Mitochondrial Proteolysis and Metabolic Control

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Mitochondria are metabolic hubs that use multiple proteases to maintain proteostasis and to preserve their overall quality. A decline of mitochondrial proteolysis promotes cellular stress and may contribute to the aging process. Mitochondrial proteases have also emerged as tightly regulated enzymes required to support the remarkable mitochondrial plasticity necessary for metabolic adaptation in a number of physiological scenarios. Indeed, the mutation and dysfunction of several mitochondrial proteases can cause specific human diseases with severe metabolic phenotypes. Here, we present an overview of the proteolytic regulation of key mitochondrial functions such as respiration, lipid biosynthesis, and mitochondrial dynamics, all of which are required for metabolic control. We also pay attention to how mitochondrial proteases are acutely regulated in response to cellular stressors or changes in growth conditions, a greater understanding of which may one day uncover their therapeutic potential.

More than a billion years of evolution since their fateful endosymbiotic origin have directed mitochondria to play central roles in metabolism and other cellular functions. For instance, precise regulation of mitochondrial function is required for the production of ATP by oxidative phosphorylation (OXPHOS), the maintenance of calcium homeostasis, and programmed cell death. Only 13 mitochondrial proteins (~1% of the total mitochondrial proteome) are encoded by resident mitochondrial DNA (mtDNA) with the remainder originating from the nucleus. These proteins are synthesized on cytosolic ribosomes and directed to four distinct submitochondrial compartments that are defined by the organelle's unique double membrane-bound structure. The mitochondrial matrix is surrounded by the cristae-forming

inner mitochondrial membrane (IMM), which is separated from the cytosol by the intermembrane space (IMS) and outer mitochondrial membrane (OMM). Proteostasis in each subcompartment is maintained by a number of mitochondrial proteases (mitoproteases) that promote protein quality control by degrading misfolded proteins and by processing newly imported proteins to facilitate their biogenesis (Quiros et al. 2015). Mitochondrial proteolysis is therefore a frontline mitochondrial quality-control mechanism that combines with others, including reactive oxygen species (ROS) scavenging and mitophagy, to maintain overall mitochondrial fitness.

Acting as quality-control enzymes, all mitoproteases are likely to impact metabolism because mitochondria represent a core metabolic

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hub. Nevertheless, recent advancements have demanded that we look beyond this classical description of mitoproteases. It is now clear that mitochondrial proteolysis offers a valuable node for the regulation of specific mitochondrial functions and can be modulated by an array of signaling inputs. Consequently, dysfunctional mitoproteases contribute to a plethora of human diseases, including cancer and neurodegeneration and are strongly associated with the aging process. The loss of mitoprotease function in these diseases impairs mitochondrial function and causes metabolic-associated phenotypes. In this review, we will focus on the close and often reciprocal relationship between mitoproteases and metabolic control. We will highlight the impact of mitoproteases on pathogenesis and health span, a greater understanding of which may one day propel these enzymes forward as therapeutic targets.

PROTEOLYSIS ORGANIZES THE MITOCHONDRIAL PROTEOME

Mitochondria contain at least 20 intrinsic and catalytically functional proteases, which are divided into three catalytic classes: 12 metalloproteases, seven Ser proteases, and one Cys protease (Quiros et al. 2015). The intrinsic mitoproteases are distributed between the submitochondrial compartments in which they contribute to mitochondrial function and homeostasis by cleaving or completely degrading target substrates. A common feature of mitoproteases is their pleiotropic nature, which arises from the fact that a protease may have multiple diverse substrates. The best examples of multisubstrate mitoproteases are those that regulate mitochondrial protein import and biogenesis.

Newly synthesised mitochondrial proteins must be imported and sorted to the correct mitochondrial subcompartment. The majority of these proteins contain an amino-terminal targeting signal, or presequence, which can be cleaved by a number of processing peptidases located in the matrix or IMM. Arguably the most common example is the essential and conserved mitochondrial-processing peptidase (MPP) complex, which is a heterodimer com-

prised of the peptidase PMCB and its noncatalytic homolog PMCA (Mossmann et al. 2012). A matrix soluble complex, MPP processes the positively charged presequence of the vast majority of newly imported proteins destined for the matrix, IMM or IMS. Discarded presequence peptides are targeted by proteases within mitochondria, such as Cym1 and Ste23 in yeast or PreP in humans, to be rapidly degraded and to prevent their potentially toxic accumulation (Stahl et al. 2002; Kambacheld et al. 2005; Falkvall et al. 2006; Mossmann et al. 2014; Taskin et al. 2017).

