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Review

Mechanistic perspective of mitochondrial fusion: Tubulation vs. fragmentation ☆

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ABSTRACT

Mitochondrial fusion is a fundamental process driven by dynamin related GTPase proteins (DRPs), in contrast to the general SNARE-dependence of most cellular fusion events. The DRPs Mfn1/Mfn2/Fzo1 and OPA1/Mgm1 are the key effectors for fusion of the mitochondrial outer and inner membranes, respectively. In order to promote fusion, these two DRPs require post-translational modifications and proteolysis. OPA1/Mgm1 undergoes partial proteolytic processing, which results in a combination between short and long isoforms. In turn, ubiquitylation of mitofusins, after oligomerization and GTP hydrolysis, promotes and positively regulates mitochondrial fusion. In contrast, under conditions of mitochondrial dysfunction, negative regulation by proteolysis on these DRPs results in mitochondrial fragmentation. This occurs by complete processing of OPA1 and via ubiquitylation and degradation of mitofusins. Mitochondrial fragmentation contributes to the elimination of damaged mitochondria by mitophagy, and may play a protective role against Parkinson's disease. Moreover, a link of Mfn2 to Alzheimer's disease is emerging and mutations in Mfn2 or OPA1 cause Charcot–Marie–Tooth type 2A neuropathy or autosomal-dominant optic atrophy. Here, we summarize our current understanding on the molecular mechanisms promoting or inhibiting fusion of mitochondrial membranes, which is essential for cellular survival and disease control. This article is part of a Special Issue entitled: Mitochondrial dynamics and physiology.

1. Introduction

Mitochondria are very dynamic organelles, whose morphological changes are achieved by constantly occurring fusion and fission events [1]. Loss of mitochondrial fusion is characterized by the presence of fragmented mitochondria, produced by ongoing fission, which contrasts to the characteristic network of most cellular types. Mitochondrial fission not only allows proper distribution of mitochondria, for example to cope with local ATP demands, but also contributes to selective removal of damaged organelles. Fusion of mitochondrial membranes, in turn, allows organelle content mixing and prevents mitochondrial DNA loss, facilitating maximal ATP production. Mitochondrial fusion is particularly important in the nervous system, helping neurons to meet the high energy demands for proper neuronal function, by diluting out injury and dysfunction to which each individual mitochondrion is subject [2,3]. Mitochondrial fusion therefore plays a protective role, preventing these deficiencies from damaging the entire neuron while maintaining an adequate level of bioenergetic capacity [4,5].

Many studies have implicated impairment of mitochondrial function as a contributor to both common and rare neurodegenerative diseases. Early discoveries showed the direct role of the central fusion components in the autosomal-dominant optic atrophy (ADOA) and in the Charcot–Marie–Tooth type 2A neuropathy (CMT2A) [6–9]. More recently, a link between mitochondrial fusion and the most common neurodegenerative diseases of the aging population, and also a link with cardiopathies, were observed [10–12]. Interestingly, the ubiquitylation of mitofusins by the Parkin E3 ligase (often mutated in Parkinson's disease patients) appears to contribute in targeting damaged mitochondria for degradation, which could protect against Parkinson's disease [13–15]. Because these topics are detailed in accompanying reviews in this issue, here we focus on the molecular mechanisms that either prevent or promote mitochondrial fusion. This leads to the opposite outcomes of mitochondrial tubulation or fragmentation. In this respect, it is interesting to notice that mitochondrial fusion recently joined a group of fundamental processes, such as transcription or cellular trafficking, which are controlled by ubiquitylation.

Mitochondrial fusion occurs if two tips or one tip and one tubule come together. The field emerged essentially with pioneer observations of fusion events and with the identification of the first protein required for fusion, Fzo, in fly [16–18]. Studies initially based on genetic screens, coupled with observations of mitochondrial morphology, allowed the identification of most proteins involved in mitochondrial

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fusion [19,20]. Subsequently, topological studies and analysis of the physical interactions between the different proteins have allowed the field to progress. In addition, the development of cell free in vitro tethering and fusion assays was of outmost importance [21–25]. Interestingly, the discovery of the yeast and mammalian homologs of Fzo [18,26–28] was an early indicator for common mechanisms and indeed the basic principles in mitochondrial fusion have proven to be conserved.

2. The key mediators of mitochondrial fusion

While the vast majority of the membrane fusion events in a cell are performed by SNAREs, fusion of mitochondria and of the endoplasmic reticulum (ER) depends on dynamin-related proteins (DRPs) [29–32]. DRPs are a special class of GTPases, which provide the mechanical forces necessary for membrane remodeling [33,34]. They are best known for their role in membrane scission events, particularly during endocytosis and certain other membrane budding events, and also perform fission in mitochondria and peroxisomes [35,36]. The key DRPs involved in mitochondrial fusion are conserved in yeast, worms, flies, mice and humans (Table 1).

2.1. Mitofusins, the DRPs in the OM

Mitofusins, the DRPs that mediate fusion of mitochondrial outer membranes (OM), are termed Mfn1 and Mfn2 in mammals, Fzo and Marf/Dmfn in flies, FZO-1 in the worm and Fzo1 in budding yeast. In contrast to the founding member identified, FZO, shortly expressed during fly spermatid development [18], all the other mitofusins are constitutively expressed ubiquitous mitochondrial proteins, in both males and females [26,28,37–40]. Loss of Fzo1 in yeast leads to loss of mitochondrial DNA and consequently loss of mitochondrial translation, resulting in respiratory incompetence [26,27]. In flies Fzo loss leads to male sterility [18], and in mice Mfn1 KO or Mfn2 KO is embryonic lethal due to a placental defect [4]. Moreover, if Mfn2 is depleted only after placental formation, mice show impaired cerebella development and lethality at day 1 post-birth [5]. In contrast, mice are normal if depleted of Mfn1 only after placental formation [5], suggesting that after this stage Mfn1, but not Mfn2, is dispensable. Isolated cells lacking both Mfn1 and 2 showed severe cellular defects, including poor cell growth, widespread heterogeneity of mitochondrial membrane potential and decreased cellular respiration, whereas single Mfn KO escaped major cellular dysfunction [41]. In addition, Mfn2 depletion leads to a loss or reduction of membrane potential [4,42].

