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COMMENTARY

OPA1 processing in cell death and disease – the long and short of it

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ABSTRACT

The regulation of mitochondrial dynamics by the GTPase OPA1, which is located at the inner mitochondrial membrane, is crucial for adapting mitochondrial function and preserving cellular health. OPA1 governs the delicate balance between fusion and fission in the dynamic mitochondrial network. A disturbance of this balance, often observed under stress and pathologic conditions, causes mitochondrial fragmentation and can ultimately result in cell death. As discussed in this Commentary, these morphological changes are regulated by proteolytic processing of OPA1 by the inner-membrane peptidases YME1L (also known as YME1L1) and OMA1. Long, membrane-bound forms of OPA1 are required for mitochondrial fusion, but their processing to short, soluble forms limits fusion and can facilitate mitochondrial fission. Excessive OPA1 processing by the stress-activated protease OMA1 promotes mitochondrial fragmentation and, if persistent, triggers cell death and tissue degeneration *in vivo*. The prevention of OMA1-mediated OPA1 processing and mitochondrial fragmentation might thus offer exciting therapeutic potential for human diseases associated with mitochondrial dysfunction.

KEY WORDS: Mitochondria, Mitochondrial cristae, Mitochondrial dynamics, Mitochondrial fusion, OPA1, OPA1 processing

Introduction

Mitochondria fulfill numerous essential functions in eukaryotic cells. Classically described as the ATP-generating powerhouses, they also play a central role in Ca^{2+} signaling, phospholipid metabolism and apoptosis. In mammals, mitochondria are utilized by each cell to meet the varying demands of a specific tissue. These demands are great in tissues such as the heart and brain, where a dysfunction of mitochondria has been associated with an increasing number of human diseases. Mitochondrial activity must therefore be tightly monitored and optimized to meet any changes in cellular metabolism and physiology. One of the fundamental ways by which healthy cells achieve such a control over their mitochondrial population is through the versatile regulation of mitochondrial morphology and dynamics (Labbé et al., 2014; Mishra and Chan, 2014).

The unmistakable morphology of mitochondria has fascinated researchers ever since their ultrastructure was revealed by electron microscopy. Mitochondria contain an outer membrane (OMM) and a protein-rich inner membrane (IMM) that is folded into the matrix to form distinctive compartments called cristae; these harbor the components required for oxidative phosphorylation (OXPHOS) and ATP production. Beyond this textbook depiction of mitochondrial ultrastructure, recent advances have unveiled the highly dynamic nature of these organelles. Using live-cell imaging, we have been able to witness mitochondria fusing, dividing and trafficking, both in cultured cells and *in vivo*. Mounting evidence now directly links mitochondrial dynamics to the function and fate of the cells and organisms in which they reside and is propelling the research into the regulation of mitochondrial morphology. A key player of both mitochondrial structure and dynamics is the IMM-localized protein dynamin-related GTPase optic atrophy type 1 (OPA1), which will be in the focus of this Commentary. Before exploring the various roles of OPA1 in mitochondria, we shall first place it in the landscape of mitochondrial dynamics and briefly discuss the core components of the mitochondrial dynamics machinery.

Mitochondrial dynamics – a balance of fission and fusion

The continual division and fusion of mitochondria is integral to their quality control and adaptation to metabolic changes (Wai and Langer, 2016; Youle and van der Bliek, 2012). Accordingly, mitochondrial morphology and dynamics vary greatly between tissues and cell types to meet cellular demands.

Mitochondrial fusion ensures the distribution of mitochondrial (mt)DNA and is associated with an increased respiratory efficiency (Labbé et al., 2014; Mishra et al., 2015). Elongation of the mitochondrial network can be achieved by stimulating fusion or by suppressing mitochondrial fission. Maintaining a network of hyper-fused mitochondria by limiting fission rates protects the organelle from degradation by autophagy during starvation (Gomes et al., 2011; Rambold et al., 2011). Conversely, a stimulation of fission is required to spatially distribute mitochondria for their correct distribution during mitosis (Mishra and Chan, 2014). Stimulated fission, combined with a reduced fusion capacity, can also help to isolate individual mitochondria from the network to facilitate their degradation by the selective autophagic process termed mitophagy (MacVicar and Lane, 2014; Twig et al., 2008). Mitochondrial dysfunction or pathogenic insults block fusion, thus resulting in unopposed fission and fragmentation of the network for as long as the insult persists.

Mitochondrial fission – distinct mechanisms for both membranes?

The delicate balance between fission and fusion is struck by the tight regulation of several dynamin-related proteins that are localized at the IMM and OMM (Fig. 1). They all share a highly conserved GTPase domain and possess the ability to self-assemble, hydrolyze GTP and remodel membranes (Labbé et al., 2014). At the OMM, mitochondrial fission is orchestrated by the mitochondrial recruitment and assembly of cytosolic dynamin-related protein 1 (DRP1, also known as DNM1L) into oligomers at sites of scission (Otera et al., 2013). Mitochondrial recruitment of DRP1 is regulated

