Review

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Regulation of mitochondrial plasticity by the *i*-AAA protease YME1L

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Abstract: Mitochondria are multifaceted metabolic organelles and adapt dynamically to various developmental transitions and environmental challenges. The metabolic flexibility of mitochondria is provided by alterations in the mitochondrial proteome and is tightly coupled to changes in the shape of mitochondria. Mitochondrial proteases are emerging as important posttranslational regulators of mitochondrial plasticity. The *i*-AAA protease YME1L, an ATP-dependent proteolytic complex in the mitochondrial inner membrane, coordinates mitochondrial biogenesis and dynamics with the metabolic output of mitochondria. mTORC1-dependent lipid signaling drives proteolytic rewiring of mitochondria by YME1L. While the tissue-specific loss of YME1L in mice is associated with heart failure, disturbed eye development, and axonal degeneration in the spinal cord, YME1L activity supports growth of pancreatic ductal adenocarcinoma cells. YME1L thus represents a key regulatory protease determining mitochondrial plasticity and metabolic reprogramming and is emerging as a promising therapeutic target.

Keywords: cancer; *i*-AAA protease; LIPIN1; mitochondria; mitochondrial plasticity; mitochondrial proteases; mTORC1; YME1L.

Introduction

Mitochondria are long recognized as central metabolic hubs, which carry out a broad range of life-sustaining tasks within cells, ranging from ATP production and bioenergetic functions to the metabolism of cellular building blocks, such as amino acids, nucleotides, or cofactors of enzymes. Accordingly, mitochondria are essential for the proliferation and survival of almost all eukaryotic cells and, if damaged, are associated with aging and various diseases, including cardiomyopathies, neurodegenerative disorders, or metabolic diseases. Mitochondria also serve as signaling hubs and can trigger inflammation and apoptotic cell death by releasing proinflammatory or proapoptotic factors. It is therefore not surprising that surveillance mechanisms are in place to monitor the functional integrity of mitochondria. Mitochondrial proteases ensure the selective removal of dysfunctional proteins, whereas irreversibly damaged organelles are removed by mitophagy (Tatsuta and Langer, 2008; Youle and van der Bliek, 2012).

The metabolic demand on mitochondria varies greatly between different cells and tissues and can change dynamically during development, cell differentiation, or in response to stress conditions and other environmental cues. The functional diversity of mitochondria is reflected in different shapes of mitochondria and alterations in the mitochondrial proteome between cells and tissues, which is composed of >1200 proteins. Different metabolic demands likely also explain why diseases associated with mitochondrial deficiencies show an extraordinary cell and tissue specificity. Multiple mechanisms are in place to regulate the biogenesis of mitochondria and their metabolic output, including transcriptional, post-transcriptional, and translational programs (Rugarli and Langer, 2012). Moreover, an increasing number of post-translational mechanisms are recognized to determine mitochondrial plasticity (Escobar-Henriques and Langer, 2014; Opalinska and Meisinger, 2015; Lim et al., 2016). Key factors regulating mitochondrial functions at a post-translational level are mitochondrial proteases, which mediate the processing or turnover of mitochondrial proteins and thereby control mitochondrial proteostasis and a variety of mitochondrial activities (Quiros et al., 2015; Deshwal et al., 2020). Pathogenic mutations have been identified in many genes encoding mitochondrial proteases,

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highlighting the importance of mitochondrial plasticity for organismal health (Rugarli and Langer, 2012; Goard and Schimmer, 2014).

Here, after a short introduction into the proteolytic system of mitochondria, we will focus on the role of the *i*-AAA protease YME1L, which is emerging as a central regulator of mitochondrial proteostasis. Proteolysis by YME1L ensures protein quality control and couples mitochondrial shape and metabolic function.

The proteolytic system of mitochondria: quality control and regulatory functions

Mitochondria harbor an independent proteolytic system, which allows the complete degradation of proteins to amino acids. At least 23 peptidases are exclusively localized within mitochondria, whereas others were found to shuttle between the cytosol and mitochondria (Quiros et al., 2015). Many mitochondrial proteases serve as processing peptidases and mediate the maturation of nuclear-encoded mitochondrial proteins, which are synthesized at cytosolic ribosomes and targeted to different mitochondrial compartments. More than half of mitochondrial proteins harbor positively charged, N-terminal mitochondrial targeting sequences, which can form amphipathic helices and must be removed upon import into mitochondria to ensure functionality (Mossmann et al., 2012; Poveda-Huertes et al., 2017). Accordingly, loss of central processing peptidases severely impairs mitochondrial functionality, causes embryonic lethality in mice, and is associated with a variety of diseases in humans (Gakh et al., 2002; Vogtle et al., 2018).

While processing peptidases were among the first mitochondrial peptidases to be identified, the need for efficient protein quality surveillance within mitochondria is apparent, considering the plasticity of the mitochondrial proteome and the complexity of mitochondrial biogenesis, which depends on the coordinated expression of mitochondria- and nuclear-encoded proteins. The potential accumulation of excess, nonassembled proteins poses therefore a constant challenge to the functional integrity of mitochondria. Moreover, mitochondria are a major site for the production of reactive oxygen species (ROS), which are formed as a by-product of mitochondrial respiration and, if not detoxified by the oxidative defense system, can cause protein, lipid, and DNA damage in mitochondria (Murphy, 2016). Many mitochondrial proteases comprise an efficient quality control system in various mitochondrial compartments and specifically degrade damaged and unfolded proteins to prevent their possibly deleterious accumulation (Figure 1) (Quiros et al., 2015; Deshwal et al., 2020). ATP-dependent proteases are core components of the mitochondrial quality control system and can specifically recognize unfolded proteins, which are degraded to peptides in a processive manner (Levytskyy et al., 2017). These peptides are either released from mitochondria or further degraded to amino acids by mitochondrial

