Curr Genet (2016) 62:291–294
DOI 10.1007/s00294-015-0542-6

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REVIEW

Regulation of mitochondrial inner membrane fusion: divergent evolution with similar solutions?

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Received: 10 November 2015 / Accepted: 11 November 2015 / Published online: 27 November 2015
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Abstract Continuous mitochondrial fusion and fission define the dynamic shape of mitochondria. One essential player of mitochondrial fusion is the conserved inner membrane dynamin-like GTPase Mgm1/OPA1. Limited proteolysis of this protein has been proposed as a mechanism to separate and subsequently eliminate dysfunctional parts from the mitochondrial network. Here, I briefly summarize our current knowledge about the underlying proteolytic processing steps in mammals, baker's yeast, *Schizosaccharomyces pombe*, *Drosophila melanogaster* and *Aspergillus fumigatus*. The apparent great diversity in Mgm1/OPA1 processing among the analyzed species indicates a surprising mechanistic heterogeneity in the regulation of mitochondrial inner membrane fusion.

Keywords OPA1 · Mgm1 · Pcp1 · PARL · Msp1 · Rhomboid · dOpa1 · OMA1 · Mitochondria · Fusion · Fission · Mitochondrial dynamics · Mitochondrial quality control · Alternative topogenesis

News and views

Mitochondria play a pivotal role in eukaryotic organisms. Many well-conserved metabolic and biosynthetic pathways such as oxidative phosphorylation or iron–sulfur cluster biosynthesis depend on the functionality of this organelle. Remarkably, the shape of mitochondria is highly variable and significantly depends on the cell type and cellular condition. Mitochondria may present as long and interconnected tubules as well as short, eventually fragmented vesicles. Most importantly, their shape is highly dynamic: continuous mitochondrial fusion and division (fission) events combined with intracellular movement along the cytoskeleton lead to changes in appearance from tubular to fragmented and vice versa within minutes (Youle and van der Bliek 2012; Escobar-Henriques and Anton 2013). Two distinct machineries facilitate fusion and fission of mitochondria. Many core subunits of these multiprotein complexes are well conserved from fungi to mammals and were subject of extensive reviews (Westermann 2010; van der Bliek et al. 2013).

In the recent years it became increasingly clear that mitochondrial dynamics is linked to important cellular processes, including mitophagy, apoptosis, mitochondrial DNA maintenance and quality control (Westermann 2010; Nunnari and Suomalainen 2012). It is therefore an intriguing question how fusion and fission are regulated. The first mechanistic model that linked mitochondrial functionality to alterations in mitochondrial dynamics was the "alternative topogenesis" model described by Herlan et al. in 2003 for *Saccharomyces cerevisiae* (Herlan et al. 2004). At this time it was shown that the inner membrane fusion GTPase Mgm1 coexists in two functionally non-redundant isoforms that originate in parallel from the same precursor protein (McQuibban et al. 2003; Herlan et al. 2003; Zick et al. 2009). The first, long isoform (l-Mgm1) is the processing product of the matrix processing peptidase (MPP) that removes the N-terminal mitochondrial targeting signal of proteins. While being imported in a TIM23 translocase-dependent manner l-Mgm1 is laterally inserted as type 1 transmembrane protein in the inner membrane, the active

Communicated by M. Kupiec.

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domains facing the mitochondrial intermembrane space. The second, short isoform (s-Mgm1) is the product of a second N-terminal processing step shortly after the MPP processing site. This is executed by the mitochondrial rhomboid protease Pcp1 and removes the hydrophobic stretch that serves as inner membrane anchor of l-Mgm1. As consequence, s-Mgm1 is a soluble intermembrane space protein (McQuibban et al. 2003; Herlan et al. 2003).

Herlan et al. showed that generation of s-Mgm1 depends on the ATP-dependent TIM23 import pathway and that the absence of s-Mgm1 effectively blocks mitochondrial fusion (Herlan et al. 2003, 2004). According to their model (Fig. 1a), mitochondrial dysfunctions that affect ATP synthesis and TIM23 translocase-dependent import would indirectly inhibit mitochondrial fusion by preventing generation of s-Mgm1 (Herlan et al. 2004). The dysfunctional organelle stays separated from the mitochondrial network and might subsequently undergo degradation.