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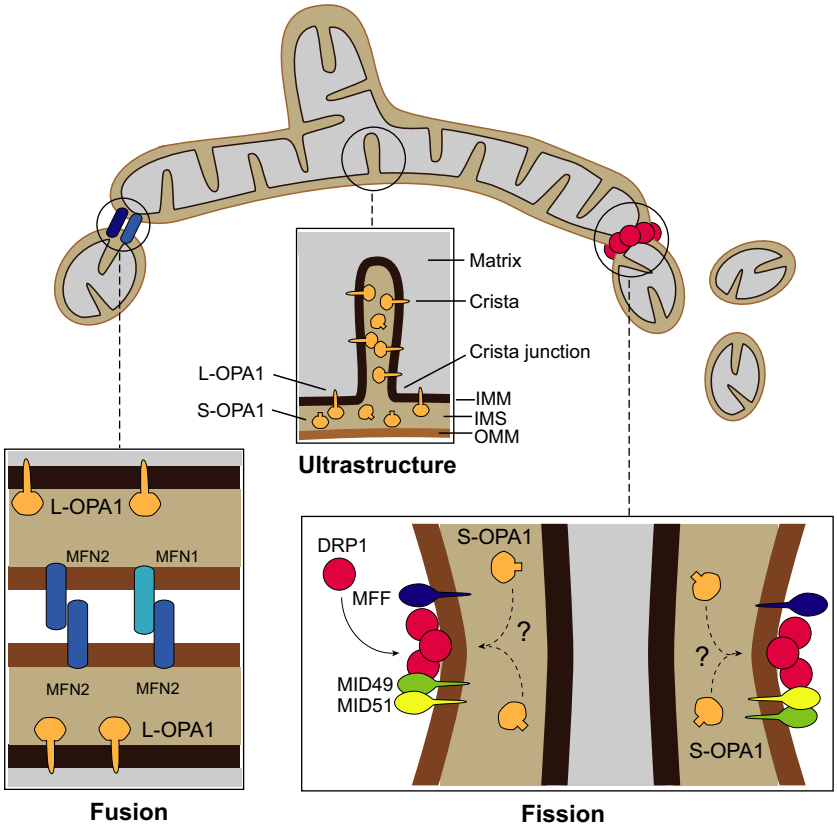

Fig. 1. OPA1 regulates mitochondrial morphology and dynamics. OPA1 regulates mitochondrial structure and dynamics. Long OPA1 (L-OPA1) is tethered to the inner mitochondrial membrane (IMM), whereas proteolytic processing releases the soluble short form (S-OPA1) into the intermembrane space (IMS). OPA1 populates the folded cristae and is required for cristae morphogenesis. Mitochondrial fusion is governed at the outer membrane (OMM) by the mitofusins MFN1 and MFN2, and at the IMM by L-OPA1. Mitochondrial fission involves the recruitment and oligomerization of cytosolic DRP1; this is facilitated by OMM adapter proteins mitochondrial fission factor (MFF), as well as MID49 and MID51. Cleaved S-OPA1 might also facilitate fission.

by a remarkable number of post-translational modification pathways, including phosphorylation, ubiquitylation and SUMOylation, whereas its targeting to fission sites is facilitated by adapter proteins at the OMM, namely mitochondrial fission factor (MFF), mitochondrial dynamics proteins of 49 and 51 kDa (MID49 and MID51, also known as MIEF2 and MIEF1, respectively) and mitochondrial fission protein (FIS1) (Loson et al., 2013; Palmer et al., 2013). Furthermore, several reports have elegantly proposed that ER tubules and actin fibers provide the constrictive force required to promote the scission of both the OMM and IMM (Friedman et al., 2011; Ji et al., 2015; Korobova et al., 2013; Li et al., 2015). Our understanding of OMM fission is therefore extending far beyond the core GTPase-dependent function of DRP1, with new components or regulators of the OMM fission machinery continuing to be discovered.

In contrast, the understanding of IMM fission remains extremely limited owing largely to the fact that a bona fide IMM fission machinery member has not been identified. Thus, the question arises as to whether the OMM fission machinery is sufficient to drive IMM fission. Alternatively, IMM fission factors might exist in mammalian mitochondria, at the very least acting as intra-mitochondrial signals to communicate with the fission machinery at the OMM.

The IMM mitochondrial protein of 18 kDa (MTP18, also known as MTFP1) might be one such factor. Overexpressed MTP18 has been shown to colocalize with DRP1 and to promote DRP1-dependent fission, whereas its suppression by RNA interference (RNAi) results in mitochondrial tubulation (Tondera et al., 2005, 2004). Another recently proposed fission factor emerging from the IMM is the soluble, cleaved fragment of OPA1, termed short-OPA1 (S-OPA1). As discussed in the later sections, overexpressed S-OPA1 colocalizes with DRP1 and other members of the OMM fission machinery, such as MID49 and MID51, at distinct punctae along the mitochondrial network (Anand et al., 2014). Importantly, it is likely that both MTP18- and S-OPA1-induced mitochondrial fission requires DRP1 and the OMM fission machinery. It thus remains a possibility that constriction of mitochondria by DRP1 and actin is indeed sufficient to drive both OMM and IMM fission simultaneously.

The mitofusins and fusion of the outer membrane

Cells must balance mitochondrial fission with fusion to preserve mitochondrial integrity. The existence of specific pathologies arising from disturbed mitochondrial fusion is an indicator of the importance of the mitochondrial fusion machinery, although alternative roles for the major fusion factors that are independent of mitochondrial fusion must also be considered.

The complete fusion of two mitochondria requires merging of first the OMM and then the IMM. At the OMM, fusion is regulated by the mitofusin GTPases Mfn1 and Mfn2 (Chen et al., 2003; Santel and Fuller, 2001). Both Mfn1 and Mfn2 are required for an elongated mitochondrial network in mouse embryonic fibroblasts (MEFs). Mfn1 and Mfn2 have redundant and distinct functions (Chen et al., 2003). Expression of Mfn1 can rescue fusion to some degree in *Mfn2*$^{-/-}$ cells, whereas Mfn2 expression only restores fusion to some extent in *Mfn1*$^{-/-}$ cells (Chen et al., 2003). Indeed, Mfn1 and Mfn2 display different mitochondrial tethering abilities and GTPase activities (Ishihara et al., 2004), and only Mfn1 is required for mitochondrial fusion promoted by OPA1 in the IMM (Cipolat et al., 2004). The whole-body knockout of either *Mfn1* or *Mfn2* causes embryonic lethality in mice, but both Mfn1 and Mfn2 have been associated with functions that are independent of each other, which might explain their varied tissue expression pattern (Bertholet et al., 2016). Mutation of *MFN2* in humans causes

approximately 20% of Charcot–Marie–Tooth (CMT) type 2 cases, an inherited peripheral neuropathy (Verhoeven et al., 2006; Züchner et al., 2004). Interestingly, no disease has been associated with mutations in *MFN1*. Crucially, it is not clear whether the axonal-specific degeneration associated with *MFN2* mutation in CMT is due to disturbed mitochondrial fusion, or rather because of a defect in other *MFN2* functions, for example in axonal trafficking, Ca^{2+} signaling or mitophagy (Schrepfer and Scorrano, 2016).