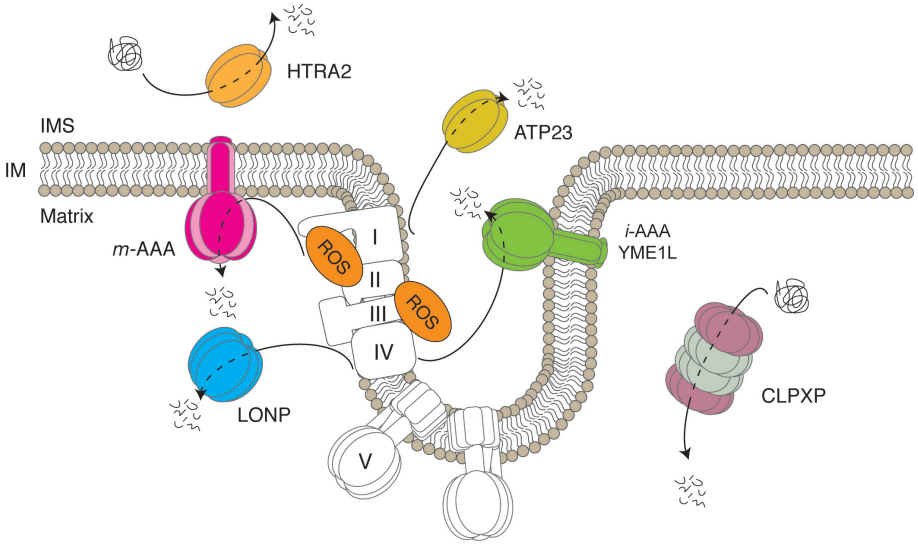

Figure 1: Quality control proteases in mitochondria.
Mitochondrial proteases, which have been demonstrated to degrade damaged and misfolded proteins in mammalian mitochondria, include the ATP-dependent *i*-AAA and *m*-AAA, LONP, and CLPXP proteases, as well as the ATP-independent proteases ATP23 and HTRA2. IMS, intermembrane space; IM, inner membrane; I, II, III, IV, respiratory complexes 1-IV; V, F_1F_0 ATP synthase.

oligopeptidases (Teixeira and Glaser, 2013; Taskin et al., 2017). Notably, ATP-dependent proteases exert chaperone-like properties and a degenerate substrate specificity (Leonhard et al., 1999; Leonhard et al., 2000). The involvement of specific quality control proteases appears to be largely determined by the location or membrane topology of substrate proteins. Nevertheless, recent studies indicate that the presence of a degenerate amino acid motif acts as a degradation signal required for the *i*-AAA–mediated degradation (Shi et al., 2016; Rampello and Glynn, 2017). Similarly, sequences recognized by *m*-AAA seem to contain some characteristic hydrophobic residues (Ding et al., 2018).

The functional analysis of mitochondrial proteases revealed dual activities of many of them in mitochondria. Besides serving as quality control enzymes, they regulate diverse mitochondrial processes by proteolytic processing or by limiting the life span of short-lived, regulatory proteins. Mitochondrial proteases were found to broadly affect mitochondrial functions and regulate almost every aspect of mitochondrial homeostasis, including protein import and lipid homeostasis, mitochondrial gene expression, and oxidative phosphorylation (OXPHOS) complex assembly to mitochondrial dynamics, calcium signaling, mitophagy, and cell death (Quiros et al., 2015; Deshwal et al., 2020). The loss of mitochondrial proteases thus may not only cause the accumulation of possibly deleterious misfolded proteins but also can broadly impair mitochondrial activities. The multiple functions of mitochondrial proteases have to be considered to understand the pathogenesis of the many diseases associated with these peptidases.

The *i*-AAA protease YME1L in the inner membrane

The inner membrane of mitochondria (IM) is considered as the protein-richest cellular membrane. It harbors OXPHOS and ATP synthase complexes and is the major site of mitochondrial ROS production. Two membrane-bound, ATP-dependent proteases, the *m*- and the *i*-AAA protease, serve as quality control enzymes in the IM and regulate membrane-associated processes (Figure 2A). They are built up of homologous and highly conserved subunits, which form hexameric, cylinder-shaped complexes, allowing proteolysis to occur in a sequestered cavity (Leonhard et al., 2000; Puchades et al., 2019). The *i*-AAA protease is composed of YME1L subunits and is active in the intermembrane space (IMS) (Leonhard et al., 2000; Puchades et al., 2017). The paralogous *m*-AAA protease, on the other hand, exposes its catalytic domains to the matrix and can exist in a homo-oligomeric form composed of AFG3L2 (in mice also AFG3L1) subunits or a hetero-oligomeric form harboring AFG3L2 (in mice also AFG3L1) and paraplegin (SPG7) subunits (Koppen et al., 2007). In agreement with the endosymbiotic theory, homologous proteases are present in the bacterial plasma membrane (FtsH) or the thylakoid membrane in plants (Tomoyasu et al., 1993; Lindahl et al., 1996).

All subunits of FtsH-like proteases harbor an ATPase domain characteristic of the AAA family of ATPases (Puchades et al., 2020), which is fused to a metallopeptidase domain of the M48 family (Figure 2C). Aminoterminal transmembrane regions anchor AAA protease subunits to the IM. FtsH-like AAA proteases recognize both peripheral and integral membrane proteins as substrates. They bind to solvent-exposed protein segments of at least 10 amino acids or larger domains of membrane proteins, if they are unfolded (Leonhard et al., 2000). The energy derived from ATP hydrolysis is used to extract membrane proteins from the membrane and transport them into the inner cavity of the protease (Tatsuta et al., 2007). Different substrate-binding modes were described for yeast Yme1l, where various substrates initially bind to different helices at the surface of the AAA and proteolytic domains (Graef et al., 2007). Substrate proteins are translocated through the central pore of hexameric complexes into the proteolytic chamber for proteolysis (Figure 2C, D). Genetic studies on the yeast *m*-AAA protease revealed that ATP binding to one AAA subunit inhibits ATP hydrolysis by the neighboring subunit, leading to a coordinated ATP hydrolysis within the AAA ring (Augustin et al., 2009). Conserved, *trans*-acting arginine fingers and an intersubunit signaling cascade via a conserved intersubunit signaling motif couple coordinated ATP hydrolysis around the AAA ring to movements of loop regions exposed to the central pore. These loops bind substrate proteins and drive their translocation through the central pore into the proteolytic chamber of AAA proteases (Puchades et al., 2020). In agreement with their critical role in substrate handling, the pore loop 1 typically harbors conserved aromatic amino acid residues, whose mutation impaired proteolysis (Graef et al., 2007; Puchades et al., 2017). The nucleotide state correlates with the conformation of the pore loop 1, which binds to substrates in the ATP-bound but not the ADP-bound state. These studies suggested a hand-over-hand model for substrate translocation through the central pore (Augustin et al., 2009; Kress et al., 2009), which was recently further supported by elegant structural studies (Puchades et al., 2017). Cryo-electron microscopy of the