Mitoproteases mediating the processing of newly imported proteins often affect the subsequent sorting of those proteins within mitochondria. For example, the inner mitochondrial membrane peptidases (IMMPs) release soluble proteins into the IMS by cleaving off their hydrophobic sorting signals. The regulation of protein import and sorting by proteolysis therefore has a huge impact on mitochondrial metabolism, for instance by ensuring the assembly of OXPHOS complexes to drive the generation of ATP. Consequently, mutations in MPPs cause a number of diseases in humans, with neurodegenerative disorders being the most prevalent. Mutation of either MPP subunit gene, *PMPCA* or *PMPCB*, causes similar early-onset neurological phenotypes in patients, with the cerebellum being particularly affected (Jobling et al. 2015; Choquet et al. 2016; Joshi et al. 2016; Vogtle et al. 2018). Mutations within *PMPCB* appear to produce more severe symptoms, perhaps because mutation of the catalytic subunit disturbs MPP processing activity to the greater extent (Vogtle et al. 2018). Furthermore, and to highlight the range of diseases caused by processing peptidase defects, mutation of IMMP subunit 2-like protease (*IMMP2*) is associated with developmental disorders such as Tourette syndrome, whereas mutation of *MIPEP*, encoding the mitochondrial intermediate peptidase (MIP/Oct1), causes infant heart failure (Petek et al. 2001; Bertelsen et al. 2014; Eldomery et al. 2016). Specific diseases associated with mitochondrial dysfunction (broadly termed as mitochondrialopathies) are also triggered by mutations in the presequences of mitochondrial proteins. For exam-

ple, impaired MPP processing of mutant Fra-taxin leads to disturbed iron homeostasis and the neurodegenerative disorder Friedreich's ataxia (Gakh et al. 2002), and a homozygous mutation at the MPP-processing site of the protease YME1L was recently found to impair maturation and destabilize the protein resulting in neuropathy and optic atrophy (Hartmann et al. 2016).

Proteases can also modulate mitochondrial protein import by regulating components of the import machinery. For instance, carboxy-terminal processing of the transmembrane protein Mgr2 by Imp (yeast IMMP) is required for proper assembly and stabilization of the translocase of inner mitochondrial membrane 23 (Tim23/TIMM23) complex (Ieva et al. 2013). Curiously, in comparison to yeast and fungal Mgr2, the mammalian homolog ROMO1, which has been attributed to diverse mitochondrial functions besides protein import, lacks an IMMP cleavage motif and has a truncated carboxy terminus (Ieva et al. 2013).

Import machinery subunits are also targeted for complete degradation by proteases in regulatory and quality-control pathways. TIMM17A is an essential component of the TIMM23 complex and is a substrate of the ATP-dependent *i*-AAA or yeast mtDNA escape 1-like (YME1L1 or YME1L) protease. YME1L can limit mitochondrial import by degrading TIMM17A at the IMM in response to a number of insults including endoplasmic reticulum (ER) stress (Rainbolt et al. 2013). Furthermore, Yme1 in yeast has also been shown to degrade small Tim proteins Tim9 and Tim10, which function as chaperones assisting the assembly of newly imported membrane inserted carrier translocases (Baker et al. 2012; Rampello and Glynn 2017). Many of these carrier proteins transfer metabolite precursors across the membrane and thus directly impact mitochondrial metabolism. The proteolytic maintenance of mitochondrial import by processing peptidases coupled with the degradation of specific translocase machinery can therefore broadly impact mitochondrial function. For the remainder of this review, we will draw attention to the proteolytic control of other specific mitochondrial func-

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tions that directly impact mitochondrial and cellular metabolic pathways.

PROTEOLYTIC CONTROL OF MITOCHONDRIAL RESPIRATION

The production of ATP by OXPHOS can be modulated by regulating the activities of metabolic enzymes, including the assembly of the respiratory chain, the uptake of mitochondrial respiratory substrates, or by altering overall mitochondrial mass. Nuclear and mitochondrial gene expression and protein synthesis machinery must be synchronized and synergized to allow the synthesis and assembly of functional OXPHOS complexes. Their coordination allows mitochondria to respond to ever-changing cellular energy demands and substrate availability. Mitochondrial biogenesis, the maintenance of mtDNA and its replication are under the control of nuclear transcription factors and nuclear-encoded mitochondrial proteins (Michel et al. 2012). However, mitochondrial proteases have been shown to participate in both the regulation of mtDNA maintenance and of mitochondrial gene expression to modulate the respiratory activity of cells.

Mitochondrial Genome Maintenance and Gene Expression under the Control of Proteases

One of the key regulators of mtDNA in mammals is mitochondrial transcription factor A (TFAM). TFAM is essential for mtDNA replication and transcription (Larsson et al. 1998; Shi et al. 2012) and functions as a histone-like protein packing the mtDNA molecule into mitochondrial nucleoids (Kaufman et al. 2007). The mtDNA copy number faithfully follows levels of TFAM and vice versa. Overexpression of TFAM increases mtDNA levels and TFAM mutation or deletion leads to mtDNA depletion (Ekstrand et al. 2004). Different TFAM:mtDNA ratios have also been postulated to regulate the rate of gene expression. TFAM is phosphorylated by cAMP-dependent protein kinase A (PKA) and degraded by mitochondrial matrix AAA⁺ peptidase Lon (LONP) (Lu et al. 2013).