OPA1 and fusion of the inner membrane

The dynamin-like GTPase OPA1 not only mediates IMM fusion but also controls cristae morphogenesis. Consequently, OPA1 has been identified as a major player in the regulation of key mitochondrial functions, including apoptosis and respiratory capacity, and is therefore the subject of rigorous cellular regulation (Olichon et al., 2006).

OPA1 was named so after its genetic mutation was shown to be the main cause of autosomal dominant optic atrophy (ADOA) (Alexander et al., 2000; Delettre et al., 2000). This optic neuropathy is characterized by a destruction of retinal ganglion cells and the optic nerve, resulting in progressive vision loss. Although OPA1 is highly expressed in the retina, it is broadly expressed throughout the body and this might reflect the multiple disorders that have presented themselves in patients harboring heterozygous mutations of *OPA1*, including deafness and dementia (Carelli et al., 2015). Until recently, all *OPA1* mutations found in patients have been identified as heterozygous, with the only homozygous *OPA1* mutation causing early-onset encephalomyopathy, cardiomyopathy and death during infancy (Spiegel et al., 2016). In mice, homozygous *Opa1* mutants die *in utero*, but heterozygous mutants reflect the main features of human ADOA, as well as additional phenotypes including cardiomyopathy (Alavi et al., 2007; Chen et al., 2012; Davies et al., 2007). In both human patients and mouse models, *Opa1* mutation leads to an abnormal mitochondrial morphology and mitochondrial dysfunction, for example OXPHOS defects and mtDNA instability. Research therefore continues to focus on the mechanisms by which OPA1 promotes mitochondrial fusion and organizes mitochondrial cristae. It is now understood that regulated proteolysis is a key facet of OPA1 regulation.

Box 1. Processing of yeast Mgm1

Membrane-bound Mgm1, denoted as long (L)-Mgm1, is processed by the mitochondrial rhomboid protease Pcp1 to release the soluble short (S)-Mgm1 into the IMS (Herlan et al., 2004, 2003). *In vitro* assays indicate that mitochondrial fusion requires the heterooligomeric assembly of both forms of Mgm1 and depends on the GTPase activity of S-Mgm1, which facilitates L-Mgm1-mediated structural changes in the IMM (DeVay et al., 2009; Rujiviphat et al., 2015; Zick et al., 2009). Pcp1-deleted yeast strains are unable to generate S-Mgm1 and have a fragmented mitochondrial network with disordered cristae; they also lose mtDNA and have reduced respiratory competence (Herlan et al., 2003; McQuibban et al., 2003). These phenotypes can be partially rescued by the expression of S-Mgm1 in Δ*pcp1* cells (Herlan et al., 2003). Curiously, however, mitochondrial fusion assays conducted in mated Δ*pcp1* cells that express mitochondrial proteins with different fluorescent tags indicate that mitochondrial fusion is actually intact in the fragmented network of Δ*pcp1* cells, whereas no mixing of mitochondrial content can be observed in Δ*mgm1* pairs (Sesaki et al., 2003). This suggests that yeast can retain some fusion capability in the absence of Mgm1 processing despite the requirement for S-Mgm1 GTPase activity *in vitro*.

OPA1 biogenesis and its proteolytic regulation

Human OPA1 was uncovered as a homolog of the dynamin-related proteins Mgm1 and Msp1 in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively (Delettre et al., 2000). Characterization in budding yeast revealed that Mgm1 promotes mitochondrial fusion and normal cristae morphology at the IMM. Its absence results in mitochondrial fragmentation that is mediated by Dnm1 (the yeast orthologue of DRP1) and a subsequent loss of mtDNA (Griparic et al., 2004; Meeusen et al., 2006; Wong et al., 2000, 2003). Yeast Mgm1 and mammalian OPA1 share a conserved GTP-binding domain, GTP-effector domain and middle domain (Delettre et al., 2000), as well as an N-terminal mitochondria-targeting sequence. This leader sequence permits the import of Mgm1 and OPA1 precursor proteins into mitochondria where they are subsequently processed by the mitochondrial processing peptidase (MPP, a complex of PMPCA and PMPCB) and tethered to the IMM by their N-terminal transmembrane domain, with the GTPase domains exposed to the inner mitochondrial space (IMS). Electron microscopy studies have revealed that Mgm1 and OPA1 are present in cristae, but also moderately enriched in the inner boundary membrane, the part of the IMM that is aligned with the OMM (Griparic et al., 2004; Vogel et al., 2006). Once anchored at the IMM, Mgm1 and/or OPA1 are subjected to proteolytic processing, the nature of which differs substantially from yeast to mammals (Box 1).

From transcription to its proteolytic processing, the biogenesis and regulation of OPA1 in mammals is more complex than Mgm1. The alternative splicing of three of the 30 OPA1 exons results in the synthesis of at least eight mRNA OPA1 isoforms in humans (Delettre et al., 2001). After MPP processing, membrane-bound long (L)-OPA1 forms can be processed at two protease cleavage sites, S1 and S2, to generate short (S)-OPA1 (Ishihara et al., 2006). In humans, every L-OPA1 polypeptide contains an S1 site and half of them also contain an S2 site (Song et al., 2007). Strikingly, whereas yeast Mgm1 is processed by the rhomboid protease Pcp1 (Box 1), the ortholog of yeast Pcp1, PARL, does not cleave OPA1 (Duvezin-Caubet et al., 2007; Ishihara et al., 2006). Rather, the presence of two cleavage sites permits the constitutive and inducible proteolytic processing of OPA1 by two IMM proteases, which have their catalytic sites exposed to the IMS: the ATP-dependent protease yeast mitochondrial DNA escape 1-like [YME1L (also known as YME1L1) or *i*-AAA protease], which acts at S2 and the zinc metalloprotease overlapping with *m*-AAA protease (OMA1) (Käser et al., 2003) at S1 (Fig. 2). Double knockout *Yme1l*$^{-/-}$*Oma1*$^{-/-}$ MEFs and cardiomyocytes show no cleavage of L-OPA1 (Anand et al., 2014; Wai et al., 2015). The activity of these two IMM proteases establishes a near equimolar equilibrium of long and short OPA1 forms under basal conditions and, crucially, can tip this balance in response to metabolic changes or mitochondrial dysfunction (Wai and Langer, 2016). Notably, although these proteases are evolutionarily conserved, neither of the yeast orthologs (Yme1 or Oma1) process Mgm1 (Bohovych et al., 2014; Herlan et al., 2003; McQuibban et al., 2003). The remarkable switch in proteases involved in processing of Mgm1 in yeast and of OPA1 in mammals might thus reflect the increased need for regulation in higher eukaryotes.

