ISSN 0006-2979, Biochemistry (Moscow), 2015, Vol. 80, No. 11, pp. 1457-1464. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © M. V. Patrushev, I. O. Mazunin, E. N. Vinogradova, P. A. Kamenski, 2015, published in Biokhimiya, 2015, Vol. 80, No. 11, pp. 1673-1682.

REVIEW

Mitochondrial Fission and Fusion

M. V. Patrushev[1,2]*, I. O. Mazunin[2], E. N. Vinogradova[1], and P. A. Kamenski[1,2]*

*[1]Lomonosov Moscow State University, Faculty of Biology, 119991 Moscow, Russia;
fax: +7 (495) 939-4309; E-mail: piotr.kamenski@gmail.com
[2]Immanuil Kant Baltic Federal University, Institute of Chemistry and Biology,
236038 Kaliningrad, Russia; fax: +7 (4012) 595-595; E-mail: maxpatrushev@gmail.com*

Received July 6, 2015
Revision received July 22, 2015

Abstract—Mitochondria are key cellular organelles responsible for many different functions. The molecular biology of mitochondria is continuously subject to comprehensive studies. However, detailed mechanisms of mitochondrial biogenesis are still unclear. Fusion and fission are among the most enigmatic processes connected with mitochondria. On the other hand, it has been shown that these events are of great biological importance for functioning of living cells. In this review, we summarize existing molecular data on mitochondrial dynamics and discuss possible biological functions of fusion and fission of these organelles.

DOI: 10.1134/S0006297915110061

Key words: mitochondria, fusion, fission

Fusion of membranes is one of the most important processes that occur in every cell. This phenomenon is exemplified by the fusion of vesicles with plasma membranes, which occurs during exocytosis and synaptic plasticity, the fusion of gametes during fertilization, and in viral infection that includes the fusion of the viral envelope with the endosomal membrane. The mechanisms and molecular apparatus involved in the fusion of different cellular membranes are significantly different. Nevertheless, general principles can be described. Thus, fusion is also associated with bringing two membranes together with subsequent local induction of lipid bilayer curvature and its destabilization. For complete or partial fusion of lipid bilayers, activities of special proteins are required, such as a soluble N-ethylmaleimide-sensitive factor more frequently colocalized with protein receptors, viral hemagglutinin, etc. Proteins involved in the fusion of bilayers contain in their structure heptad repeats that form inter- and intramolecular hairpin structures mediating the appearance of close intermembrane contact. These proteins are also characterized by the presence of hydrophobic domains, which penetrate into the fusing membranes to destabilize the bilayers [1].

The fusion of mitochondria is a more complicated process because these organelles have two membranes, and mitochondrial fusion is really the fusion of four membranes in pairs. GTPase from the dynamin family was the first identified component of the mitochondria fusion apparatus. This GTPase is necessary for uniting the inner membranes of mitoplasts within a mitochondrion. This protein was initially identified in yeast and was termed mitochondrial genome maintenance protein (Mgm1p), and it was shown that mutants in the corresponding gene were characterized by disorders in the maintenance of mitochondrial genome functioning [2]. This protein is synthesized in the cytosol, contains an *N*-terminal signal for import into mitochondria, and is imported into them (to make acquaintance with the mechanisms of protein import into mitochondria, we recommend review [3]). However, Mgm1p does not penetrate into the mitochondrial matrix: the protein contains an *N*-terminal transmembrane domain promoting its insertion into the inner membrane, whereas its major part is located in the intermembrane space [4]. Further studies revealed that in the yeast cell Mgm1p is present in two isoforms produced as a result of proteolytic cleavage of the protein by the membrane rhomboid proteinase Pcp1p [5, 6] (see also review [7] which describes in detail the mechanism of functioning of rhomboid proteinases). The elongated product of proteolytic cleavage of Mgm1p contains the above-mentioned *N*-terminal transmembrane domain, which promotes the insertion of the protein into the inner membrane; the shorter isoform lacks the anchoring transmembrane part. Both isoforms contain

* To whom correspondence should be addressed.