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TFAM binds mtDNA in its dephosphorylated state but the phosphorylated, non-DNA-bound TFAM is degraded by LONP (Fig. 1A). In the case of severe mtDNA depletion, LONP can recognize DNA-free TFAM and degrade it independently of the phosphorylation status of TFAM (Lu et al. 2013). LONP degrades misfolded, oxidized, and damaged proteins (Pinti et al. 2015) and, as new findings reveal, it also degrades functional proteins in a regulatory manner. The yeast Lon protease was found to degrade Abf2 (the yeast TFAM homolog), mtDNA maintenance protein Mgm101, and human mitochondrial replicative helicase Twinkle as well as large ribosomal subunit protein MrpL32 in vitro (Kunova et al. 2017). In addition to its wide range of protein substrates, LONP has also been shown to directly bind mtDNA. In mammalian cells, LONP can bind to the control region of mtDNA that contains promoter regions and an origin of replication in a sequence-specific manner (Lu et al. 2013). In *Drosophila* cells, LONP was shown to regulate TFAM and mtDNA levels (Matsushima et al. 2010). How the binding of LONP to mtDNA affects the abundance, organization, or reading of mtDNA is still unknown. Regardless, LONP controls mitochondrial genome maintenance in healthy normal cells in addition to its quality-control function degrading damaged or oxidized proteins.

Proteolytic Control of Mitochondrial Protein Synthesis

Different mitoproteases regulate mitochondrial protein synthesis at multiple sites. Their substrates include proteins that affect messenger RNA (mRNA) processing, ribosomal assembly, and nascent polypeptide chain modifications as well as ribosomal subunits or newly synthesized mitochondrial proteins (Fig. 1B).

Mitochondrial matrix protease complex CLPP is formed by protease CLPP (ATP-dependent caseinolytic protease) and a chaperone CLPX (ATP-dependent caseinolytic protease X, chaperone subunit). This protease complex has been shown to degrade misfolded or aggregated proteins (Kang et al. 2002; Flynn et al. 2003;

Fischer et al. 2016) in the mitochondrial matrix and it also participates in the mitochondrial unfolded protein response (UPR^{mt}) in *Caenorhabditis elegans* (Haynes et al. 2007). CLPP has also recently been shown to have an unexpected role in controlling mitochondrial translation and ribosomal assembly in mice (Szczepanowska et al. 2016). CLPP degrades 12S ribosomal RNA (rRNA) chaperone ERAL1 in a timely manner, thus allowing the assembly of ribosomes to promote translation (Fig. 1B). Moreover, CLPP was also shown to regulate mitochondrial protein synthesis at the mRNA level. In *Drosophila* cells, CLPP degrades leucine-rich pentatricopeptide repeat domain-containing protein 1 (LRPPRC1), which is needed for polyadenylation of mt-mRNAs, for example, when transcription is inhibited (Matsushima et al. 2017). CLPP deficiency causes a disease characterized by ovarian failure and hearing loss called Perrault syndrome, which is also associated with mutations in the mitochondrial translation and transcription machinery (Perrault et al. 1951; Pierce et al. 2011, 2013; Morino et al. 2014; Chatzispayrou et al. 2017). CLPP depletion in the mouse mimics the human disease, leading to infertility and hearing loss (Gispert et al. 2013; Szczepanowska et al. 2016). It is tempting to speculate that the reported symptoms of these mice, and possibly also humans, are caused by mitochondrial translation defects and the concomitant respiratory deficiency. Indeed, impaired mitochondrial protein synthesis has profound consequences both in mice and human (Boczonadi and Horvath 2014). It should be noted that decreased translation rates in mitochondria lacking CLPP can also have beneficial consequences. The loss of aspartyl aminoacyl tRNA synthetase (DARS2) in the heart leads to cardiomyopathy and early death in mice (Dogan et al. 2014) and deletion of CLPP in this model alleviates cardiac hypertrophy possibly caused by a reduced mitochondrial translation rate (Seiferling et al. 2016).

Another protease that ensures mitochondrial translation is the *m*-AAA protease, which forms a hexameric complex in the IMM with its catalytic domains facing the matrix. In humans, the *m*-AAA protease is a homo-oligomeric

Figure 1. Proteases controlling mitochondrial gene expression and oxidative phosphorylation (OXPHOS) assembly. (A) Peptidase Lon (LONP) regulates mitochondrial genome maintenance and transcription by binding to mitochondrial DNA (mtDNA) and degrading mitochondrial replicative helicase Twinkle and transcription factor A (TFAM). (B) Several proteases control mitochondrial protein synthesis. LONP degrades ribosomal subunit MRPL32 and CLPXP 12S ribosomal RNA (rRNA) chaperone ERAL1 and messenger RNA (mRNA) polyadenylation enzyme leucine-rich pentatricopeptide repeat domain-containing protein 1 (LRPPRC1). Matrix AAA^+ ($m\text{-AAA}^+$) protease cleaves newly synthesized mitochondrial translation products and MRPL32. (C) OMA1, ATP23, $m\text{-AAA}$, YME1L, LONP, and CLPXP are all participating in the degradation of OXPHOS subunits.

