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Mitochondrial Division: Molecular Machinery and Physiological Functions

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Abstract

Mitochondrial division has emerged as a key mechanism for this essential organelle to maintain its structural integrity, intracellular distribution, and functional competence. An evolutionarily conserved dynamin-related GTPase, Dnm1p/Drp1, interacts with other proteins to form the core machinery involved in mitochondrial division. We summarize recent progress in understanding how the division machinery assembles onto mitochondria and how mitochondrial division contributes to cellular physiology and human diseases.

Introduction

Mitochondrial shape, size, and number are regulated by cycles of division and fusion [1–3]. These dynamic processes control mitochondrial distribution and function. In many cell types, these organelles form short tubules, continuously dividing and fusing to exchange their soluble and membrane components, which include DNA, proteins, and lipids (Fig. 1). Dynamic remodeling of mitochondrial structure in response to physiological and environmental cues is important to accommodate different demands on mitochondrial function in various cell types during growth, differentiation, and maintenance[4,5]. In the last decade, many proteins involved in mitochondrial division and fusion have been identified[6,7]. Current challenges in the field include understanding the mechanistic functions of each protein and deciphering the *in vivo* functions of mitochondrial dynamics in mammals. In this review, we aim to cover recent advances in these topics.

Formation of mitochondrial division machinery

A key component of the mitochondrial division machinery is a dynamin-related GTPase called Dnm1p (for yeast)/Drp1 (for mammals)[8–13]. With the majority present in the cytosol, Dnm1p/Drp1 is recruited to the outer membrane for mitochondrial division. This protein has been shown to polymerize into highly ordered oligomers that most likely wrap around mitochondrial tubules with a diameter of approximately 500 nm[14,15]. In contrast to classical dynamin GTPases, which assemble onto the narrow neck of coated pits during endocytosis, Dnm1p/Drp1 convenes onto much wider mitochondrial tubules. A recent cryo-EM study by Mears et al. (2010)[16] showed that Dnm1p can form spirals with a diameter of approximately 100 nm, which is much larger than the classical dynamin GTPase which assembles at a diameter of approximately 20 nm. In addition, Dnm1p/Drp1 binds, constricts, and fragments membrane liposomes *in vitro*. Therefore, Dnm1p/Drp1 may work to pinch off wider tubular structures directly. However, Dnm1p/Drp1 helices were only observed when mitochondrial tubules are highly constricted at presumably later stages of mitochondrial division, suggesting the presence of other factors that may make the diameter of mitochondria shorter to help Dnm1p/Drp1 assemble *in vivo*. Nevertheless, the ability of

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Dnm1p/Drp1 to assemble is a key mechanism for mitochondrial division. Once assembled, GTPase activity is stimulated and leads to conformation changes necessary for severing membranes.

In cells, mitochondrial recruitment of Dnm1p/Drp1 requires other proteins present on the organelle surface. In yeast, the integral outer membrane protein Fis1p provides an anchoring site through two functionally overlapping WD40 domain-containing adaptor proteins, Mdv1p and Caf4p (Fig. 2)[17–29]. Lackner et al. (2009)[30] demonstrated that Mdv1p functions as a structural nucleation factor that promotes Dnm1p assembly on membranes and ultimately its GTPase activity. Mdv1p-dependent nucleation onto mitochondria could serve as a key regulatory step for mitochondrial division. Koirala et al. (2010)[31] solved the X-ray structure of the Mdv1p coiled-coil domain located in the middle of the protein, and showed that this domain forms intermolecular anti-parallel helices and mediates dimerization. This domain also affects interactions with Dnm1p, which are mediated by a WD-40 domain at the C-terminus of Mdv1p (Koirala et al., 2010). As Lanckner et al. (2009) suggested, Mdv1p further assembles into oligomers with Dnm1p, indicating that the coiled-coil domain of Mdv1p is also required for oligomerization and therefore regulation of its interactions with Dnm1p. Compared to Mdv1p, Caf4p plays a relatively minor role in Dnm1p assembly. Mammalian homologs of Mdv1p and Caf4p have not been identified.