GTPase and effector domains and several heptad repeats. The amount of the short isoform of Mgm1p in a yeast cell is determined by the level of ATP in the mitochondrial matrix (see below) and by the state of the functional apparatus of protein import into these organelles [8]. The biological role of each isoform is still unclear, but it is known that for effective fusion of outer mitochondrial membranes both products are necessary for the proteolytic processing of the initial polypeptide chain [9].

It should be noted that, in addition to its major function, Mgm1p also participates in the protection of mitochondrial DNA against damages [10] and in the maintenance of the normal morphology of cristae of the inner mitochondrial membrane [11]. Later, analogs of the yeast Mgm1p were described for many other organisms. The mammalian protein OPA1 (from optic atrophy, this disease arises in animals with the corresponding gene deficiency) is best studied [12, 13]. Based on the essential likeness of this protein amino acid sequence with that of the yeast Mgm1l, it was identified as a mitochondrial dynamin. However, if mechanisms of OPA1 and Mgm1p functioning seem to be similar (see below), the processing of these two proteins is very different. Although in earlier experiments on OPA1 processing this protein was shown to be degraded also by the rhomboid proteinase, subsequent works did not confirm this observation: in mammalian cells lacking functional rhomboid proteinases, the set of OPA1 isoforms was just the same as in wild-type cells [14, 15].

In mammalian cells, there are eight OPA1 isoforms that are products of alternative splicing and are characterized by different combinations of three small exons located in the *N*-terminal part of the molecule, between the transmembrane and GTPase domains [16]. Depending on the set of exons, one to three sites of proteolytic cleavage can be formed in different OPA1 isoforms. These sites are usually termed S1, S2, and S3. Site S1 is present in all eight isoforms, whereas each of the S2 and S3 sites are present in four isoforms.

Cleavage at the S2 and S3 sites occurs constitutively, and this cleavage results in approximately equal amounts of the cleaved and non-cleaved variants of each OPA1 isoforms, which is similar to the situation with dynamin Mgm1p in yeast (see above). This cleavage is realized by AAA-protease of the intermembrane space Yme1L [15, 17]. As in the case of yeast, the biological significance of the existence of different length OPA1 isoforms is still unclear.

On the contrary, the cleavage of OPA1 isoforms at the S1 site occurs only under certain conditions, namely, on decrease in ATP concentration in the matrix, in the absence of membrane potential, and with disorders in normal functioning of mechanisms responsible for control of mitochondrial quality [18, 19]. In this case, the polypeptide chain is processed by the inner membrane zinc proteinase termed OMA1 (from overlapping with m-AAA protease, which suggests that the activity of this protein is partially overlapping with that of mitochondrial AAA-proteases) [20, 21]. It is interesting that OMA1 starts to degrade all OPA1 isoforms literally within some tens of seconds after the induction of stress conditions, and the further fusion of mitochondrial inner membranes becomes impossible [15, 17]. Thus, prevention of this process is the first response of mitochondria to stress, which is ahead of all other stages of organelle quality control described to date. The biological necessity of the virtually instantaneous inhibition of mitochondria fusion in response to stress is still an enigma for researchers.

