. Conclusions and perspectives

In this review, we summarize the emerging roles of mitochondrial proteolysis in stress responses. Because we could not include the

recent dramatic advance in mtUPR, another important mitochondrial proteolysis-related stress response, please refer to the excellent reviews on this topic [64,65]. Accumulating evidence supports the importance of defects in mitochondria proteolysis-mediated stress responses to some pathophysiological disorders, including neuronal degeneration [47]. Uncovering the molecular mechanisms of stress-dependent mitochondrial proteolysis is also important for further understanding the developing mechanisms of these diseases and discovering new drug targets.

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