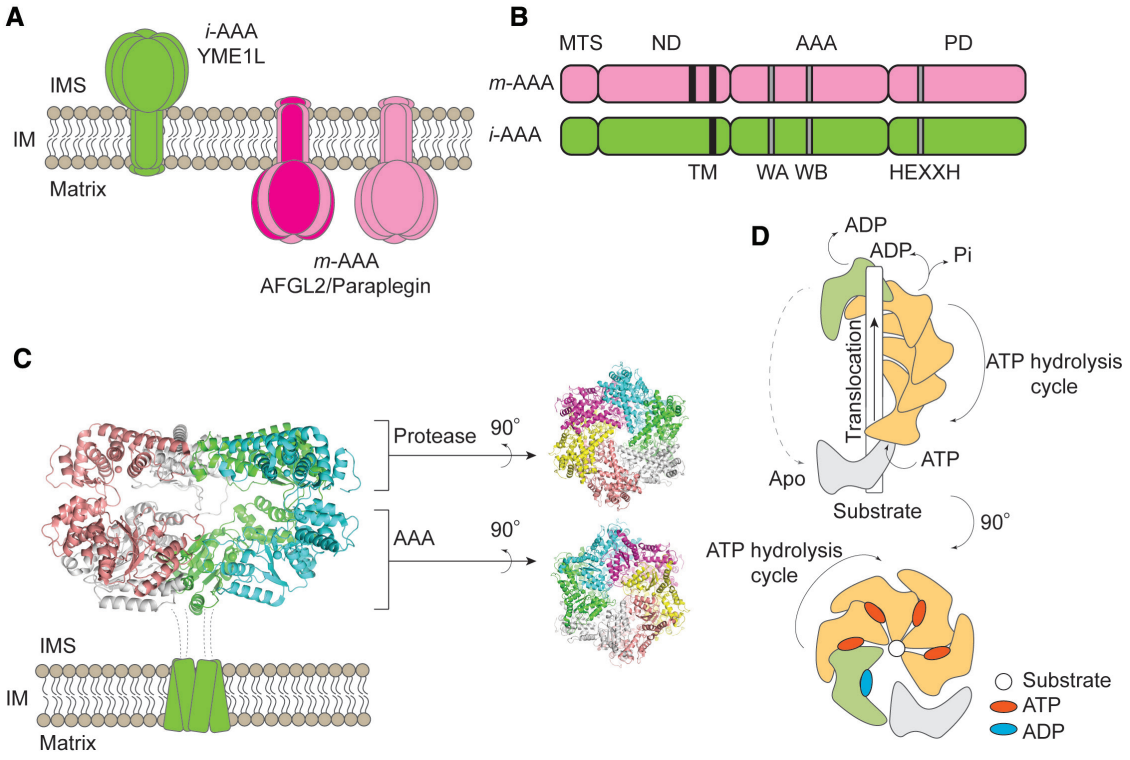

Figure 2: Architecture of AAA proteases.
(A) Topology of hexameric *i*- and *m*-AAA proteases in the IM. The *i*-AAA protease is composed of YME1L subunits, whereas homo-oligomeric AFG3L2 complexes or hetero-oligomeric complexes composed of AFG3L2 and paraplegin represent different *m*-AAA protease isoforms. (B) Domain structure of AAA protease subunits. MTS, mitochondrial targeting sequence; ND, N-terminal domain; AAA, AAA domain; PD, proteolytic domain; TM, transmembrane region; WA, Walker A motif; WB, Walker B motif. (C) Available structural information on the *i*-AAA protease YME1L is shown (PDB ID: 6AZ0) (Puchades et al., 2017). Four of six subunits are shown in the left panel. (D) Proposed mechanism of ATP-dependent substrate translocation. Six subunits assemble into a spiral staircase surrounding the translocating substrate. ATP binding allows substrate interaction with pore loops. Consecutive ATP hydrolysis within the AAA staircase promotes substrate translocation through the pore.

catalytic domains of human YME1L and AFG3L2 ring complexes demonstrated a spiral-staircase arrangement of the central pore loops and revealed how the nucleotide state of YME1L or AFG3L2 subunits allosterically controls their interaction with translocating substrates (Puchades et al., 2017; Puchades et al., 2019).

Regulation of mitochondrial dynamics by YME1L

Mitochondria constantly fuse and divide forming dynamic, reticulated networks in many cells. Fusion and fission rates—and thereby the morphology of mitochondria—are intimately coupled to the function of mitochondria and are adjusted to altered metabolic demands and environmental cues (Tilokani et al., 2018; Dorn, 2019). Reduced fusion or increased mitochondrial fission cause mitochondrial fragmentation, which is associated with the removal of dysfunctional mitochondria by mitophagy or cell death if mitochondria are irreversibly damaged (Youle and van der Bliek, 2012). The dynamin-like GTPase OPA1 (Mgm1 in yeast) mediates mitochondrial fusion at the level of the IM (MacVicar and Langer, 2016). Moreover, it is required to maintain cristae morphogenesis and respiration (Pernas and Scorrano, 2016). Therefore, inactivation or loss of OPA1 triggers mitochondrial fragmentation and severely affects mitochondrial activities (Olichon et al., 2006). Mutations in *OPA1* are associated with dominant optic atrophy, the most common inherited form of blindness in humans, which is characterized by degeneration of retinal ganglion cells (Alexander et al., 2000; Delettre et al., 2000). Moderate overexpression of OPA1, on the other hand, was found to protect against apoptosis in cultured cells and ameliorate the phenotype of two different mouse models for mitochondrial diseases related to OXPHOS dysfunction (Civiletto et al., 2015).