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structure composed of AFG3L2 (AFG3-like matrix AAA peptidase subunit 2) subunits or a hetero-oligomeric complex consisting of AFG3L2 and highly homologous SPG7 (Paraplegin) subunits. Mutations in either of these proteins cause OXPHOS dysfunction and neurodegeneration with overlapping but distinct pathologies: spinocerebellar ataxia, in the case of AFG3L2, and spastic paraplegia, in the case of SPG7 (Casari et al. 1998; Di Bella et al. 2010). AFG3L2 mutations in mouse models mimic the human symptoms of severe motor dysfunction and axonal loss (Maltecca et al. 2008, 2009). AFG3L2 controls ribosomal assembly by regulating Mrpl32 maturation in yeast (Nolden et al. 2005). Similarly, the loss of AFG3L2 in mammalian cells impairs mitochondrial ribosome assembly and protein synthesis (Almajan et al. 2012). Together, these studies show that proteases take part in the intrinsic regulatory pathway of mitochondrial gene expression (Fig. 1B). In addition, the *m*-AAA protease also exerts quality-control functions (Arlt et al. 1998; Leonhard et al. 2000) and modulates mitochondrial calcium homeostasis by regulating the assembly of the mitochondrial calcium uniporter (MCU) (Konig et al. 2016; Patron et al. 2018).

Proteases Acutely Control OXPHOS Complex Assembly and Activity

The IMM accommodates mitochondrial respiratory chain complexes (complex I–IV) that function as electron carriers creating a proton-motive force of pH and membrane potential across the IMM that allows ATP production by the F_1F_0 ATP synthase (complex V) (Chaban et al. 2014). Mitoproteases are emerging as central regulators of the assembly of these complexes and thereby control cellular ATP production (Fig. 1C).

The formation of a functional respiratory chain depends on the coordinated assembly of nuclear-encoded subunits, which must be imported into mitochondria after their synthesis on cytosolic ribosomes, with mt-encoded respiratory chain subunits. Various mitoproteases degrade nonassembled subunits and thus prevent potential damaging effects (Arlt et al. 1998;

Hornig-Do et al. 2012; Stiburek et al. 2012; Richter et al. 2015). Moreover, they play direct roles during the assembly of OXPHOS complexes and of the F_1F_0 ATP synthase. In yeast, the processing peptidase Atp23 was shown to be essential for ATPase F_1F_0 assembly by controlling both the maturation Atp6 subunits synthesized within mitochondria and their subsequent assembly (Osman et al. 2007; Zeng et al. 2007). The zinc metalloprotease Oma1 degrades selectively Cox1 subunits if the assembly of cytochrome *c* oxidase (COX) is impaired in yeast mutant cells (Khalimonchuk et al. 2012; Bohovych et al. 2015). Heme is an essential cofactor for respiratory chain enzymes and its synthesis is regulated by CLPXP. CLPXP degrades the rate-limiting enzyme in heme synthesis, ALAS1, which curbs heme accumulation in mitochondria (Kardon et al. 2015; Kubota et al. 2016). Mutations in CLPX causes erythropoietic protoporphyria in human patients (Yien et al. 2017).

Proteases are also required to regulate OXPHOS function acutely when a cell needs to adapt to sudden metabolic changes. Hypoxia is one example of a situation in which mitochondrial metabolism must be matched with altered cellular metabolism. Hypoxia-inducible factor (HIF-1 α) is a transcriptional activator that promotes a metabolic shift during hypoxia by up-regulating glycolysis and down-regulating mitochondrial respiration, a hallmark of many cancers. *LONP* mRNA is up-regulated on HIF-1 α stabilization and its subsequent accumulation leads to enhanced degradation of the complex IV subunit COX4-1 (Fukuda et al. 2007). This occurs alongside the transcriptional up-regulation of an alternative subunit, COX4-2, which may optimize O_2 usage during hypoxia to suppress oxidative stress (Fukuda et al. 2007; Sepuri et al. 2017). Interestingly, *LONP* expression is enhanced in some tumors and its down-regulation can consequently promote cancer cell death in vitro and block melanoma metastasis in vivo (Hu et al. 2005; Bernstein et al. 2012; Quiros et al. 2014). Although a complete loss of *LONP* causes embryonic lethality, heterozygous mice are viable and are protected from colon carcinoma and skin tumors (Quiros et al. 2014). The

remodeling of complex IV by LONP may be central to its apparent oncogenic potential because it supports the shift toward glycolysis. Additionally, LONP and CLPP together specifically degrade the peripheral arm of complex I in response to a loss of membrane potential and increasing levels of ROS in mammalian cells (Pryde et al. 2016). This partial degradation of complex I limited further ROS production and cellular damage under these conditions.