OPA1 also performs in the cell additional functions similar to those of its yeast ortholog Mgm1p. About 90% of the total cellular production of OPA1 is localized inside the cristae of the mitochondrial inner membrane, and only 10% is in the ring-like structures produced on the fusion of the inner membranes. The absence of OPA1 leads to serious structural disorders in cristae [22]. Thus, we can assert that OPA1, similarly to Mgm1p, participates in the maintenance of normal structure of mitochondrial cristae. Possibly, the participation in structuring of the inner mitochondrial membrane should not be considered as a noncanonical function of mitochondrial dynamins. A viewpoint has been repeatedly published suggesting that the correct structure of the cristae can be fundamentally important for fusion of the inner membranes. Thus, the two apparently independent functions of OPA1 (and its orthologs) can occur as sides of the same medal. It is interesting that for normal functioning of OPA1, the presence of only one of two human mitofusins (Mfn) is critical, that is, of Mfn1 but not of Mfn2 [23]. Another protein participating in the fusion of mitochondrial membranes was identified in 1997 from studies on spermatogenesis in drosophila and was termed FZO (from "fuzzy onions", because just such associations were induced by the shape of drosophila's mitochondria in [24]). Similar proteins were found later in other organisms (yeast, worms, and mammals); they were combined in the protein family of mitofusins [25-27]. All proteins of this family have GTPase activity and are similar in domain organization: they inevitably have two transmembrane regions crossing the outer mitochondrial membrane and a small loop in the intermembrane space, with the major part exposed into the cytosol [28, 29]. All mitofusins also have in their sequences a great number of heptad repeats, which are extremely important for fusion of various membranes.

As mentioned above, in mammals two mitofusin isoforms, Mfn1 and Mfn2, were found. Mfn1 was shown to play the more important role, because the suppression of the expression of its gene resulted in more pronounced fragmentation of mitochondria than the suppression of the gene encoding Mfn2. However, Mfn1 and Mfn2 can functionally replace each other. Thus, induction of Mfn2 overexpression in cells deprived of Mfn1 completely abolished the effect of the absence of Mfn1 [30].

Mitofusins and Mgm1-like proteins are convincingly shown to be key components of the protein apparatus responsible for mitochondria fusion in various organisms. Thus, cells with mutant forms of these proteins were shown to contain many fragmented mitochondria [16, 31]. It was also shown that intact mitochondria introduced into cells deprived of the above-mentioned proteins were unable to exchange contents of their matrix, which indicated the absence of fusion [23, 30]. Experiments performed by Meeusen and colleagues revealed that mitochondria of yeast with *FZO1* and *MGM1* gene knockout were unable to undergo *in vitro* fusion. Moreover, mitofusins Mgm1 and OPA1 contain domains that seem to be components of the so-called fusogene, a multienzyme complex capable of performing energy-dependent biomechanical processes overcoming the energy barrier of membrane contact formation [32].

In addition to mitofusins, OPA1 is also a necessary component of the mitochondria fusion system. Suppression of expression of its gene using RNA interference technology leads to fragmentation of mitochondria because of impossibility of their fusion and to changes in the structure of mitochondrial cristae [33]. Analysis of OPA1 overexpression revealed that, depending on the tissue type, it could result either in fragmentation of mitochondria or in their complete fusion [34]. Therefore, full comprehension of the molecular mechanisms of the functioning of OPA1 and, in particular, of its participation in mitochondrial dynamics is absent. Nevertheless, the interpretation of functions of individual components of mitochondrial dynamics suggests a hypothetical model for fusion of these organelles. The first stage is the drawing of mitochondria closer and appearance of an intermolecular conjunction produced by the *C*-terminal heptad repeats of mitofusin 1. This process (also called docking) results in mutual orientation of mitochondria, their step-by-step closing, and at last fusion of the outer membrane bilayers. During this process mitofusins seem to form a "docking contact" between the two organelles with their gradual "dragging" to each other. The fusion of outer mitochondrial membranes does not occur without the involvement of mitofusin contacts [35].