YME1L and mitochondrial fusion

Eight different OPA1 isoforms are expressed in human (four isoforms in mice) that are generated by alternative splicing of exons 4, 4b, and 5b. Newly synthesized OPA1 is matured upon import into mitochondria by the mitochondrial processing peptidase MPP and anchored to the IM by an aminoterminal transmembrane segment (L-OPA1). The maintenance of mitochondrial morphology depends on further proteolytic processing of L-OPA1 (or L-Mgm1 in yeast) to a shorter variant, S-OPA1, which lacks the membrane-anchoring domain (MacVicar and Langer, 2016; Del Dotto et al., 2018). L-OPA1 and S-OPA1 accumulate in a stoichiometric manner and assemble into large complexes in mitochondria (Frezza et al., 2006). Processing of OPA1 by two peptidases occurs at two neighboring cleavage sites (Figure 3A): OMA1 cleaves OPA1 at S1 encoded by exon 5, whereas YME1L cleavage occurs at S2 encoded by exon 5b (Ishihara et al., 2006; Anand et al., 2014); however, the functional difference between OMA1- and YME1L-derived S-OPA1 is unclear. Notably, the yeast ortholog of OPA1, Mgm1, is processed by the rhomboid protease Pcp1 and not the conserved, orthologous peptidases Oma1 and Yme1 (Herlan et al., 2003; McQuibban et al., 2003; Herlan et al., 2004; Bohovych et al., 2014). Accordingly, OPA1 can replace Mgm1 functionally only if the aminoterminal region harboring the transmembrane segment and the proteolytic cleavage site of Mgm1 is preserved (Nolli et al., 2015). It is an attractive hypothesis that cleavage of OPA1 by

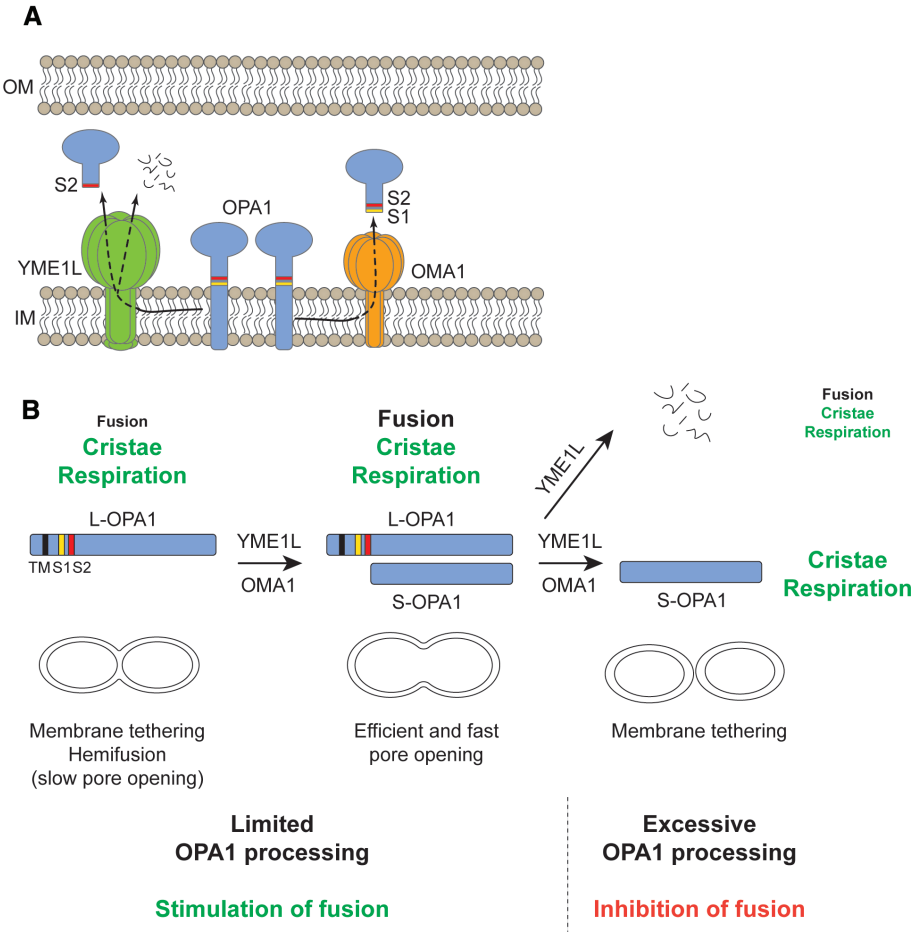

Figure 3: Proteolytic control of OPA1 functions.
(A) OPA1 processing is mediated by OMA1 (at site 1) and YME1L (at site 2) converting membrane-bound L-OPA1 into soluble S-OPA1. YME1L can mediate the complete proteolytic breakdown of OPA1. (B) OPA1 processing regulates mitochondrial fusion but not respiration or cristae morphogenesis. L- or S-OPA1 is sufficient to maintain respiration and cristae morphogenesis and allow membrane tethering. L-OPA1 is fusion-active, but efficient pore opening after hemifusion depends on the balanced accumulation of L- and S-OPA1. YME1L mediates OPA1 processing and determines the steady-state level of OPA1 and thus adjusts mitochondrial fusion, respiration, and cristae morphology to metabolic demands.

two different peptidases provides cells with the regulatory flexibility required to adapt the morphology of mitochondria to different metabolic and environmental demands. Whereas processing of OPA1 by YME1L has been observed to increase upon shift from glycolysis to OXPHOS (Mishra et al., 2014), various stress conditions, including mitochondrial depolarization, oxidative stress, heat stress, and hypoxia, lead to increased processing of OPA1 by OMA1 (An et al., 2013; Baker et al., 2014; Jones et al., 2017).

Although recognized to maintain normal mitochondrial morphology, a role for OPA1 processing in mitochondrial fusion is discussed. The analysis of cells expressing only individual OPA1 variants or of cells lacking both OPA1 processing peptidases revealed that L-OPA1 is fusion-competent (Del Dotto et al., 2017; Del Dotto et al., 2018). However, short mitochondrial tubules accumulated in *Oma1*$^{-/-}$*Yme1l*$^{-/-}$ cells harboring exclusively L-OPA1 (Anand et al., 2014), and the normal tubular mitochondrial morphology in *Opa1*$^{-/-}$ mouse embryonic fibroblasts was completely restored only upon coexpression of multiple OPA1 isoforms (Del Dotto et al., 2017). Moreover, an increased mitochondrial fusion rate was observed under respiratory growth conditions and shown to depend on OPA1 processing by YME1L (Mishra et al., 2014). Reconstitution experiments in liposomes provided further insight into the role of OPA1 processing for membrane fusion. While some fusion occurred upon heterotypic interaction of L-OPA1 with cardiolipin, the presence of S-OPA1 stimulated liposome fusion (Ban et al., 2017). Total internal reflection fluorescence microscopy revealed that L-OPA1 is sufficient to allow membrane tethering and hemifusion, but subsequent opening of the fusion pore was stimulated by the presence of S-OPA1 (Ge et al., 2020). Together, these experiments suggest that, while L-OPA1 is fusion-competent, optimal fusion depends on the presence of equimolar concentrations of L- and S-OPA1. Excessive OPA1 processing, however, further limits the accumulation of L-OPA1 and inhibits fusion, resulting in mitochondrial fragmentation (Figure 3B).