PROTEASES TUNE THE MITOCHONDRIAL DYNAMICS MACHINERY TO REGULATE CELLULAR METABOLISM

Another key feature of mitochondrial biology that exists under close proteolytic control is mitochondrial dynamics. The modification of mitochondrial morphology represents an important facet of mitochondrial quality control and is intricately coupled to the metabolic status of cells. The fusion of mitochondria into elongated networks is balanced by fission events that can isolate individual mitochondria or, if unopposed, can lead to a complete fragmentation of the mitochondrial network (Youle and van der Bliek 2012). The elongation of a mitochondrial network is associated with high demands for OXPHOS, whereas metabolic shifts toward glycolysis correlate with a fragmented mitochondrial morphology (Mishra and Chan 2016; Wai and Langer 2016). The physiological importance of this relationship between mitochondrial morphology and metabolism has been highlighted, for example, during tumorigenesis, stem cell differentiation and T-cell programming (Buck et al. 2016; Chen and Chan 2017). The fragmentation of mitochondria also facilitates the removal of dysfunctional or redundant organelles by the quality-control pathway called mitophagy (discussed in the following section). Differing metabolic inputs and demands can rapidly alter the morphology of mitochondria by targeting their conserved dynamics machinery via posttranslational modification pathways. Mitochondrial proteolysis has emerged as a central regulator of the mitochondrial dynamics machinery at both membranes.

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Proteolytic Control of OMM Dynamics

Mitochondrial fusion and fission are orchestrated by a conserved set of dynamin-related GTPases. Fusion of the OMM is mediated by mitofusin 1 and 2 (MFN1/2); homologous proteins that are anchored to the OMM. Hetero- or homotypic interactions between mitofusins of opposing mitochondria promotes their tethering (Daumke and Roux 2017). Ablation of *MFN1* or *MFN2* leads to mitochondrial fragmentation and specific mutations in *MFN2* cause the recessive peripheral neuropathy Charcot-Marie-Tooth type 2A (Cartoni and Martinou 2009). Mitofusins are ubiquitinated, which can either promote MFN1/2-mediated fusion or target them for degradation by the 26S proteasome. The OMM localized cysteine ubiquitin-specific protease 30 (USP30), which is currently the only known integral OMM protease, negatively regulates mitochondrial fusion by cleaving profusion ubiquitin moieties from the mitofusins (Fig. 2) (Nakamura and Hirose 2008; Yue et al. 2014). Indeed, the suppression of USP30 by knockdown or chemical inhibition stimulates mitochondrial fusion and can restore the mitochondrial network and the OXPHOS capacity of *Mfn1*^{-/-} and *Mfn2*^{-/-} cells (Yue et al. 2014).

Mitochondrial fission at the OMM is also regulated by proteolysis but may involve proteases that are not constitutively present at mitochondria. Fission is driven by the recruitment of the large cytoplasmic GTPase DRP1 to sites marked by fission receptor proteins MFF, MiD49, and MiD51 (Kraus and Ryan 2017). Subsequent oligomerization at these sites facilitates mitochondrial constriction and ultimately leads to scission in collaboration with another dynamin family member, dynamin-2 (Lee et al. 2016). DRP1 activity is coupled to cellular energy status, for example, starvation conditions block DRP1 recruitment to mitochondria to preserve an elongated mitochondrial network, whereas the regulation of DRP1 by the circadian clock supports a rhythmic relationship between mitochondrial dynamics and ATP production (Gomes et al. 2011; Rambold et al. 2011; Schmitt et al. 2018). Consequently, much focus has been placed on the posttranslational regulation of

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Figure 2. Proteolytic control of mitochondrial dynamics. Mitochondrial fusion (*upper*) and fission (*lower*) depend on the regulation of mitochondrial dynamics factors by proteolysis. MFN1 and MFN2 promote outer mitochondrial membrane (OMM) fusion and they are negatively regulated by the deubiquitinase USP30. YME1L and OMA1 process OPA1 to regulate inner mitochondrial membrane (IMM) fusion. Excessive processing of membrane-bound long-form (L)-OPA1 by OMA1 limits fusion and promotes mitochondrial division.

DRP1, predominantly by phosphorylation, but also by SUMOylation and ubiquitylation. Phosphatases, deubiquitylases and deSUMOylases thus may modulate DRP1 fission activity in response to specific metabolic and stress stimuli. The deSUMOylase SENP3, for instance, has been shown to promote DRP1 interaction with MFF and to drive mitochondrial fission and cell death in response to oxidative stress (Guo et al. 2013, 2017). A seemingly contradictory role for another peptidase, SENP2, in the regulation of DRP1 SUMOylation highlights the need for further work to understand the proteolytic control of mitochondrial fission at the OMM (Fu et al. 2014).

Proteolytic Control of IMM Dynamics and Ultrastructure

Mitochondrial fusion at the IMM is elaborately regulated by the proteolytic processing of the pro-fusion GTPase optic atrophy 1 (OPA1) (MacVicar and Langer 2016). OPA1 mediates both IMM fusion and cristae biogenesis, which places it as a key regulator of mitochondrial dynamics, apoptosis, and respiration (Olichon et al. 2006). Loss of OPA1 in cells causes complete mitochondrial fragmentation, mtDNA loss, and respiratory dysfunction. As its name indicates, OPA1 mutation leads to autosomal-dominant optic atrophy, a neuropathy that presents

a progressive deterioration of retinal ganglion cells and the optic nerve (Alexander et al. 2000; Delettre et al. 2000).