It seems that the major purpose of mitochondrial fusion is the exchange by contents of their matrices (mainly the genetic material). It has been shown in many experiments that this process is mainly coordinated depending on the inner membrane geometry, which in its turn depends on the type of cells containing the mitochondria [36]. The inner mitochondrial membrane is a rather complicated structure. One part of it directly adjoins to the outer membrane, whereas the other part forms tube-like or plate-like structures termed cristae. The majority of researchers think that the fusion of inner membranes occurs in their regions located nearer to the outer membrane. A high degree of coordination of the fusion in a pair of outer and inner membrane has been observed in many experiments [37-39]. However, under some experimental conditions, independence of these processes can be observed, and this indicates that the fusion is mediated by different mechanisms for the inner and outer membranes. Thus, it has been shown that for fusion of the outer membranes in yeast, the mitochondria drawing closer, low level of GTP, and proton gradient on the inner membrane are required [32]. On the contrary, for fusion of the inner membrane a high level of GTP is necessary. It is still not clear what role is played in fusion processes by the potential on the inner membrane. It was shown that the addition to mammalian cell cultures of K^+ and H^+ ionophores dissipating membrane potential suppressed fusion of mitochondria [40]. Additional careful studies revealed that the ionophores suppressed the fusion of the inner but not of the outer membranes [41]. Based on the totality of data in this field, it is supposed that coordination between fusions of the inner and outer membranes should not be as strict as many authors formerly believed.

FISSION OF MITOCHONDRIA

Mitochondria of eukaryotic cells are not assembled *de novo*, but are produced by fission of preexisting organelles. As a result, all biological molecules are distributed between the daughter mitochondria. The fission of mitochondria is strictly regulated, and cells always maintain a fine balance between their fusion and fission [42]. Mitochondrial fission is mainly controlled by the cytosol. The fission is mainly regulated by representatives of a large family of dynamin-like GTPases that are designated as dynamin-related protein 1 (DRP1) in mammals, Dnm1p (from dynamin) in yeast, and DRP3A/B in plants. Note that the major protein apparatus of mitochondrial fusion is also represented by dynamin-like GTPases. The dynamin-like GTPases involved in the fission of mitochondria have a unique ability to oligomerize, which results in spiral-like structures apparently girdling the mitochondria. Hydrolysis of GTP by GTPase oligomers mediates compression of the multiple protein complex, which finally results in the separation of membranes [43-45]. Cells deprived of functional Dnm1p or DRP1 contain developed, dense, thick, fine mitochondrial networks produced from constant fusion when mitochondrial fission is impossible [46]. It should be noted that an additional splice-form DRP1 specific for brain was detected in humans, and it contains an insertion between the middle and the GTPase domain [47]. The functional significance of this protein insertion is still unknown.

As in the case of the fusion of mitochondria, mechanisms of their fission are best studied for yeast. The translocation of Dnm1p from the cytosol and formation of the girdling multiple protein structure on the mito-

chondria surface depends on the involvement of two other proteins designed as adaptor proteins: Fis1p (from "fission") and Mdv1p (from the "mitochondrial division protein"). Fis1p is a small protein anchored in the outer mitochondrial membrane with its *N*-terminal domain turned to the cytosol by a six-spiral region, which includes tetratricopeptide repeats (TPR) responsible for interaction with protein Mdv1p. This protein, in turn, consists of an *N*-terminal Fis1p-binding site, a heptad repeat responsible for homo-oligomeric interactions, and a *C*-terminal repeat WD40 directly interacting with Dnm1p [48].

Model studies on isolated proteins allowed assessment of the role of Mdv1p in the assembly of the Dnm1p superstructures. Mdv1p is joined to the GTP-binding form of Dnm1p, which stimulates its self-assembly to a superstructure. In other words, Mdv1p is a factor of Dnm1p stabilization in definite conformations favorable for its oligomerization. Studies *in vitro* have shown that the GTP-binding Dnm1p can also spontaneously assemble in a superstructure. The diameter of the ring consisting of joined Dnm1p molecules is 100 nm, which is equal to the diameter of the fission sites of mitochondria [43].

Thus, the general scheme of mitochondria fission is as follows: Fis1p captures Mdv1p dissolved in the cytosol. The Fis1p–Mdv1p complex is located on the membrane. The membrane-bound Mdv1p joins Dnm1p-GTP oligomers mediating their assembly in a superstructure, which in turn forms a spiral "girdling" the mitochondrion in the fission site. Then conformational changes occur in the spiral superstructure, presumably its compression accompanied by hydrolysis of GTP and the "cutting" of the mitochondrial membrane.