While proteolytic regulation allows rapid adaptation of mitochondrial morphology, it bears the problem as to how the response can be terminated. OMA1 undergoes autocatalytic degradation upon stress activation (Baker et al., 2014) and degrades YME1L if ATP is limiting (Rainbolt et al., 2015; Rainbolt et al., 2016). In hypoxia, YME1L degrades itself, as well as OMA1, thus limiting L-OPA1 processing and allowing fusion to proceed (MacVicar et al., 2019). Notably, prolonged hypoxia leads to mitochondrial fragmentation by increased OPA1 processing (An et al., 2013).

In contrast to mitochondrial fusion, both L- and S-OPA1 are able to preserve cristae morphogenesis and respiration when expressed individually in *Opa1*$^{-/-}$ cells (Del Dotto et al., 2017; Lee et al., 2017). Thus, processing of OPA1 apparently does not regulate the formation of cristae or the maintenance of respiratory chain complexes. Notably, YME1L was found to degrade OPA1 without affecting OPA1 processing and the relative ratio of L-OPA1 to S-OPA1, when cells are exposed to hypoxia and shift from an OXPHOS-dependent to glycolytic growth (MacVicar et al., 2019). Thus, an additional level of proteolytic regulation of OPA1 by YME1L exists, which apparently adjusts all functions of OPA1 for mitochondrial dynamics, cristae morphogenesis, and respiration to altered metabolic demands (Figure 3A, B). It will be of interest to define the parameters determining whether OPA1 is processed or completely degraded by YME1L.

Tissue-specific consequences of the loss of YME1L

In agreement with the role of YME1L in regulating mitochondrial fusion, loss or inactivation of YME1L causes mitochondrial fragmentation in various cells and tissues (Anand et al., 2014; Wai et al., 2015; Sprenger et al., 2019). Mechanistically, this is explained by increased processing of OPA1 by OMA1 in the absence of YME1L, which inhibits fusion and triggers mitochondrial fragmentation by DRP1-mediated mitochondrial fission (Anand et al., 2014). A homozygous mutation in *YME1L* found in a pedigree of Saudi Arabian descent caused a multisystemic mitochondriopathy associated with various neurological symptoms and fragmentation of the mitochondrial network (Hartmann et al., 2016). Similarly, increased OPA1 processing and mitochondrial fragmentation are an early phenotype of mice lacking YME1L specifically in the heart or the nervous system (Wai et al., 2015; Sprenger et al., 2019). Notably, mitochondria in YME1L-deficient cardiomyocytes and neurons exhibit normal cristae morphogenesis. However, despite similar effects on mitochondrial morphology, the consequences of deletions of *Yme1l* differ between tissues. YME1L is essential for embryonic development and impairs cardiac function culminating in heart failure when deleted in adult cardiomyocytes. Stabilization of L-OPA1 upon concomitant deletion of *Oma1* restored cardiac function, suggesting deleterious effects of mitochondrial fragmentation in the heart (Wai et al., 2015). Deletion of *Yme1l* in *Drosophila* causes age-associated locomotor deficiency and ocular dysfunction (Qi et al., 2016). Loss of YME1L in the mammalian

nervous system has striking cell type–specific consequences despite widespread mitochondrial fragmentation (Sprenger et al., 2019). It impairs eye development and is associated with axonal degeneration specifically in the dorsolateral tracts of the spinal cord. Ablation of *Oma1* stabilized L-OPA1 and restored mitochondrial tubulation but, in contrast to cardiomyocytes, aggravated phenotypic deficiencies in the absence of YME1L (Sprenger et al., 2019). These observations pointed to additional, tissue-specific functions of YME1L (or OMA1) that may be related to the different metabolic demands of cardiomyocytes and neurons. Consistently, metabolic interventions and alterations in systemic glucose homeostasis were found to suppress cardiac deficiencies in the absence of YME1L without restoring a tubular mitochondrial morphology (Wai et al., 2015). Indeed, recent studies revealed a key role of YME1L in the regulation of the metabolic output of mitochondria.

Regulation of mitochondrial metabolism by YME1L

A plethora of metabolic reactions occurs within mitochondria reaching far beyond their bioenergetic function (Spinelli and Haigis, 2018). OXPHOS-dependent ATP production is eminent in tissues with high energy demands such as cardiac and skeletal muscle and the brain. However, mitochondria are also the site of many anabolic reactions and ensure cell proliferation by providing amino acids, nucleotides, or fatty acids as building blocks of macromolecules. During cell differentiation or in adaptation to various stressors, such as nutrient deprivation or hypoxia, cells adjust the abundance of mitochondria regulating their biogenesis as well as their turnover by mitophagy. Moreover, the metabolic function of mitochondria is tailored to utilize glutamine, the most abundant amino acid in the cytoplasm, to fuel the tricarboxylic acid cycle and preserve the synthesis of macromolecules under glycolytic growth conditions. This metabolic repurposing of mitochondria critically depends on YME1L (Figure 4).