OPA1 regulation is complex. Eight alternatively spliced mRNA isoforms exist in humans and after import and tethering to the IMM, long (L)-OPA1 forms are proteolytically processed to release short (S)-OPA1 into the IMS (Fig. 2). Homeostasis between L-OPA1 and S-OPA1 is maintained by YME1L and OMA1 and these integral IMM membrane proteases target OPA1 at distinct individual processing sites exposed to the IMS (Ishihara et al. 2006). Unlike its yeast homolog Mgm1, which is only processed by a single protease, the rhomboid protease Pcp1 (Herlan et al. 2003, 2004), the cleavage of L-OPA1 by two proteases allows both constitutive and inducible proteolytic control of OPA1. The analysis of cells expressing exclusively L- or S-OPA1 variants as well as cells lacking the OPA1-processing peptidases YME1L and OMA1 revealed that L-OPA1 is necessary and sufficient to promote mitochondrial fusion (Anand et al. 2014; Del Dotto et al. 2017; Lee et al. 2017). In contrast, expression of S-OPA1 was found to be sufficient to stabilize mtDNA and maintain cristae morphogenesis in *Opa1*^{-/-} cells (Del Dotto et al. 2017; Lee et al. 2017). Thus, proteolytic processing by YME1L or OMA1 offers an intriguing possibility to acutely modulate the fusion activity of OPA1 in response to mitochondrial stressors and altered metabolic demands.

The zinc metalloprotease OMA1 is activated by mitochondrial dysfunction, resulting for instance from IMM depolarization, mitochondrial ATP depletion, or by the loss of other IMM proteins such as YME1L, DNAJC19, and the *m*-AAA protease (Anand et al. 2014; Baker et al. 2014; Richter-Dennerlein et al. 2014; Rainbolt et al. 2016). The subsequent cleavage of L-OPA1 and accumulation of S-OPA1 limits mitochondrial fusion and facilitates fission, resulting in fragmentation of the mitochondrial network and an increased sensitization to apoptosis (Anand et al. 2014). Similarly, various stress conditions, including ROS, heat stress and apoptotic stimuli can trigger OMA1 activation and mitochondrial fragmentation (Baker

et al. 2014; Jiang et al. 2014; Faccenda et al. 2017; Silic-Benussi et al. 2018). It remains to be properly defined how the loss of this response contributes to the relatively mild metabolic phenotypes present in *Oma1*^{-/-} mice (Quiros et al. 2012). Conversely, OMA1 hyperactivation has been shown to cause tissue atrophy in models of neurodegeneration, cardiomyopathy, and renal injury (Xiao et al. 2014; Wai et al. 2015; Korwitz et al. 2016). Indeed, targeting OMA1 holds significant therapeutic potential. For instance, OMA1 ablation is cardioprotective in four mouse models of heart failure and the overexpression of its substrate OPA1 also protects against degeneration in a number of tissues including the heart (Civiletto et al. 2015; Varanita et al. 2015; Wai et al. 2015; Acin-Perez et al. 2018). Targeting the protease itself would arguably be more clinically applicable and may avoid unwanted side effects attributed to high levels of OPA1 overexpression in vivo (Civiletto et al. 2015; Acin-Perez et al. 2018).

Although OMA1 can be regarded as a stress-inducible OPA1-processing peptidase, the ATP-dependent *i*-AAA protease YME1L is regulated by different metabolic inputs. YME1L was reported to process OPA1 more readily in mitochondria with high levels of OXPHOS, correlating with an increase in network connectivity (Mishra et al. 2014). In addition, YME1L is required to promote mitochondrial hyperfusion in cells undergoing acute ER stress (Rainbolt et al. 2013; Lebeau et al. 2018). However, during these conditions, OPA1 processing remains unaltered and perhaps YME1L-dependent processing or degradation of one or multiple additional substrate(s) may be of significance in this context to facilitate mitochondrial morphology adaptation.

MITOCHONDRIAL PROTEOLYSIS AND LIPID METABOLISM

The regulation of membrane lipid accumulation is another emerging role of mitoproteases that is intimately linked to cellular metabolism. Mitochondria participate in lipid homeostasis by synthesizing and degrading phospholipids, sphingolipids, and sterols (Horvath and Daum