In some works, it was shown that in yeast the role of Mdv1p could be performed by the chromatin assembly protein factor (Caf4p) (this term initially given to the protein by mistake is used in yeast protein nomenclature) that has similar domain structure [49]. Moreover, protein Num1p (from nuclear migration, which reflects the major function of this protein) is also involved in the regulation of mitochondrial fission. During mitochondrial fission, Num1p interacts with Dnm1p; this process is controlled by the protein Mdm36p (from "mitochondrial distribution and morphology"). The Num1p–Mdm36p complex regulates the localization of mitochondria during fission, as well as their orientation with respect to the cytoskeleton, which allows the cell to control the localization of the mitochondrial network [50]. Such close interaction with the cytoskeleton suggests that a dependence should exist between mitochondrial fission and movement of cells. The situation with adaptor proteins of mitochondrial fission in cells of higher eukaryotes seems to be much more complicated. No Dnm1p orthologs were found in these cells, and the detected ortholog of Fis1p is not required for the fission of mitochondria [51]. On the other hand, in mammals several proteins were identified whose changes in contents in the cells resulted in disorders in mitochondrial fission (see the review [9]).

Yeast were shown to have another potential participant in mitochondrial fission – protein Mdm33p characterized by the presence of heptad repeats suggesting it as a component of the mitochondria fission apparatus. Functional features of this protein have not been described, but its overexpression results in strong fragmentation of mitochondria, whereas its shortage in the cell led to formation of dense mitochondrial networks [52]. It should be noted that no structural analog of Mdm33p has been found in higher eukaryotes, whereas the separation of the inner membrane in these organisms is contributed to by mitochondrial protein MTP18. Over- and underexpression of this protein result in the same consequences as in the case with Mdm33p [53].

REGULATION OF FUSION AND FISSION OF MITOCHONDRIA

Taking into account that fusion and fission of mitochondria are opposite processes, balance between them must be maintained in the cell. This balance has to promote both the maintenance of morphology and functions of mitochondria and their rapid adaptation on changes in physiological conditions. The above-mentioned major components of fusion and fission in yeast (Fzo1p, Mgm1p, and Dnm1) and mitofusins OPA1 and DRP1 in mammals are junctions in the regulation of mitochondrial dynamics.

The level of Fzo1p is controlled by the protein Mdm30p. It was shown experimentally that mutants lacking Mdm30p accumulate Fzo1p and contained mitochondria unable to fuse. Mdm30p is a subunit of the multisubunit ubiquitin ligase complex that mediates the ubiquitinylation and degradation of Fzo1p in proteasomes. The activity of this complex is extremely important for maintaining the required level of Fzo1p.

A hypothesis about dependence on ATP level of Mgm1p processing by Pcp1p protease is now under discussion. Newly synthesized Mgm1p is imported into mitochondria under the influence of translocases located in both the inner and outer mitochondrial membranes. During import, the mitochondrial localization signal is detached from Mgm1p by the mitochondrial processing peptidase (Mpp1p). Concurrently, the first hydrophobic region of Mgm1p is inserted into the inner membrane and anchors the long isoform of this protein. Mgm1p can be transferred into the matrix, which allows Pcp1p to cleave another hydrophobic region and thus to generate a short isoform that is released into the intermembrane space. Considering that Mgm1p import depends on ATP, the ATP level in the mitochondrial matrix has to determine the ratio of long and short isoforms of Mgm1p. At low ATP level, the import will be slow, and this will allow the

long isoform to "fasten" in the inner membrane faster than Pcp1p will be able to process it. At high ATP level, import will be rapid, and that will promote Pcp1p processing and cause higher content of short Mgm1p isoforms [8].