While this model might hold true for *S. cerevisiae*, the situation in mammals is very different. OPA1, the mammalian ortholog of Mgm1, also exists as long membrane-anchored and short soluble isoforms. However, the short OPA1 isoforms primarily originate from processing events catalyzed by proteases other than the mitochondrial rhomboid protease PARL (the mammalian Pcp1 ortholog) (Duvezin-Caubet et al. 2007; Chan 2012; Chan and McQuibban 2013; Anand et al. 2014). Most importantly, it was shown in several independent studies that mitochondrial dysfunctions that negatively affect the bioenergetic state cause a turnover of the long OPA1 isoforms to short isoforms by the metalloprotease OMA1, concomitant with mitochondrial fragmentation (Ishihara et al. 2006; Duvezin-Caubet et al. 2006; Ehses et al. 2009; Head et al. 2009; Anand et al. 2014; Baker et al. 2014; Zhang et al. 2014). This led to a model for mitochondrial quality control in mammals (Fig. 1b) that was initially proposed in 2006 by Ishihara et al. as well as by Duvezin-Caubet et al.: in mammalian cells dysfunctional mitochondria inactivate fusion by processing the long OPA1 isoforms to short isoforms (Ishihara et al. 2006; Duvezin-Caubet et al. 2006; Twig et al. 2008). Together with the yeast "alternative topogenesis" model this indicates the evolution of two distinct but similar mechanisms in which mitochondrial fusion and thereby mitochondrial quality control depend on shifting the balance of long and short Mgm1/OPA1 isoforms.

Beside in mammals and baker's yeast, processing of the mitochondrial fusion GTPase Mgm1/OPA1 was also subject of investigation in the fly *Drosophila melanogaster*, in the fission yeast *Schizosaccharomyces pombe* and, very recently, in the opportunistic pathogenic mold *Aspergillus fumigatus* (Whitworth et al. 2008; Leroy et al. 2010; Rahman and Kylsten 2011; Neubauer et al. 2015). The involvement of the *D. melanogaster* mitochondrial rhomboid protease Rhomboid-7 in processing of dOpa1, the OPA1/Mgm1 ortholog, is controversial. On the one hand, overexpression of Rhomboid-7 appears to positively affect generation of short dOpa1 isoforms (Rahman and Kylsten 2011). On the other hand, the dOpa1 processing pattern, i.e., the abundance of long and short isoforms, is not significantly altered in *rhomboid-7* null mutant flies (Whitworth et al. 2008). Taken together, this suggests that Rhomboid-7 is involved but not essential for processing dOpa1 to form short isoforms. Similar results were obtained in *S. pombe* (Leroy et al. 2010). Deletion of the gene coding the mitochondrial rhomboid protease, Rhomboid 1, clearly does not