Although YME1L and OMA1 are the only OPA1-processing peptidases that act under basal conditions, the proteolytic control of OPA1 appears to be more complex in response to particular metabolic demands. Upon mammalian target of rapamycin complex 1 (mTORC1) inhibition *in vivo* and *in vitro*, an unidentified cysteine protease has been reported to cleave all OPA1 forms at sites within the middle domain to yield C-terminal fragments (CTFs) that are

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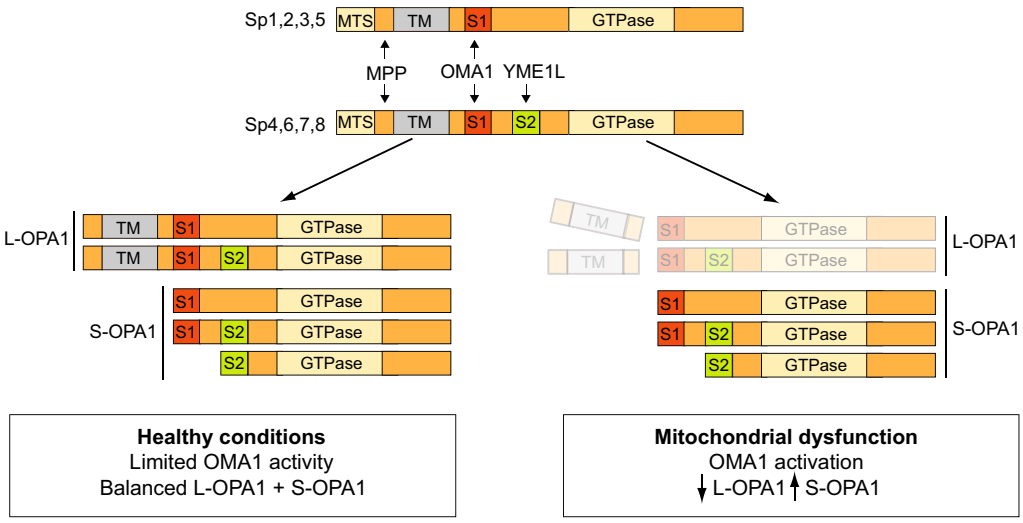

Fig. 2. OPA1 proteolysis by YME1L and OMA1. *OPA1* is alternatively spliced to yield eight variants (denoted Sp1–Sp8). In humans, four of the splice variants contain both S1 and S2 proteolytic sites, whereas the other four contain S1 alone. Constitutive processing of L-OPA1 by YME1L at S2 and by OMA1 at S1 generates a mix of L-OPA1 and S-OPA1 in healthy cells. Mitochondrial dysfunction enhances OMA1 activity and results in the enhanced processing of L-OPA1 and generation of S-OPA1 forms. MPP, mitochondrial processing peptidase; MTS, mitochondrial targeting sequence; TM, transmembrane domain.

GTPase dead (Sood et al., 2014). Cleavage likely results in loss of OPA1 activity and correlates with reduced cristae density and mitochondrial fragmentation in mouse liver with suppressed mTORC1 signaling (Sood et al., 2014).

OPA1 processing by YME1L and OMA1 – limiting mitochondrial fusion

The identification of YME1L and OMA1 as OPA1-processing peptidases allowed for the first time an unambiguous examination of how OPA1 proteolysis affects mitochondrial dynamics. Deletion of *Oma1* abolishes OPA1 cleavage at S1, with cells maintaining an elongated and reticulated mitochondrial network (Anand et al., 2014; Quirós et al., 2012). Loss of YME1L, by contrast, impairs processing of OPA1 at S2 and triggers mitochondrial fragmentation in cultured cells and in cardiomyocytes *in vivo* (Griparic et al., 2007; Song et al., 2007; Stiburek et al., 2012; Wai et al., 2015). This was initially interpreted to be a consequence of impaired OPA1 cleavage, but this view changed dramatically when it was recognized that complete inhibition of OPA1 processing upon ablation of *Oma1* in these cells restored tubular mitochondria *in vitro* and mitochondrial morphology *in vivo* (Anand et al., 2014; Wai et al., 2015). Indeed, mitochondria lacking YME1L, or both YME1L and OMA1, retain the ability to fuse, as measured by live-cell photoactivatable fusion assays and polyethylene glycol (PEG) fusion assays (Anand et al., 2014; Ruan et al., 2013). Thus, OPA1 processing is dispensable for fusion, and the fragmented network in YME1L-deficient cells in fact results from increased fission.

How is this brought about? A study of the OPA1 processing pattern in *Yme1l*-knockout MEFs revealed an accumulation of S-OPA1 forms, indicating accelerated OMA1-mediated processing of OPA1. Deletion of *Oma1* prevents the formation of S-OPA1 in these cells and restores tubular mitochondria, whereas mitochondria fragment upon re-expression of S-OPA1 in double knockout *Yme1l*$^{-/-}$*Oma1*$^{-/-}$ MEFs (Anand et al., 2014) or upon over-expression of S-OPA1 in wild-type cells (Ishihara et al., 2006). These data link uncleaved L-OPA1 to mitochondrial fusion and cleaved S-OPA1 to mitochondrial fission. The colocalization between S-OPA1 constructs and OMM fission machinery further strengthens this link (Anand et al., 2014).