In dividing cells, the state of mitochondria depends on the cell cycle. During the S-phase, mitochondria demonstrate a high degree of fusion, whereas during division they are strongly fragmented. Before the beginning of S-phase, the enzymatic anaphase-promoting complex/cyclosome (APC/C) stimulates the degradation of Drp1p, weakening the fusion of mitochondria and leading to accumulation of cyclin E, which acts as a signal for initiating S-phase [54]. Thus, the decondensation of fused mitochondria preceding cyclin E accumulation indicates the most important role of mitochondrial dynamics in the cell cycle. During S-phase, the APC/C complex is inactivated, which results in the recovery of Drp1p level and hence in the establishment of the correct balance between the fusion and fission of mitochondria. During the subsequent stages, Drp1p is phosphorylated by the Cdk1/cyclin B system on Ser585, which leads to a rapid and massive fragmentation of mitochondria during the cell entrance into mitosis [55]. The premitotic fragmentation of mitochondria is necessary for their equal distribution between the daughter cells. The fusion of mitochondria during mitosis can lead to damage to cellular DNA and to instability of the genome, and this is additional evidence of the importance of mitochondrial dynamics in the cell cycle [56].

Fusion and fission of mitochondria have key significance on both cell and organism level. It was shown in some works that knockout of any dynamin-like protein led to the death of mouse embryos [57, 58] due to multiple disorders in cell differentiation. Thus, Drp1 knockout resulted in strong changes in the Notch-cascade that, in turn, made impossible normal differentiation of embryonic stem cells. These changes were caused by the inability of mitochondria in the absence of Drp1 to regulate intracellular calcium level that is crucial for such signaling pathways [59]. It should be emphasized that this work was the first demonstration of a definite connection between mitochondrial dynamics and calcium-dependent signaling cascades.

Stress, which is frequently manifested by damage to DNA and by inhibition of transcription or translation, can strongly modulate the state and functions of cells. Stress also influences mitochondrial dynamics. During stress, the fusion of mitochondria can increase in response to accumulation of oxidized glutathione [60]. There are also indirect indications that cells with great numbers of fused mitochondria are more viable, which seems to be due to increase in ATP production associated with the "hyperfusion" of mitochondria [61].

Special attention should be given to the mutual regulation of mitochondrial dynamics and apoptosis. In 2006, the journal *Nature* published a work that first showed that the proapoptotic proteins Bax and Bak of the Bcl2 family induced fusion of mitochondria in healthy cells [62]. In other work, Bax was shown to specifically modulate homotypical Mfn2 complexes through a direct interaction and to be the first effector of Drp1 fusion [63]. In the case of stress-induced activation of apoptosis, fragmentation of mitochondria and their fission were increased, which could be explained by permeabilization of the outer membrane. This resulted in the release of pro-apoptotic factors into the cytosol [64]. The permeabilization could be caused by formation of a homo-oligomeric pore in the outer membrane consisting of the Bax and Bak proteins [65].

A special role in apoptotic regulation of mitochondrial dynamics belongs to posttranslational modification of proteins involved in fusion or fission of mitochondria. During apoptosis, Drp1 is phosphorylated and undergoes the action of small ubiquitin-related modifiers (SUMO). The Drp1 protein contains three serine residues that are phosphorylated, and effects of their modification are different. Phosphorylation of Ser616 activates fission of mitochondria, whereas phosphorylated Ser637 induces suppression of this process [66]. The third regulatory serine residue (Ser693) is phosphorylated only during apoptosis and results in the inhibition of mitochondrial fission [67]. The phosphorylated protein Mfn2 demonstrates increased affinity for ubiquitin ligases, which induces its instability. This plays an important role in mitochondrial apoptotic pathways. Note that Bax oligomers are colocalized with Drp1 and Mfn2 in apoptotic cells, which is believed to influence the activity of mitochondrial dynamin-like proteins [68].