In addition to the Fis1p-Mdv1p/Caf4p mechanism, a cortical protein, Num1p, also provides a separate binding site for Dnm1p on mitochondria and functions in mitochondrial division (Cervený et al., 2007)[32,33]. Hammermeister et al. (2010)[34] showed that another outer membrane protein, Mdm36, facilitates stable association of Dnm1p and Num1p. In addition to division, this Num1p-Mdm36p-Dnm1p mechanism also links mitochondria to the cell cortex as, without this mechanism, mitochondria leave their usual location at the cell periphery and relocate to the cellular center. This cortical association likely participates in equal inheritance of mitochondria during cell division[34]. Mitochondria in cells lacking both Num1p and Dnm1p cannot stay in mother cells, and thus move to daughter cells during cell division under conditions such as high temperature[32]. Since mitochondria in yeast are transported along and anchored to actin filaments in the cortex[35], the Num1p-Mdm36p-Dnm1p mechanism may provide a connection to the actin cytoskeleton. This cytoskeletal connection may also promote efficient mitochondrial division by mechanically stretching and thinning mitochondrial tubules.

Fis1 was identified in mammals based on sequence homology to yeast Fis1p and has been suggested to recruit Drp1 to mitochondria[21,36–38]. However, surprisingly, Otera et al. (2010)[39] showed that mammalian Fis1 is dispensable for Drp1 recruitment. To demonstrate this, the authors generated Fis1-null cells and found no major mitochondrial division defects and normal Drp1 localization to mitochondria. This study also showed that Fis1 knockdown by siRNA used in previous studies has an offtarget effect, as it still elongates mitochondrial tubules in Fis1-null cells[39]. These findings are consistent with a previous study in which *C. elegans* lacking two Fis1 homologs does not exhibit mitochondrial division defects [40]. A recently identified integral outer membrane protein, mitochondrial fission factor (Mff), was found to play a major role in Drp1 recruitment, presumably through direct interactions[39,41]. It appears that mitochondria have switched anchoring and assembly mechanisms for Dnm1p/Drp1 during evolution. Given the relatively weak interactions of Mff with Drp1, there may be additional proteins that facilitate their stable connection during mitochondrial division, such as yeast Mdv1p and Caf4p. Mitochondrial recruitment of Drp1 from the cytosol is regulated by a variety of post-translational modifications, including phosphorylation, sumoylation, and ubiquitination[42]. In light of these new findings, it would be interesting to determine whether these modifications regulate the interactions between Drp1 and Mff.

Physiological functions of mitochondrial division

Many studies using *in vitro* cell culture systems and relatively simple eukaryotic model organisms such as yeast, *Drosophila*, and *C. elegans* have demonstrated the involvement of mitochondrial division in such cellular functions as organelle shape, distribution, energy metabolism, apoptosis, and calcium signaling. However, until recently, its physiological function in mammals was unknown due to a lack of mouse models. Recent studies by Wakabayashi et al. (2009)[43] and Ishihara et al. (2009)[44] have generated and characterized complete and brain-specific Drp1 knockout mice. Drp1 was found to be required for embryonic development as complete knockouts die at E11.5, which is when the placenta develops. Embryonic fibroblasts derived from the knockout mice showed highly connected mitochondrial tubules and greatly decreased mitochondrial division rates. Despite dramatic changes in mitochondrial structure and dynamics, these knockout MEFs displayed normal respiratory activities and ATP production.

Analysis of Drp1-null MEFs also suggested that mitochondrial division regulates fusion. A balance between division and fusion is critical for the maintenance of mitochondria; therefore, it is logical to propose that these activities are synchronized. One possible explanation of this coordination is that the intrinsic properties of the fusion and division machineries determine their frequency independent of each other. Another possibility is that cells, upon sensing one activity, subsequently adjust the other to maintain a homeostatic balance between division and fusion. In support of the latter model, the study of Drp1-null MEFs, as well as other studies using Drp1-specific siRNA, revealed that loss of this protein leads to decreases in the steady state levels of Mfn1 and 2 [43–45]. These findings strongly suggest a model in which the abundance and/or activity of the mitochondrial division machinery regulates fusion. Further supporting this model, some factors that influence mitochondrial division, such as ubiquitin and Bcl-xL, also have the ability to modulate fusion[5,42,46–50].