Taken together, the analysis of the function of YME1L and OMA1 led to a new view for the role of OPA1 processing in the regulation of mitochondrial dynamics: cleavage of L-OPA1 limits mitochondrial fusion and, together with the generation of fission-associated S-OPA1, tips the balance towards mitochondrial fragmentation (Fig. 3). This model is in agreement with previous observations demonstrating that overexpression of L-OPA1, which lacks an S1 cleavage site, alone is sufficient to preserve a filamentous mitochondrial network in HeLa cells in which OPA1 has been silenced by small interfering RNA (siRNA) (Ishihara et al., 2006), and to promote mitochondrial hyperfusion in response to certain stress stimuli (Duvezin-Caubet et al., 2006; Ishihara et al., 2006; Tondera et al., 2009). However, a number of questions surrounding the precise and relative contribution of the different OPA1 forms to fission and fusion remain (Box 2).

YME1L and OMA1 – two regulated proteases at the IMM

Balancing mitochondrial fusion and fission through proteolytic processing of OPA1 by two peptidases offers intriguing possibilities for regulation. Indeed, it can be envisaged that YME1L and OMA1 have evolved in higher eukaryotes to permit the processing of L-OPA1 in response to physiological conditions, which perhaps are less relevant in lower eukaryotes. Ultimately, these proteases provide a regulatory node to alter the balance between L-OPA1 and S-OPA1 and to consequently adapt mitochondrial morphology. According to this scenario, the various S-OPA1 forms, which only differ in a few N-terminal amino acids, are generated by YME1L or OMA1 in response to different input signals; but ultimately they are expected to have identical functions in balancing fusion and fission. Given that the S1 site is found in every translated OPA1 isoform, whereas S2 is present in only half, only activation of OMA1 can result in a complete conversion of L-OPA1 to S-OPA1, which is likely of particular relevance under stress conditions (see below).

Relatively little is known regarding how and to what degree cleavage by YME1L is regulated (Griparic et al., 2007; Song et al., 2007). YME1L-mediated processing of OPA1 can be metabolically stimulated by increased OXPHOS activity in cells grown on ketogenic carbon sources (Mishra et al., 2014). Conversely, YME1L activity might be suppressed during oxidative stress because the protease was recently found to be degraded in ATP-depleted cells treated with mitochondrial poisons (Rainbolt et al., 2015). Although the degradation of YME1L in response to H_2O_2 perturbs the YME1L-dependent proteolysis of TIMM17A, a mitochondrial import machinery substrate, it is not yet clear how YME1L degradation under these conditions relates to OPA1 function (Rainbolt et al., 2015).

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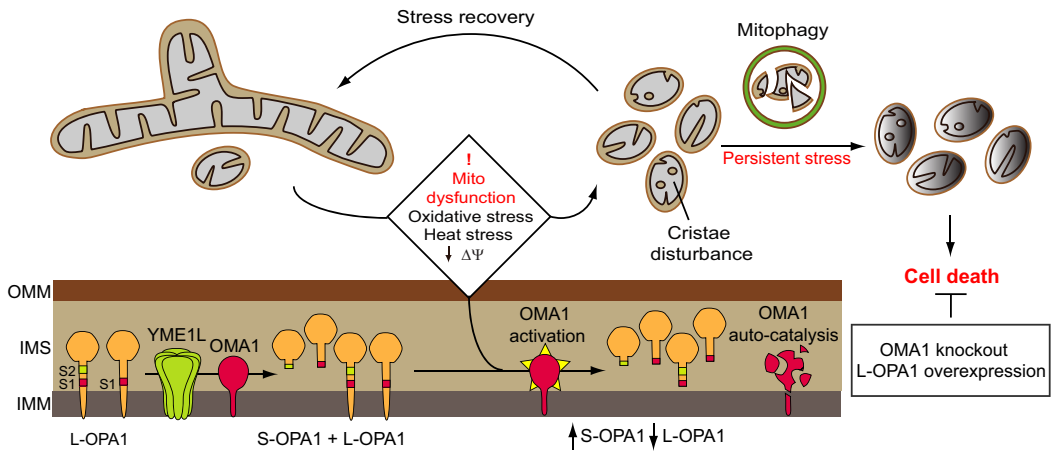

Fig. 3. OMA1 activation causes mitochondrial fragmentation and cell death. Uncleaved L-OPA1 mediates IMM fusion and preserves cristae structure. Constitutive processing of L-OPA1 by YME1L and OMA1 results in an equilibrium between L-OPA1 and S-OPA1; this balances fusion and fission and maintains a reticulated network. Stress conditions or mitochondrial dysfunction including a decrease in membrane polarization ($\Delta\Psi$) activates OMA1 and results in the enhanced cleavage of L-OPA1 and the release of S-OPA1. Loss of L-OPA1 limits mitochondrial fusion and disturbs cristae structure, whereas S-OPA1 appears to facilitate mitochondrial fission. Mitochondrial fragmentation facilitates the sequestering and degradation of mitochondria by mitophagy. Activated OMA1 also undergoes autocatalysis and this permits the recovery of the mitochondrial network if stress conditions are alleviated. If mitochondrial dysfunction persists, mitochondrial fragmentation results in cell death via apoptosis or necrosis.

In contrast to YME1L, OMA1-mediated cleavage of OPA1 at S1 is strongly enhanced by a number of conditions that are associated with mitochondrial dysfunction and respiratory deficiencies. OMA1 was revealed to be the protease that mediates this rapid L-OPA1 cleavage and concomitant fragmentation of the mitochondrial network, identifying mammalian OMA1 as a stress-activated metalloprotease (Ehses et al., 2009; Head et al., 2009). An increasing number of stress paradigms have been shown to depolarize mitochondria and enhance OMA1 activity. For example, defective protein quality control in the absence of the *m*-AAA protease subunit AFG3L2 results in the accumulation of unfolded polypeptides at the IMM and dissipation of the membrane potential, leading to OMA1-dependent OPA1 processing and mitochondrial fragmentation (Ehses et al., 2009; Richter et al., 2015). Importantly, however, some stressors, including heat stress, have been demonstrated to enhance OMA1 activity independent of a gross depolarization of mitochondria (Baker et al., 2014). OMA1 is also enhanced following the genetic ablation of several IMM proteins, such as DnaJ homolog subfamily C member 19 (DNAJC19), prohibitin membrane scaffolds or YME1L (Anand et al., 2014; Ehses et al., 2009; Merkwirth et al., 2012; Richter-Dennerlein et al., 2014). Interestingly, the knockout of *Yme1l* in *Drosophila* [where it is known as dYME1L (CG3499)] results in an increase in reactive oxygen species (ROS), and OMA1 is indeed activated in response to some oxidative stressors *in vitro* (Baker et al., 2014; Qi et al., 2016). Mitochondrial and cytosolic ROS scavenging experiments could address whether ROS is indeed a signal for the activation of OMA1 upon YME1L depletion. Thus, despite the fact that novel stimulatory conditions have been unearthed, the exact mechanism by which OMA1 is activated remains enigmatic.