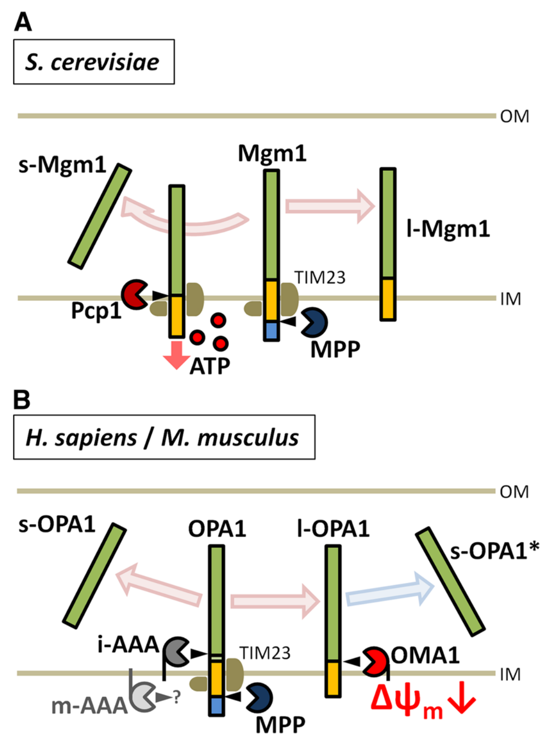

Fig. 1 Simplified models of Mgm1 and OPA1 processing in mitochondria (**a**, **b**). Baker's yeast Mgm1 and mammalian OPA1 precursor proteins are substrates of the TIM23 translocase. After having passed the mitochondrial outer membrane (OM) and partial import over the inner membrane (IM), the N-terminal targeting signals (*blue*) are removed by the matrix processing peptidase (MPP). The long isoforms of Mgm1 (l-Mgm1) and OPA1 (l-OPA1) are laterally inserted in the inner mitochondrial membrane, the N-terminal hydrophobic stretches (*orange*) serve as transmembrane domains. **a** In baker's yeast (*Saccharomyces cerevisiae*), matrix ATP levels drive further import (*red arrow*) of the Mgm1 precursor. This enables the mitochondrial rhomboid protease Pcp1 to cleave Mgm1 and generate the short isoform (s-Mgm1). **b** In mammalian cells (*Homo sapiens*, *Mus musculus*) other proteases process OPA1. The regular short OPA1 isoforms (s-OPA1) are generated in parallel to l-OPA1 by the i-AAA protease and, possibly, by the m-AAA protease. Mitochondrial dysfunction, e.g., dissipation of the mitochondrial membrane potential ($\Delta\psi_m\downarrow$), induces a proteolytic turnover of l-OPA1 to short OPA1 isoforms (s-OPA1*) by the metalloprotease OMA1

abolish generation of the short isoform of the OPA1/Mgm1 ortholog Msp1. However, the ratio of long to short isoforms is shifted towards the long isoforms in this mutant. The authors propose that Rhomboid 1 processes Msp1, but a second unknown protease features a similar activity and generates s-Msp1 in the absence of Rhomboid 1 (Leroy et al. 2010). Alternatively, the impact of mitochondrial rhomboid proteases on processing of *D. melanogaster* dOpa1 and *S. pombe* Msp1 could be indirect, i.e., by affecting pathways that modulate the actually responsible protease.

Recently, we analyzed mitochondrial dynamics in the filamentous fungus *A. fumigatus* (Neubauer et al. 2015). We could show that the mitochondrial rhomboid protease, AfPcp1, is solely responsible for generation of the short isoform of the OPA1/Mgm1 ortholog AfMgm1 in this fungus. But surprisingly, neither AfPcp1 nor the short AfMgm1 isoform is required for mitochondrial fusion (Neubauer et al. 2015). This is in marked contrast to the situation in baker's yeast where s-Mgm1 as well as Pcp1 is essential for fusion (McQuibban et al. 2003; Herlan et al. 2003; Zick et al. 2009). Yet, it could partially reflect the situation in mammalian cells. Here, it was recently shown that the long OPA1 isoform is sufficient to promote mitochondrial fusion (Anand et al. 2014). However, in contrast to the OMA1-dependent OPA1 processing in mammalian cells, dissipation of mitochondrial membrane potential does not induce a turnover of the long to short AfMgm1 isoforms (Neubauer et al. 2015). This indicates that in *A. fumigatus* neither the "alternative topogenesis" model proposed for baker's yeast nor the inverse model proposed for mammalian cells is applicable. From an evolutionary perspective regulation of the mitochondrial inner membrane fusion in *Aspergillus* could indicate the transition state between baker's yeast and mammals. While the short isoform became dispensable for fusion, the long isoform is not yet proteolytically eliminated upon mitochondrial dysfunction. Alternatively, regulation of inner membrane fusion in *Aspergillus* could represent the ancestral situation or a further branch of evolution. In any case, the cardinal question remains how *A. fumigatus* realizes exclusion of dysfunctional organelles from the mitochondrial network.

Several other recent studies emphasize a regulation of mitochondrial dynamics that is independent of Mgm1/OPA1. For example, certain tumor cells' growth specifically depends on the induction of mitochondrial fission via Erk2 MAP kinase-dependent phosphorylation, thereby activation of the mitochondrial fission GTPase dynamin-related protein-1 (Taguchi et al. 2007; Kashatus et al. 2015). And the yeast as well as the mammalian mitochondrial outer membrane fusion GTPases (mitofusins; *H. sapiens*: the partially redundant paralogs Mfn1 and Mfn2; *S. cerevisiae* Fzo1) undergo ubiqitinylation which can not only result in proteolytic breakdown of the mitofusins and inactivation of fusion but, surprisingly, also stimulate fusion (Anton et al. 2013; Escobar-Henriques 2014). This provides a foretaste of how complex the overall regulation of mitochondrial dynamics might turn out in future studies. At the same time this identifies mitochondrial dynamics as a promising target structure to tweak the cellular physiology, the first pharmacologic modulators of fusion and fission currently being under investigation (Cassidy-Stone et al. 2008; Wang et al. 2012; Qi et al. 2013; Yue et al. 2014).

Acknowledgments This work was supported by the German Research Foundation (DFG – WA 3016/2-1).

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