During brain development, neurons establish an elongated shape with long axons and branched dendrites. Proper distribution of mitochondria within these cells is crucial. Indeed, *in vitro* studies have shown that the frequency of mitochondrial division becomes greater than that of fusion with smaller mitochondria distributed to different neuronal regions, including synapses, during development[50–52]. Wakabayashi et al. (2010) deleted Drp1 in the developing cerebellum since this protein is expressed highly in Purkinje cells. This study showed that loss of Drp1 blocked normal cerebellar development. Interestingly, due to ongoing mitochondrial fusion without division, the Purkinje neurons contained giant round mitochondria, which are distinct from the highly interconnected, elongated tubules seen in Drp1-null fibroblasts (Fig. 1). Thus, mitochondrial division appears to regulate the overall shape of this organelle. Unlike Purkinje cells, granule cells have normal short tubular mitochondria in the absence of Drp1, suggesting cell type-specific requirements of this protein for mitochondrial division. Mitochondrial division may occur via a Drp1-independent mechanism in granule cells. Ishihara et al. (2010) also found similar developmental defects when Drp1 was deleted in the brain using *nestin*-Cre, which is expressed in a wider region of the central and peripheral nervous system. Culture of neurons from Drp1 knockouts *in vitro* showed defects in synapse formation and increased sensitivity to apoptotic stimuli. Together, these studies have demonstrated the physiological importance of mitochondrial division for mouse embryonic and brain development, as well as generated tools to further study mitochondrial division in more physiological contexts.

Mitochondrial division in human diseases

Further supporting its physiological importance, altered mitochondrial division has been associated with several diseases, especially those affecting neurons [53–55]. For example, a mutation (A395D) in Drp1 causes postnatal death due to brain developmental defects[56]. A recent biochemical study has shown that this disease mutation blocks higher order assembly of Drp1 and its mitochondrial localization[57]. In addition, Charcot-Marie-Tooth type 4A is caused by mutations in GDAP1, an outer membrane protein involved in mitochondrial division; however, its molecular mechanisms are less well understood[58–62].

A recent study described that the pathogenesis of Alzheimer's disease, one of the most common age-related diseases, may also involve changes in mitochondrial dynamics. In this disease, mitochondrial dysfunction is the earliest known event and fragmentation of this organelle and its altered distribution have been reported [63–65]. Cho et al. (2009) provided interesting evidence for the activation of Drp1-mediated mitochondrial division in Alzheimer's disease[66]. In this study, the authors found that S-nitrosylation of Drp1 was increased in Alzheimer's patients, leading to activation of Drp1 GTPase activity, excess mitochondrial division, and ultimately neural injury. How excess division leads to neural injury remains to be determined. However, Bossy et al. (2010) have challenged this model[67]. Further studies are required to resolve this important issue.

Similarly, Huntington's disease has also been linked to mitochondrial dysfunction. In particular, mitochondrial division has been implicated as increased Drp1 levels were observed in the brain of these patients [68]. Activation of Drp1-dependent mitochondrial division by phosphorylation/dephosphorylation is also suggested to be involved in hypersensitivity to apoptosis in cellular models for Huntington's disease [69]. Another study also showed that fragmentation of mitochondria due to excess division over fusion contributes to cell death in Huntington's cellular models [70].

In addition, by using hypertensive rats with hypertension-induced brain injury, Qi et al. (2010)[71] showed that, upon oxidative stress, Drp1 becomes phosphorylated by delta protein kinase C and induces excess mitochondrial division, resulting in neuronal injury. These studies suggest a common theme in that hyperactivation of mitochondrial division, like its complete loss, leads to physiological abnormalities and disease. Therefore, well-balanced mitochondrial dynamics is critical for cellular health.

Mitochondrial DNA mutations and deletions have been implicated as underlying causes for normal aging and aging-related disease. Mitochondrial DNA is highly susceptible to oxidative stress and can accumulate mutations and deletions over time. A balance between division and fusion is critical for maintaining the integrity of mitochondrial DNA and therefore contributes to these processes. For example, excess fragmentation of mitochondria increases mutations and loss of mitochondrial DNA[72]. Also inhibition of mitochondrial division results in increased oxidative damage and loss of mitochondrial DNA[73]. Interestingly, separation of damaged mitochondria from healthy ones by division may help target them for degradation, which is a possible cellular quality control strategy[74]. Defects in this process have also been associated with Parkinson's disease, another age-related neurodegenerative syndrome. This topic is discussed in another review within this issue.