The identification of a stress-sensing domain in OMA1 might pave the way to a better understanding of OMA1 activation (Baker et al., 2014). Yeast Oma1 does not target Mgm1 in this organism even under stress conditions (Bohovych et al., 2014). A comparison of yeast and mammalian OMA1 sequences has revealed that an additional N-terminal hydrophobic stretch of amino acids followed by positively charged residues has evolved in mammals; these residues were found to be crucial for stress-induced OMA1 mediated proteolysis of OPA1 (Baker et al., 2014). The N-terminal extension of OMA1 therefore appears to have been attained during evolution for further control of mitochondrial dynamics through OPA1 processing.

An important feature of OMA1 activity is its autocatalysis upon activation. The C-terminal self-cleavage of OMA1 in response to mitochondrial stress has been proposed to promote its activity (Zhang et al., 2014), whereas its complete self-degradation permits

Box 2. What is the role of S-OPA1 in mitochondrial dynamics?

Uncleaved L-OPA1 mediates mitochondrial fusion, which is limited upon OPA1 processing. Increasing evidence suggests that S-OPA1 generated by proteolysis of L-OPA1 facilitates mitochondrial fission. Mechanistically, S-OPA1 might stimulate the assembly of the OMM fission machinery or directly affect IMM fission, with a mild contribution of its GTPase activity to this role (Anand et al., 2014). The overexpression of different S-OPA1 forms, generated by either YME1L or OMA1, drive mitochondrial fission to the same extent, arguing that the functions of S-OPA1 forms are identical in balancing mitochondrial dynamics (Anand et al., 2014). This does not exclude differences between S-OPA1 forms originating from different splice variants in other functions associated with OPA1, for example cytochrome *c* release (Olichon et al., 2007). Regardless, the relative amounts of L- and S-OPA1 determine the balance between fusion and fission. Accordingly, the loss of L-OPA1 and the concomitant accumulation of S-OPA1, for instance upon stress-activation of OMA1, induces mitochondrial fragmentation. The role of S-OPA1 in steady-state mitochondrial dynamics is enigmatic but it should be noted that OMA1 is constitutively active and that OMA1-deficient cells harbor an elongated mitochondrial network, which might reflect impaired fission due to a decrease in the accumulation of S-OPA1 (Ehses et al., 2009; Quiros et al., 2012). By contrast, the mitochondria in cells lacking both YME1L and OMA1 (and thus completely lacking S-OPA1) are not as reticular as those in wild-type cells. Therefore, there might be a requirement for basal levels of S-OPA1 to fully restore mitochondrial morphology in these cells. Indeed, YME1L-mediated OPA1 processing was found to be required for increased fusion rates in cells grown on ketogenic carbon sources, and, therefore, YME1L-depleted cells might be lacking a pro-fusion contribution of YME1L (Mishra et al., 2014). Systematically studying the contribution of all OPA1 forms along with the regulatory proteases YME1L and OMA1 to mitochondrial dynamics under basal and physiological stress conditions will improve our understanding of how S-OPA1 regulates mitochondrial dynamics.

the recovery of L-OPA1 forms and a restoration of mitochondrial morphology upon stress conclusion (Baker et al., 2014). Notably, OMA1 was recently found to be degraded by YME1L in depolarized mitochondria containing high levels of ATP (Rainbolt et al., 2016), suggesting that proteolysis of OMA1 can be regulated differently in different physiological contexts. The stress-induced degradation of OMA1 and of YME1L is thus emerging as an important feature of the adaptive and reversible regulation of mitochondrial dynamics. Cells can manipulate the instability of OMA1 and YME1L under various conditions in order to minimize the processing of L-OPA1 and to limit S-OPA1 production, perhaps not only upon alleviation of mitochondrial stress but also in response to altered metabolic conditions.

Mitochondrial-dysfunction-induced OPA1 processing causes cell death and disease

Stress-induced OPA1 processing by OMA1 has been observed in different tissues and in response to different mitochondrial dysfunctions (included in Table 1). A fragmentation of the mitochondrial network often occurs in disease, raising the question about the physiological requirement for OMA1 activation, L-OPA1 cleavage and S-OPA1 generation. It could be envisaged that a transient activation of OMA1 fragments mitochondria in order to promote mitochondrial recycling by mitophagy and support cell survival. However, excessive mitochondrial fragmentation has also been directly associated with cell death and can thus be detrimental to the cell.

The analysis of two genetic mouse models for tissue-specific mitochondrial dysfunction revealed deleterious effects of OPA1 processing by OMA1. The deletion of *Yme1l* in cardiomyocytes in the heart and the loss of IMM prohibitin membrane scaffolds in the forebrain trigger mitochondrial fragmentation and lead to cardiomyopathy and neurodegeneration, respectively (Korwitz et al., 2016; Merkwirth et al., 2012; Wai et al., 2015). Here, both the cardiac-specific knockout of *Yme1l* and neuronal-specific ablation of prohibitin 2 (*Phb2*) cause accelerated OPA1 processing, disturbed mitochondrial morphology and cell death. Remarkably, when L-OPA1 was genetically preserved by the additional deletion of *Oma1*, tissue atrophy was reduced in both models and this correlated with a significant improvement in fitness and prolonged lifespan. In another study, *Oma1*-knockout mice were shown to have improved renal function after ischemic kidney injury, an acute insult that also promotes OPA1 processing (Xiao et al., 2014). These reports therefore demonstrate that the suppression of OMA1 is protective in pathological models that are caused by persistent mitochondrial stress.