Proteolytic rewiring of hypoxic mitochondria by YME1L

Cell growth in hypoxia or upon amino acid starvation is accompanied by increased proteolysis by YME1L, which broadly reshapes the mitochondrial proteome to sustain cell proliferation (MacVicar et al., 2019). More than 40 proteins located in the IM or the IMS were identified as putative substrates of YME1L (Figure 4) including previously described proteins (Potting et al., 2013; Rainbolt et al., 2013; Saita et al., 2018; Richter et al., 2019). The intramitochondrial lipid transfer proteins STARD7, PRELID1, and PRELID3B, as well as TRIAP1, which forms heterodimers with PRELID1 and PRELID3B, are substrates of YME1L. Whereas STARD7 binds phosphatidylcholine (PC) in the IMS, PRELID1-TRIAP1 and PRELID3B-TRIAP1 complexes shuttle phosphatidic acid (PA) and phosphatidylserine (PS), respectively, across the IMS (Potting et al., 2013; Saita et al., 2018; MacVicar et al., 2019). Increased YME1L-mediated proteolysis thus reduces mitochondrial phospholipid biogenesis. Similarly, the import of newly synthesized mitochondrial proteins is limited by YME1L-dependent degradation of subunits of protein translocases in the IM, the TIM23 and TIM22 complexes (Rainbolt et al., 2013; MacVicar et al., 2019). Among others, YME1L degrades

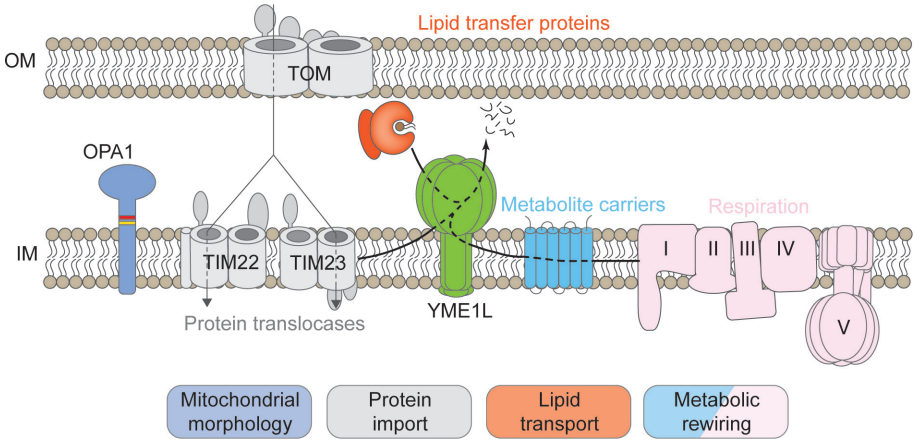

Figure 4: Regulatory substrates of YME1L.
YME1L mediates the processing and degradation of multiple IM and IMS proteins, involved in the regulation of mitochondrial morphology, protein import and lipid transport, and metabolic functions of mitochondria.

the TIM23 subunit ROMO1, which is required for efficient import of newly synthesized YME1L itself pointing to an intriguing regulatory feedback loop (Richter et al., 2019). Thus, the activation of YME1L in hypoxia acutely inhibits mitochondrial protein and phospholipid biogenesis in response to hypoxia and nutrient starvation. Other YME1L substrates include various metabolite carriers in the IM and other metabolic enzymes, whose turnover likely contributes to the metabolic rewiring of mitochondria required to ensure efficient glutamine utilization and support glycolytic cell growth (MacVicar et al., 2019).

These experiments demonstrated that YME1L-mediated proteolysis broadly preserves mitochondrial proteostasis under normoxia and reshapes the mitochondrial proteome in response to hypoxia to adjust mitochondrial function to the altered metabolic demands. YME1L therefore couples mitochondrial morphology and function by, on the one hand, balancing fusion and fission of mitochondria via OPA1 turnover and processing and, on the other hand, regulating the metabolic output via degradation of a broad range of substrates in response to nutrient availability and cellular stress.

mTORC1-dependent regulation of YME1L

Increased protein degradation by YME1L upon oxygen and nutrient deprivation depends on hypoxia-inducible factor 1α (HIF1α), the major transcription factor driving the cellular response to hypoxia (Semenza, 2017). However, HIF1α does not modulate the transcription of YME1L or of genes encoding YME1L substrates. Rather, HIF1α controls YME1L-mediated proteolysis by inhibition of the mTORC1 kinase complex, which regulates cell growth in response to numerous endogenous and exogenous signals (Saxton and Sabatini, 2017). mTORC1 can modulate mitochondrial biogenesis and dynamics at the transcriptional and translational level (Morita et al., 2015) but regulates YME1L posttranslationally. Inhibition of mTORC1 in hypoxia or upon amino acid starvation was found to inhibit a cellular lipid signaling cascade, which ultimately reduces phosphatidylethanolamine (PE) levels in the IM-activating proteolysis by YME1L (Figure 5) (MacVicar et al., 2019). The PA phosphatase LIPIN1, which converts PA to diacylglycerol and thereby regulates the synthesis of glycerophospholipids and triacylglycerides, is a direct target of the mTORC1 kinase complex (Peterson et al., 2011). mTORC1 inhibition activates LIPIN1 and decreases PA accumulation. As PA activates the synthesis of PC by CTP: phosphocholine cytidylyltransferase alpha (CCTα) (Jacquemyn et al., 2017), LIPIN1 activation decreases PC levels and reduces its conversion

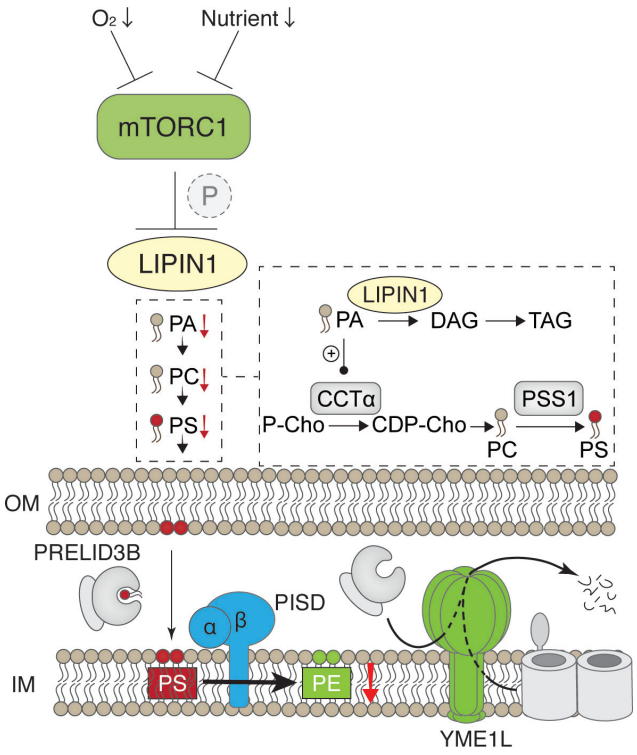

Figure 5: mTORC1-dependent lipid signaling regulates YME1L-mediated proteolysis.
mTORC1 inhibition upon oxygen or nutrient deprivation results in dephosphorylation and activation of LIPIN1. The decreased PA level limits the PS supply to mitochondria and ultimately results in decreased PE levels in mitochondrial membranes and enhanced YME1L-mediated proteolysis. See text for details.
OM, outer membrane; IM inner membrane; DAG, diacylglycerol; TAG, triacylglycerol; P-Cho, phosphocholine; CDP-Cho, cytidine diphosphate-choline; PA, phosphatidic acid; PE, phosphatidylethanolamine; PS, phosphatidylserine.