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2013). Mitochondria are able to synthesize some lipids independently, such as phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylglycerol (PG), and cardiolipin (CL), the latter of which is specific to mitochondria but is still highly dependent on precursor lipids, which are synthesized in the ER and transported to mitochondria and across the IMS (Tatsuta and Langer 2017). Shuttling of phospholipids across the IMS is mediated by conserved lipid transfer proteins in a lipid-specific manner, all of which turn out to be under proteolytic control. Mitoproteases thus regulate the phospholipid composition of mitochondrial membranes by limiting the accumulation of lipid transfer proteins in the IMS. Members of the Ups/PRELI protein family, PRELID1 (Ups1 in yeast) and PRELID3B (Ups2 in yeast), are in a heterodimeric complex with TRIAP1 (Mdm35 in yeast) and are responsible for transporting PA and phosphatidylserine (PS) across the IMS, respectively. They accumulate at low levels within the IMS owing to ongoing proteolysis by Yme1 (Fig. 3) (Potting et al. 2010; Connerth et al. 2012; Watanabe et al. 2015; Aaltonen et al. 2016; Miyata et al. 2016). Studies in yeast suggest

that binding of Ups1 to the IMM and its subsequent degradation by Yme1 is modulated by CL levels in the IMM (Connerth et al. 2012). Similarly, YME1L degrades the START (StAR-related lipid transfer) domain-containing lipid transfer protein STARD7, which mediates phosphatidylcholine (PC) transfer to the IMM (Saita et al. 2018). Interestingly, STARD7 is dually localized in the IMS and the cytosol. PARL cleavage of STARD7 during its import into mitochondria allows its kinetic partitioning between the IMS and the cytosol (Saita et al. 2018). Another member of the START family of lipid transfer proteins, STARD1, transports cholesterol to mitochondria (Martin et al. 2016) and is degraded after import into the mitochondrial matrix (Granot et al. 2007).

Lipid synthesis enzymes within mitochondria are also targeted by mitoproteases. Mitochondrial PE synthesis from PS is catalyzed by mitochondrial phosphatidylserine decarboxylase 1 (Psd1) in yeast. Newly imported Psd1 is processed by MPP and the metalloendopeptidase Oct1 (Horvath et al. 2012) and its membrane insertion is accompanied by an autocatalytic cleavage event that is required for its PS

Figure 3. Mitochondrial proteases and lipid metabolism. PARL processes lipid transfer protein STARD7 and regulates its localization between mitochondria and cytoplasm. STARD1 transports cholesterol (C) to the outer mitochondrial membrane (OMM) but can be degraded by LONP in the matrix. The PRELID1–TRIAP complex and PRELID3B–TRIAP complex transport phosphatidic acid (PA) and phosphatidyl serine (PS), respectively, across the intermembrane space (IMS) and are degraded by YME1L. Phosphatidylserine carboxylase (PSD1) is cleaved by LACTB and PSD1 is also processing itself and thus switching from protease into a decarboxylase.

decarboxylase activity (Choi et al. 2012; Horvath et al. 2012). Although misfolded yeast Psd1 is degraded by Yme1 and Oma1, both exerting quality-control functions, the mammalian homolog PSD is under proteolytic control by LACTB (Keckesova et al. 2017), a mitochondrial IMS protein related to the bacterial penicillin-binding/ β -lactamase protein family. *LACTB* was identified as a novel tumor suppressor gene, whose function is linked to the PSD-PE axis highlighting the importance of mitochondrial lipid metabolism for the control of cell proliferation (Fig. 3) (Keckesova et al. 2017).

PROTEOLYSIS AND ORGANELLAR QUALITY CONTROL BY MITOPHAGY

Mitophagy regulates mitochondrial mass and quality by selectively targeting redundant or dysfunctional mitochondria for destruction in the lysosome via the autophagy machinery. Mitophagy is also regarded to be important for regulating metabolic homeostasis in a number of physiological contexts (Rodger et al. 2018). For instance, the clearance of mitochondria is required to support a switch to glycolysis during retinal ganglion cell differentiation (Esteban-Martinez et al. 2017) and to support cancer cell survival after mitotic arrest (Domenech et al. 2015). The increasing number of mitophagy regulated physiological scenarios is mirrored by the continued discovery of novel mitophagy pathways. The signaling for mitochondrial engulfment by nascent autophagosomes depends on the exposure of mitophagy receptor proteins and signals on the mitochondrial surface (Rodger et al. 2018), and IMM proteases have emerged as key players in several surveillance mechanisms that exist to target particular mitochondria for mitophagy. Indeed, the first example of this was described in yeast in which Yme1 was found to regulate mitophagy by directly processing the OMM mitophagy receptor Atg32 (Wang et al. 2013). Although a mammalian homolog for Atg32 has been proposed, it remains to be seen whether YME1L regulates mitophagy in mammals (Murakawa et al. 2015).

In mammalian cells, PINK1/Parkin-mediated mitophagy has received a great deal of atten-

tion because mutations in the kinase PINK1 and the E3-ubiquitin ligase Parkin were found to cause autosomal-recessive Parkinson's disease (Pickrell and Youle 2015). Both proteins act in the same mitophagy pathway, which begins with the stabilization of PINK1 on the OMM of dysfunctional mitochondria and is followed by the mitochondrial recruitment of Parkin and the subsequent ubiquitination of OMM proteins to recruit the autophagy machinery (Fig. 4) (Durcan and Fon 2015; Pickrell and Youle 2015). The deubiquitinase USP30 negatively regulates mitophagy by removing ubiquitin signals from Parkin target proteins (Bingol et al. 2014; Cunningham et al. 2015). Surprisingly, USP30 depletion can even enhance mitophagy in cells lacking detectable Parkin, suggesting that it deubiquitinates specific OMM proteins to restrict basal mitophagy (Marcassa et al. 2018).