When considering the importance of OMA1 activity, it is difficult to exclude the possibility that a preservation of additional OMA1 substrates might positively affect the observed phenotypes. However, Sun and colleagues used an alternative method to preserve L-OPA1 levels in a mouse model of retinal ischemia–reperfusion injury, an insult that is also accompanied by dramatic processing of L-OPA1 (Sun et al., 2016). When they overexpressed an OPA1 construct lacking the OMA1 cleavage site (OPA1ΔS1), they found that there was an almost complete protection of retinal thickness and the ganglion cell layer (Sun et al., 2016).

Together, these *in vivo* findings provide strong evidence that the excessive processing of OPA1 by OMA1 and the concomitant mitochondrial fragmentation is indeed detrimental in diverse tissues and that the preservation of L-OPA1 can be desirable. But how does the turnover of L-OPA1 by OMA1 result in such negative outcomes? The answer brings the relationship between OPA1 and cell death into sharp focus.

OPA1, cristae morphogenesis and cell death

OPA1 has long been known to play a key role in the mitochondrial regulation of programmed cell death by apoptosis, and the mechanisms by which it performs this role have been closely examined *in vitro* and *in vivo*. We will first review how OPA1 affects cell death pathways in general before exploring specifically how stress-induced OPA1 processing can facilitate cell death.

Apoptotic cells exhibit dramatic changes to mitochondrial morphology and cristae ultrastructure. Intrinsic and extrinsic

Table 1. The protection against pathologies afforded by OPA1 manipulation

Pathological model	Increased L-OPA1 processing?	Intervention	Rescue	Ref.
Kidney ischemia–reperfusion injury (mouse)	Yes	OMA1 KO	Preserved renal function	Xiao et al. (2014)
Retinal ischemia–reperfusion injury (mouse)	Yes (Loss of L-OPA1)	OPA1-ΔS1 overexpression	Protection against retinal degeneration	Sun et al. (2016)
Neurodegeneration in neuronal knockout of Phb2 (mouse)	Yes	OMA1 KO	Delayed neurodegeneration and extended lifespan	Korwitz et al. (2016)
Heart failure in cardiac-specific knockout of Yme1l (mouse)	Yes	OMA1 KO	Rescue of cardiomyopathy and extended lifespan	Wai et al. (2015)
Oxidative-stress-induced degeneration of muscle cells (differentiated myotube culture)	Yes	HT-AT treatment	HT-AT treatment prevented L-OPA1 processing in response to oxidative stress and improved myotube viability	Wang et al. (2014)
Cardiac ischemia–reperfusion injury (mouse)	Yes	OPA1 overexpression	Reduced cardiac injury	Varanita et al. (2015)
Denervation-induced muscle atrophy (mouse)	Yes (loss of L-OPA1 in denervated muscle)	OPA1 overexpression	Protection from muscle atrophy	Varanita et al. (2015)
Mitochondrial disease in Ndufs4-knockout model (mouse)	No	OPA1 overexpression	Mild improvement of motor function and lifespan	Civiletto et al. (2015)
Mitochondrial disease in Cox15-muscle-specific knockout (mouse)	No	OPA1 overexpression	Strong improvement of motor function and lifespan	Civiletto et al. (2015)

KO, knockout; HT-AT, hydroxytyrosol acetate.

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apoptotic signaling promotes DRP1-dependent mitochondrial fragmentation of the mitochondrial network and permeabilization of the OMM (Youle and van der Bliek, 2012). Mitochondrial fission and cristae remodeling are proposed to facilitate the release of proapoptotic proteins, such as cytochrome *c*, from cristae stores into the cytosol to initiate the irreversible apoptotic cascade (Pernas and Scorrano, 2016; Scorrano et al., 2002; Youle and van der Bliek, 2012). OPA1 mediates IMM fusion and preserves cristae morphogenesis; two independent functions that both protect against cell death and can be dissected genetically (Frezza et al., 2006; Patten et al., 2014). Loss of OPA1 in cultured cells increases the susceptibility to cytochrome *c* release and apoptosis (Griparic et al., 2004; Olichon et al., 2003). In the absence of OPA1, mitochondria fragment and the cristae structure is dramatically disrupted, facilitating the discharge of cytochrome *c* in response to apoptotic stimuli (Frezza et al., 2006). Upon the induction of apoptosis, the mitochondrial recruitment of the Bcl-2 homology 3 domain (BH3)-only-containing protein Bid and activation of proapoptotic Bax and Bak proteins at the OMM can disassemble OPA1 oligomers (Frezza et al., 2006; Yamaguchi et al., 2008). The stabilization of Drp1-dependent ER-mitochondrial contact sites has also been implicated as a mechanism to disassemble OPA1 oligomers prior to cell death (Prudent et al., 2015). Surprisingly, however, OPA1 oligomer destabilization has recently been shown to be insufficient for the induction of cytochrome *c* release in cells lacking core mitochondrial fission machinery (Otera et al., 2016). Interestingly, OPA1 oligomerisation is also regulated by metabolic demand and can be stimulated in order to protect cells from cell death during starvation (Patten et al., 2014). This protection conferred to starved cells has been shown to depend on the maintenance of cristae ultrastructure by OPA1 oligomers, independently of OPA1-mediated mitochondrial fusion activity (Patten et al., 2014).

Importantly, the ectopic expression of OPA1 also confers apoptotic resistance *in vitro* and *in vivo* (Costa et al., 2010; Frezza et al., 2006; Merkwirth et al., 2008; Varanita et al., 2015). The protective effect of OPA1 under basal conditions has been attributed to the ability of OPA1 oligomers to maintain cristae structure and thus confine cytochrome *c* to cristae (Cipolat et al., 2006; Frezza et al., 2006). OPA1 overexpression can conserve cristae shape without altering gross mitochondrial morphology, and this correlates with an improvement of phenotypes, such as lifespan and motor function in mice harboring an impaired respiratory chain (Civiletto et al., 2015). In summary, a loss of OPA1 sensitizes cells to apoptosis, whereas its overexpression can be protective. What is then the relative contribution of OPA1 function in fusion and cristae remodeling to cell death and pathology progression? The analysis of OPA1 processing might provide an answer.