to PS, which is transported to mitochondria and converted into PE. Genetic experiments support mTORC1 regulation of YME1L-mediated proteolysis along this lipid signaling cascade. LIPIN1 depletion abolished activation of YME1L-mediated proteolysis upon mTORC1 inhibition, but YME1L-mediated degradation was restored upon concomitant depletion of CCTα. Furthermore, reducing PE levels in the IM by depletion of the PS-specific lipid transfer protein PRELID3B or of the PS decarboxylase increased proteolysis by YME1L. Reconstitution experiments in liposomes demonstrated unambiguously the regulation of YME1L-mediated proteolysis by PE in the IM and revealed increased turnover of YME1L substrates if PE levels were decreased. Thus, mitochondrial lipid homeostasis and protein homeostasis are coupled via YME1L. As YME1L degrades the PS-specific lipid transfer protein complex PRELID3B-TRIAP1 in the IMS, increased proteolysis by YME1L further decreases PE levels in the IM pointing to a positive feedback regulation of YME1L.

How PE regulates YME1L-mediated proteolysis remains to be defined. Phosphatidylethanolamine levels are only moderately decreased in hypoxic cells or upon mTORC1 inhibition, suggesting that the local lipid environment critically determines proteolysis. Notably, AAA proteases are associated with membrane scaffolds at the opposite membrane surface, which are thought to define functional domains in the IM (Steglich et al., 1999; Wai et al., 2016). YME1L is part of a proteolytic hub in the IM, the SPY complex, which contains the rhomboid protease PARL and the membrane scaffold SLP2 at the matrix-exposed side of the IM (Wai et al., 2016). mTORC1 inhibition may therefore decrease PE levels locally in the membrane environment of YME1L, which is defined by SLP2. Phosphatidylethanolamine may directly bind to YME1L and modulate its activity. Alternatively, it is conceivable that decreased PE levels facilitate the membrane dislocation of substrate proteins during YME1L-mediated proteolysis.

Mitochondrial proteases and cancer

Mitochondria play a pleiotropic role during multiple stages of cancer by regulating bioenergetics, metabolite synthesis, redox homeostasis, and cell death (Vyas et al., 2016). The mechanisms by which mitochondria enable tumors to overcome environmental stress and therapeutic treatment are of great interest. In these scenarios, the plasticity of the mitochondrial population supports the metabolic flexibility required to drive cancer progression (Vyas et al., 2016). Regulated mitochondrial proteolysis is emerging as a mechanism by which cancer cells adapt and reprogram mitochondrial activity during tumorigenesis.

YME1L in tumorigenesis

Mitochondrial proteome rewiring in response to oxygen or nutrient deprivation bears significance for solid tumors that exist in harsh microenvironments. An examination of pancreatic ductal adenocarcinoma (PDAC) patient biopsies revealed that HIF1α stabilization is accompanied by the depletion of YME1L substrates in PDAC tumor tissues (MacVicar et al., 2019). Pancreatic ductal adenocarcinoma is one of the most hypoxic and nutrient-starved cancer types (Vaziri-Gohar et al., 2018), and PDAC cells overcome these conditions by reprogramming glutamine metabolism and HIF signaling (Guillaumond et al., 2013; Son et al., 2013). Stimulating YME1L proteolysis may optimize mitochondria to support such metabolic adaptations during PDAC development. Indeed, YME1L is required for PDAC cells to grow in culture or as xenografts in nude mice. Further analysis is required to establish which YME1L substrate(s) have a negative impact on the metabolism and growth of PDAC cells *in situ*. Conversely, the growth of cultured hepatocellular carcinoma (HCC) cell lines was not dependent on YME1L, and no depletion of YME1L substrates was observed in highly vascularized HCC tissue (MacVicar et al., 2019). It remains to be seen whether YME1L temporarily facilitates the rapid growth of hypoxic HCC nodules that readily scavenge their oxygen supply (Chen and Lou, 2017).

The demand for proteolytic rewiring by YME1L in tumorigenesis is therefore likely to be determined by the environment and metabolic requirements of each tumor. Interestingly, *YME1L* has been found to be frequently mutated in human colorectal cancer along with other cancers to a lesser degree, although the functional implications of these mutations are unknown (Srinivasainagendra et al., 2017). It is appreciated that mTORC1 is frequently activated in cancer to maintain a progrowth metabolic state (Mossmann et al., 2018), and one would therefore expect YME1L proteolysis to be inhibited in conjunction with upregulated mitochondrial biogenesis (Morita et al., 2013). In these circumstances, it may be possible to uncouple the mTORC1–YME1L axis in order to test whether enhanced YME1L proteolysis would disturb the metabolic programming engaged by hyperactive mTORC1 signaling, including glutamine catabolism (Csibi et al., 2013). Unfortunately, the therapeutic targeting of mTORC1 with active site inhibitors has had limited success in cancer patients (de la Cruz Lopez et al., 2019). The issue remains that blocking mTORC1 often has a cytostatic rather than cytotoxic effect, which has encouraged the employment of combined therapy strategies in an effort to force cancer cell death (de la Cruz Lopez et al., 2019). The downstream stimulation of YME1L-mediated proteolysis upon treatment with mTORC1 inhibitors may encourage cancer cell survival via metabolic rewiring. Alternatively, YME1L activation may support the cytoprotective effect of mitochondrial elongation that occurs in cells treated with mTORC1 inhibitors (Morita et al., 2017). Inhibition of YME1L has been shown to promote cell death *in vitro* and *in vivo*, which may further support its suitability as a combined target with mTORC1 inhibition (Stiburek et al., 2012; Wai et al., 2015).