Mitofusins are localized throughout the mitochondrial network [26,28,37,39,40]. They are anchored to the OM by two transmembrane domains, and their N-terminus and C-terminus are exposed to the cytoplasm [18,26,27,37,43] (see Table 1 and Fig. 1). The N-terminus comprises the GTPase domain, followed by one coiled-coil heptad repeat (HR1). Moreover, yeast, worm and fly mitofusins have one additional coiled-coil motif upstream of the GTPase domain (HRN) (see Table 1). Although no equivalent coiled-coil is present in mammalian mitofusins, the N-terminal region upstream of the GTPase domain in Mfn2 is also essential for its function [44]. In turn, the C-terminal domain of mitofusins possesses one additional coiled-coil heptad repeat (HR2). All HR domains are required for mitofusin function, at least in yeast [45]. Importantly, mutations in the conserved GTPase P-loop or switch motifs abolished mitochondrial fusion in all organisms, indicating that both GTP binding and hydrolysis are conserved requirements [4,18,26,45–47].

As expected for DRPs, mitofusins self-assemble [27,39] and in mammals as in yeast, GTP induced changes in the three oligomerization states detected [47,48]. P-loop mutants were exclusively present in a lower molecular weight form, being the wild-type Fzo1 or Mfn1 recovered in a complex with a middle molecular weight [47,48]. After *trans* associations, i.e. associations between two mitochondria, the middle molecular weight complex then shifted to the higher oligomerization state [47,48]. Recent studies in yeast clearly demonstrated that the smaller complex corresponds to the monomeric form of the protein, whereas the middle complex represents a dimer in *cis* (in the same mitochondria), formed upon GTP binding [47]. The oligomeric state of the bigger *trans* complex has not been clearly defined, but was compatible with the formation of a tetramer in *trans* [47].

In addition to the oligomerization of full-length mitofusins, their N-terminal HR1 and C-terminal HR2 interact with each other [37,45]. This interaction is GTPase dependent, because it is not formed in GTP binding or hydrolysis mutants [44,45], and also depends on an intact N-terminal domain [44]. Moreover, co-expression of non-overlapping halves of Fzo1 domains partially complements the wild-type protein, showing that different domains can be provided in separate molecules [45].

2.2. OPA1/Mgm1, the DRP promoting fusion in the IM

The key mediator of inner mitochondrial membrane fusion is called Mgm1 in budding yeast, Msp1 in fission yeast, eat-3 in the worm and OPA1 in flies and mammals [6,7,49–59]. OPA1 is expressed in all tissues analyzed and both in vivo and in isolated mitochondria Mgm1/OPA1 is localized to discrete foci in interface regions of the IM [22,53,60,61]. Loss of Mgm1 leads to respiratory incompetence in yeast due to mitochondrial DNA loss and OPA1 KO in mice is embryonic lethal [49,62,63]. In addition, OPA1 repression in mammals decreases cell growth and oxygen consumption [41,64–67]. Mutations in OPA1 cause ADOA, characterized by progressive bilateral blindness due to the loss of retinal ganglion cells and optic nerve deterioration [6,7,68]. Consistently, heterozygous mutant OPA1 mice show a visual impairment resembling the human ADOA [62,63]. OPA1 mutations are associated with multiple deletions in mitochondrial DNA and also with other neurological conditions adding to ADOA, called “OPA1 plus” phenotype. This consists of chronic progressive ophthalmoplegia, ataxia, sensironeuronal deafness, sensory-motor neuropathy and myopathy [68]. Moreover, in addition to its fundamental role in mitochondrial fusion, OPA1/Mgm1 is also required for cristae formation [22,65,69–72]. Loss of OPA1/Msp1 leads to cell death [64,65,73] and increases sensitivity to apoptosis, which was proposed to occur via increased release of cytochrome *c* due to widened cristae junctions [65,66,67,70,72,74–76]. The structural role of OPA1/Mgm1 in cristae formation occurs via oligomeric self-interactions and was suggested to be independent of ongoing mitochondrial fusion, because it was not abolished in the absence of Mfn1 [75]. However, inactivation of the main fission machinery component, Dnm1, reverses the cristae morphology phenotype of yeast Mgm1 mutants [69].

OPA1/Mgm1 is present in the IM with long, membrane anchored, and short soluble forms, both required for IM fusion [77,78]. These short and long isoforms constitute a complex pattern regulated both post-transcriptionally and post-translationally. In mammals, alternative splicing of the mRNA creates several long isoforms, which are processed to yield several short isoforms [67,79,80], whereas in yeast there is only one long and one short isoform [77,81,82]. All OPA1 variants possess a mitochondrial targeting sequence, cleaved upon import. This sequence is followed by a transmembrane segment that anchors the long isoforms in the inner membrane (IM), with the bulk of the protein facing the intermembrane space (IMS) (Table 1 and Fig. 1). The short isoforms are constitutively generated by further proteolytic maturation at the S2 cleavage site (Table 1) [83]. Only a fraction of the long isoforms is cleaved, thereby producing equimolar amounts of long and short isoforms. This cleavage is performed by Pcp1 in yeast [77,81,82] and was found to depend on cellular ATP levels [84]. In mammals, it was shown that constitutive processing at the S2 cleavage site depends on YME1L [78,85]. However, other