OPA1 processing – a shortcut to cell death

Mitochondria lacking both YME1L and OMA1 are able to fuse and to preserve normal cristae morphogenesis, demonstrating that OPA1 processing is dispensable for the maintenance of mitochondrial structure (Anand et al., 2014). However, the concomitant loss of L-OPA1 and accumulation of S-OPA1 upon OMA1 activation triggers mitochondrial fragmentation, disturbs cristae morphology and sensitizes cells to apoptosis *in vitro* (Anand et al., 2014; Head et al., 2009; Jiang et al., 2014; Xiao et al., 2014). An intriguing link between OMA1 and apoptosis progression has recently been uncovered whereby the activation of OMA1 was found to occur downstream of Bax–Bak oligomerization at the OMM. How Bax–Bak oligomerization can activate OMA1-dependent OPA1 proteolysis at the IMM remains unclear, but the suppression of OMA1 activity strongly prevented cytochrome *c* release into the cytosol (Jiang et al., 2014). In agreement with a pro-apoptotic role for OMA1, cells lacking the peptidase show an increased resistance to external apoptotic stimuli (Anand et al., 2014; Quirós et al., 2012). Moreover, the deletion of *Oma1* completely restores apoptotic resistance in *Yme1l*- and *Phb2*-knockout cells, which are otherwise rendered more vulnerable to apoptosis owing to augmented OPA1 processing (Anand et al., 2014; Korwitz et al., 2016). Other examples also link increased OPA1 processing to greater sensitivity to apoptosis. For instance, upon the depletion of hypoxia-induced gene domain protein-1a (HIGD1A), an interactor of L-OPA1 at the IMM, the resulting increased OPA1 processing correlates with mitochondrial fission, cristae defects and reduced cell viability (An et al., 2013). Overexpression of HIGD1A delays depolarization-induced cleavage of OPA1 and improves cell viability in response to CCCP (An et al., 2013). Similarly, the knockdown of other IMM proteins, such as ROS modulator 1 (ROMO1), AFG3L2, PHB2 or DNAJC19 enhances OPA1 processing, disrupts cristae morphology and increases the susceptibility to apoptosis (Ehses et al., 2009; Merkwirth et al., 2008; Norton et al., 2014; Richter-Dennerlein et al., 2014). Taken together, these *in vitro* data reveal that excessive OPA1 processing and the loss of L-OPA1 leads to mitochondrial fragmentation and cristae disruption, thereby facilitating cell death (Fig. 3).

The mechanisms by which OMA1 activation can promote cell death are important in order to fully understand the disease models that can be rescued by *Oma1* ablation or L-OPA1 overexpression (Table 1). Excessive OPA1 processing disrupts normal cristae organization, and one might expect that this facilitates the release of pro-apoptotic proteins, which alone is sufficient to induce cell death. Remarkably, however, OMA1 depletion and L-OPA1 stabilization can provide resistance to cell death independently of restoring cristae morphology *in vivo*. For example, *Oma1* deletion prevents brain atrophy in *Phb2*-knockout mice despite the fact that there is no improvement in neuronal mitochondria cristae structure (Korwitz et al., 2016). The cardiomyopathy caused by excessive, OMA1-dependent OPA1 processing in mice with a heart-specific *Yme1l* knockout is also independent of any changes in the cristae morphology (Wai et al., 2015). *Oma1* knockout and stabilization of L-OPA1 in this model therefore promotes cell survival without regulating cristae morphology. The importance of fission and mitochondrial fragmentation in cell death is also illustrated by the notion that mitochondrial fission mediated by DRP1 and the DRP1-receptor proteins MID49 and MID51 at the mitochondrial surface is required for apoptotic cristae remodeling (Germain et al., 2005; Otera et al., 2016). Moreover, preventing mitochondrial fragmentation by other means, such as DRP1 inhibition, has also been shown to be cyto-protective in the mouse heart and brain (Song and Dorn, 2015).

Notably, enhanced OPA1 processing by OMA1 does not cause apoptosis but rather necrotic death of cardiomyocytes in the heart. There is also evidence for OPA1 processing causing necrotic cell death in retina that has been subjected to ischaemia–reperfusion injury (Sun et al., 2016). These findings demonstrate that excessive OPA1 processing can result in either apoptotic or necrotic cell death, and that cell death can be prevented if mitochondrial fragmentation is inhibited, either by *Oma1* suppression or L-OPA1 overexpression.

Concluding remarks

The research discussed in this Commentary has firmly established OPA1 as a key modulator of mitochondrial dynamics and cell death.

Exciting progress continues to improve our understanding of how the proteolytic processing of OPA1 is central to its function. It would be fascinating to discover whether preventing stress-induced OPA1 processing by OMA1 can improve the phenotypes of other mitochondrial-dysfunction-associated pathologies. For example, it remains to be seen whether the maintenance of L-OPA1 alone is sufficient to rescue disease models with mitochondrial respiration dysfunction that have already been shown to improve upon general OPA1 overexpression. This might circumvent the undesirable induction of cancer, which has been observed in *Opa1*-transgenic mice likely due to the anti-apoptotic effect of Opa1 overexpression (Civiletto et al., 2015; Varanita et al., 2015). Targeting OMA1 for therapeutic use, however, would require greater understanding of the role of OMA1-mediated OPA1 processing for mitochondrial quality control under conditions of transient stress and would also benefit from a greater exploration of additional substrates in mammalian models. The loss of *Oma1* in mice impairs thermogenesis with only mild metabolic alterations (Quirós et al., 2012). Intriguingly, however, it is unclear to what degree OMA1-mediated mitochondrial remodeling might actually be required upon exposure to additional environmental stressors. Regardless, further steps can now be taken to explore the therapeutic potential of preventing excessive stress-induced mitochondrial fragmentation in different tissues with a goal to eventually alleviate the severe symptoms of human diseases that are associated with mitochondrial dysfunction.

Competing interests

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