Finally, Cassidy-Stone et al. (2008) identified a small molecule inhibitor of Dnm1p/Drp1, mdivi-1 [75]. Mdivi-1 has been used to suppress mitochondrial division in both cell culture and animal models [76]. Use of this inhibitor may have therapeutic potential to readjust mitochondrial dynamics in diseases in which division is overactivated or fusion is impaired.

Drp1 in Bax activation during apoptosis

Whether Drp1 is involved in apoptosis remains controversial. Since this topic has been reviewed extensively in many articles [77–82], we will only briefly summarize recent findings by focusing on Drp1 in apoptosis, especially on results obtained from *in vivo* models. Knockout studies have demonstrated that Drp1 is required for apoptosis during neural tube formation *in vivo* [43]. In support of this role, decreased Drp1 association with mitochondria, thereby leading to suppressed apoptosis, has been observed during myocardial infarction (Wang et al., 2010). Similarly, treatment of mice with a Drp1 inhibitor, mdivi-1, partially protected the heart from ischemia/reperfusion injury [83]. In contrast, the contribution of Drp1 to apoptosis *in vitro* cell culture systems using Drp1-null cells is little to none, suggesting that Drp1 involvement in apoptosis differs depending on cell type and physiological context [43,44]. Drp1 most likely promotes the efficiency of certain types of apoptosis, but its involvement is not essential.

As a function of Drp1 in apoptosis, Montesuit et al. (2010)[84] have shown that Drp1 activates Bax oligomerization, a key step in cytochrome *c* release from the mitochondrial intermembrane space. Using biochemical approaches, this study revealed that Drp1 induces Bax oligomerization in the mitochondrial outer membrane through hemifusion. Surprisingly, purified Drp1 binds to cardiolipin, a mitochondria-specific phospholipid, and promotes hemifusion of cardiolipin-containing liposomes *in vitro*. This study also showed that, while hemifusion is a key mechanism, mitochondrial division is not since mutations in the Drp1 GTPase domain, which block mitochondrial division, did not affect Bax activation. Furthermore, Drp1 can be replaced by cytochrome *c*, which can hemifuse liposomes under certain biochemical conditions. Separation of Drp1-mediated mitochondrial division and apoptosis (e.g., its GTPase activity is dispensable) has been also supported by other studies using BIK-induced cristae opening to release cytochrome *c* [85]. Also, cytochrome *c* release from isolated mitochondria without division has been shown to occur in a Drp1-dependent manner *in vitro* [75]. Further supporting a role for Drp1 in Bax oligomerization, reports indicate that both proteins colocalize on mitochondria after induction of apoptosis[86]. Upon activation, Bax promotes Drp1 sumoylation, which is proposed to stabilize Drp1 on the mitochondrial membrane, suggesting a positive feedback loop for Bax activation and Drp1 mitochondrial association [87].

Conclusions

Mitochondria face continuous oxidative damage due to respiratory activities in this organelle. As animals and humans age, such damage accumulates and mitochondria become dysfunctional. Recent research has made clearer the importance of control of mitochondrial dynamics in defense mechanisms against age-related dysfunction and environmental stress. While there has been tremendous progress in identifying the proteins involved in mitochondrial dynamics, current challenges lie in deciphering how these proteins work together biochemically and how this process functions physiologically. An *in vitro* reconstitution of mitochondrial division may be needed to reveal biochemical roles of each protein. In addition, future studies are needed to identify the physiological consequences of altered mitochondrial division in different tissues. A thorough understanding of mitochondrial division will provide valuable insight for designing strategies to improve health and well-being.

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Figure 1. Mitochondrial shape in MEFs and Purkinje neurons. Mitochondria in wildtype and Drp1-null MEFs were visualized by immunofluorescence microscopy using antibodies against Tom20. Mitochondria in Purkinje cells were visualized by electron microscopy.

Figure 2.
Mitochondrial division machinery in yeast and mammals.