Proteolytic regulation of cancer by matrix and IMS proteases

Mitochondrial function appears to be tightly regulated by proteolysis in cancer, and some mitochondrial proteases are already being explored as possible therapeutic targets. The matrix caseinolytic protease CLPP is an exciting example because its hyperactivation by treatment with

the imipridone ONC201 results in the selective death of malignant leukemia and lymphoma cells (Ishizawa et al., 2019). ONC201 is currently undergoing clinical trials and has the potential to treat acute myeloid lymphoma, as well as solid tumors (Kline et al., 2016; Arrillaga-Romany et al., 2017; Stein et al., 2017). Activation of CLPP results in the degradation of a specific subset of matrix proteins, which include respiratory chain complex subunits, leading to OXPHOS impairment (Ishizawa et al., 2019). CLPP may therefore be a particularly promising target in tumors that depend on OXPHOS for growth and survival. The maintenance of matrix proteins by CLPP is tightly balanced as the inhibition or depletion of CLPP has also been shown to be toxic in leukemic cells (Cole et al., 2015). The vulnerability of these cells to CLPP loss was also attributed to defective OXPHOS, in this case linked to the accumulation of dysfunctional respiratory chain complex II subunits (Cole et al., 2015). It should also be noted that many putative CLPP substrates are not components of the respiratory chain, and therefore hyperactivation or depletion of the protease is likely to impact cancer cell metabolism in multiple ways.

It is unclear if and how CLPP activity is dynamically regulated by cancer cells in response to changes in the microenvironment or metabolic demand. However, another resident protease of the mitochondrial matrix, the AAA+ Lon protease (LONP), is upregulated in response to stress stimuli such as hypoxia *in vitro* and *in vivo* (Fukuda et al., 2007; Quiros et al., 2014). Unlike the acute post-translational stimulation of YME1L-mediated proteolysis upon hypoxia, LONP is transcriptionally upregulated by stabilized HIF1α (Fukuda et al., 2007). *LONP* overexpression has been determined as a poor prognostic marker in a variety of human cancers, and it supports the proliferation and metastasis of tumors in mice (Liu et al., 2014; Quiros et al., 2014; Di et al., 2016). LONP broadly preserves mitochondrial fitness by degrading misfolded and oxidized substrates, and its transcriptional upregulation by HIF1α may also indicate a role in the metabolic reprogramming of glycolysis and OXPHOS. Indeed, overexpression of LONP limits complex I–dependent respiration and upregulates glycolysis (Quiros et al., 2014). Inhibiting LONP using triterpenoid compounds has been shown to cause apoptosis in cancer cell lines, but it is unclear whether LONP can be specifically targeted in tumor cells without causing profound mitochondrial dysfunction in normal tissue (Bernstein et al., 2012; Gibellini et al., 2015).

Other proteases may cooperate with YME1L to tune the proteome of the IMS and regulate tumorigenesis. Depletion of OMA1 promotes the metastatic potential of breast cancer cell lines, which indicates a possible antitumorigenic role for OMA1 in specific metabolic conditions (Daverey et al., 2019). This contrasts with the protumorigenic role of YME1L in PDAC cells described above. Considering that OMA1 is a substrate of YME1L (Rainbolt et al., 2016) and that both proteases may differentially regulate substrates in the IMS, further work will be required to carefully assess the contribution of both proteases to different aspects of tumorigenesis. The IMS serine protease, LACTB, is also a proposed tumor suppressor in breast and colorectal cancers (Keckesova et al., 2017; Zeng et al., 2018). While the direct proteolytic substrates of LACTB remain unknown, LACTB overexpression results in mitochondrial phospholipid remodeling and delays the growth of a subset of breast cancer cells in culture and in mice. The depletion of PE contributes to the poor growth of LACTB-overexpressing cells as supplementation with Lyso-PE can partially restore proliferation (Keckesova et al., 2017). Several studies have also indicated a role for the serine protease HTRA2 by reporting its differential expression between tumor and nontumor tissue (Bowden et al., 2006; Narkiewicz et al., 2008). Mechanistically, HTRA2 plays a tumor suppressive role in Ras-driven cancer cells, which depends on its release from mitochondria downstream of p53-dependent mitochondrial fragmentation. Once in the cytosol, activated HTRA2 may process numerous substrates, including β-actin, which ultimately impacts the invasive capability of these cells in culture (Yamauchi et al., 2014). Another extramitochondrial substrate of HTRA2 linked to cancer is the Wilms tumor suppressor protein WT1. Processing of WT1 by HTRA2 was shown to promote cell death by apoptosis (Hartkamp et al., 2010). Further work will be required to establish whether apoptotic induction by HTRA2 may be an applicable therapeutic strategy to target hard-to-kill cancers and whether mitochondrial localized HTRA2 features in cancer cell regulation.

Concluding remarks

The *i*-AAA protease YME1L has been identified as a key regulatory protease in mitochondria, which controls mitochondrial protein and lipid biogenesis, mitochondrial fusion, and the metabolic profile of mitochondria. YME1L-mediated proteolysis thus couples the morphology of mitochondria to their metabolic function. Rewiring of mitochondria by YME1L was found to be critical for the cellular adaptation to oxygen and nutrient starvation. While glucose-dependent respiration does not depend on YME1L, the degradation of various metabolite carriers and

metabolic enzymes by YME1L repurposes mitochondria in favor of anabolic pathways to support glycolytic cell growth. It will be of interest to examine the role of YME1L-mediated mitochondrial rewiring in other processes that involve metabolic shifts from OXPHOS dependent on glycolytic growth, as they occur, for instance, during stem cell activation or immune cell reprogramming. Similarly, YME1L-mediated proteolysis may support cellular adaptation to low OXPHOS activities, as they occur, for instance, during aging or in mitochondrial diseases affecting the respiratory chain. Current evidence supports a critical role of YME1L proteolysis in PDAC and suggests YME1L as a promising therapeutic target. Enzymes such as YME1L or other mitochondrial proteases might indeed prove to be druggable targets in cancer, but identifying their substrates or regulatory mechanisms may help target cancer-specific metabolic pathways. This would avoid the detrimental collapse in the mitochondrial integrity of healthy tissue associated with broad disruption of protein homeostasis.

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