PINK1 was the first substrate of the rhomboid protease PARL to be identified and constitutive PINK1 processing at the IMM of healthy mitochondria promotes its retro-translocation to the cytosol for subsequent degradation by the 26S proteasome (Jin et al. 2010; Meissner et al. 2011; Greene et al. 2012; Yamano and Youle 2013). However, mitochondrial damage and a loss of mitochondrial membrane potential ($\Delta\Psi_{mt}$) blocks the insertion of PINK1 into the IMM, thereby preventing its cleavage by PARL (Jin et al. 2010; Meissner et al. 2011). This results in the accumulation of PINK1 in the OMM as a positive signal for mitophagy. Intriguingly, PARL processing of PINK1 is also directly regulated by mitochondrial metabolism. During mitochondrial metabolic crisis and reduced ATP production, PARL is cleaved at the amino terminus via an autocatalytic mechanism regulated by the matrix kinase PDK2 (Sík et al. 2004; Shi and McQuibban 2017). This processed form of PARL, termed PACT (PARL carboxyl terminus), has reduced catalytic activity and may facilitate the accumulation of PINK1 on dysfunctional mitochondria (Shi and McQuibban 2017). PARL autocatalysis also releases the short amino-terminal sequence of PARL termed p β (Sík et al. 2004). The p β peptide has been reported to localize to the nucleus and to facil-

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Figure 4. Proteolytic regulation of PINK1/Parkin mitophagy. In a healthy mitochondrion with a normal membrane potential ($\Delta\Psi_{mt}$), PARL processes newly imported PINK1 at the inner mitochondrial membrane (IMM), which subsequently leaves the mitochondrion and is degraded by the proteasome. Mitochondrial dysfunction blocks PINK1 import and promotes PINK1 accumulation in the outer mitochondrial membrane (OMM). Here, PINK1 selectively recruits the E3-ligase Parkin, which ubiquitylates mitophagy receptor (R) proteins; an action suppressed in healthy mitochondria by USP30 deubiquitylase activity. These ubiquitin signals are recognized by mitophagy adaptor (A) proteins that interact with the autophagosomal protein LC3, resulting in the engulfment of the mitochondrion and delivery to the lysosome.

itate the transcriptional up-regulation of mitochondrial biogenesis (Civitaresi et al. 2010). It is certainly fascinating to consider that PARL proteolysis contributes to metabolic homeostasis by simultaneously regulating the removal and replacement of dysfunctional mitochondria.

Interestingly, other identified PARL substrates have been reported to show dual-localization, which includes the proapoptotic protein Smac/DIABLO, the lipid transfer protein STARD7 and the Ser/Thr phosphatase phosphoglycerate mutase 5 (PGAM5) (Sekine et al. 2012; Saita et al. 2017, 2018). PGAM5 dephosphorylation of the mitophagy receptor FUNDC1 at the OMM promotes mitophagy during hypoxia (Chen et al. 2014; Wu et al. 2014). In stark contrast to PINK1 processing, PARL cleavage of PGAM5 is enhanced by mitochondrial depolarization and, albeit to a lesser degree, during hypoxia (Sekine et al. 2012; Chen

et al. 2014; Saita et al. 2017). Depolarization-induced processing occurs in conjunction with enhanced OMA1-mediated cleavage of PGAM5 and results in the rapid dissociation of PGAM5 from the IMM (Sekine et al. 2012; Wai et al. 2016). How PGAM5 relocates to the mitochondrial surface after processing by PARL and how the coordinated processing of PGAM5 by PARL and OMA1 contributes to mitophagy regulation during stress remains to be explained. Furthermore, the proteolytic release of PGAM5 from the IMM is likely to influence mitochondrial metabolism in multiple ways. For example, Bernkopf et al. (2018) recently reported that stimulated PGAM5 cleavage by PARL during mitochondrial depolarization or hypoxia results in PGAM5 release into the cytosol, where it can boost mitochondrial biogenesis by dephosphorylating β -catenin ultimately resulting in enhanced nuclear gene transcription.

CONCLUDING REMARKS

This review of the multiple mitochondrial functions under proteolytic control has highlighted the importance of mitoproteases in metabolic adaptation. Given the central role of proteolysis for mitochondrial activities, it is arguably not surprising that a steadily increasing number of mitoproteases have been associated with disease and that the strong phenotypes observed due to the loss of mitoproteases share similarities with general mitochondriopathies. Importantly, these phenotypes may be altered under stress or different metabolic conditions, reflecting the plasticity of the mitochondrial proteome and the central role of proteolysis in adaptive processes. Mitoproteases therefore require tight regulation and we are only beginning to place them within cellular signaling networks. Identifying the specific stressors and metabolic conditions that regulate mitoproteases, in addition to unveiling their novel substrates, will help us to realize their therapeutic potential. Combining such findings with insights into the protein structure and working mechanisms of these enzymes will facilitate the development of mitoprotease agonists and antagonists to target specific human diseases and to support healthy aging.

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