Cellular metabolism is one of the major regulators of mitochondrial dynamics. Because every type of cell and tissue has a specific transcriptional profile, and consequently unique features of metabolic pathways, the remodeling of mitochondria can be tissue-specific. Studies on the influence of diet on mitochondrial dynamics in neurons revealed that the fat-enriched diet led to increased fission of mitochondria in one type of neurons, whereas in other neurons mitochondria manifested the tendency for fusion [69]. The possible causes of such specific reaction of the cells to a particular diet were not discussed, but the cell need for ATP seemed to play an important role in the mitochondrial dynamics. Formation of a great number of fused mitochondria seems to increase the coupling of membrane electrochemical processes with ATP synthesis. Starvation is also a strong external factor causing cellular stress, which directly influences the structural characteristics of mitochondria. In this case, the specific features depend on the character of food limitation and its duration [70]. It was shown that starvation was accompanied by inhibition of Drp1 through an unknown mechanism that resulted initially to elongation of fragmented mitochondria but

then to their fusion. During long-term starvation the mitochondrial reticulum stabilized [70, 71].

As stated above, the fusion of mitochondria provides for more stable profile of fluctuations in intracellular ATP level. This is confirmed by studies on the ultrastructure of mitochondria in animals subjected to limitations in diet. During starvation, mitochondria contained more ATP synthase dimers and were characterized by more complicated cristae structure. This seemed to be an adaptation of the cells to shortage in nutrients, which could influence the formation of membrane potential and, consequently, biosynthesis of ATP [72].

It is supposed that some metabolic diseases influence mitochondrial dynamics, which in such case can be a stage in pathogenesis. Thus, models of type 2 diabetes in animals obtained by leptin receptor knockout manifested a high acetylation degree of Opa1 in cardiomyocytes, which directly influenced the mitochondrial dynamics in these cells [73].

Mitochondrial dysfunction is known to be associated with many diseases, the majority of which are neurologic. Recent works suggest relationships between mitochondrial dynamics and pathogeneses of various neuro- and myopathies. Thus, associations were revealed between polymorphism of the gene encoding Mfn2 with Charcot–Marie–Tooth disease [74].

In this review, molecular mechanisms were considered that underlie the fusion and fission of mitochondria and regulation of these processes. Most of these mechanisms are still far from being fully understood. It seems that dynamics of mitochondria is possibly the least-studied aspect of functioning of these organelles, and studies on the corresponding processes are at a stage when every new work in this field, together with answering some questions, raises many new ones. We have no doubt that further studies on dynamics of mitochondria on the molecular level will result in many very interesting discoveries.

We are deeply grateful to our colleagues in the laboratories in Kaliningrad and Moscow for their help in preparation of this review.

The works at Moscow State University were supported by the Federal Center Program "Studies and Elaborations in Priority Lines of Development of Scientific and Technological Complex in Russia for 2014-2020" (treaty 14.604.21.0113, identifier RFMEFI60414X0113), and the works at the Baltic Federal University were supported by the Russian Foundation for Basic Research (project No. 15-04-00378).

REFERENCES

1. Martens, S., and McMahon, H. T. (2008) Mechanisms of membrane fusion: disparate players and common principles, *Nat. Rev. Mol. Cell Biol.*, **7**, 543-556.
2. Jones, B. A., and Fangman, W. L. (1992) Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin, *Genes Dev.*, **3**, 380-389.
3. Dudek, J., Rehling, P., and Van der Laan, M. (2013) Mitochondrial protein import: common principles and physiological networks, *Biochim. Biophys. Acta*, **2**, 274-285.
4. Wong, E. D., Wagner, J. A., Gorsich, S. W., McCaffery, J. M., Shaw, J. M., and Nunnari, J. (2000) The dynamin-related GTPase, Mgm1p, is an intermembrane space protein required for maintenance of fusion competent mitochondria, *J. Cell. Biol.*, **2**, 341-352.
5. Herlan, M., Vogel, F., Bornhovd, C., Neupert, W., and Reichert, A. S. (2003) Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA, *J. Biol. Chem.*, **278**, 27781-27788.
6. Esser, K., Tursun, B., Ingenhoven, M., Michaelis, G., and Pratje, E. (2002) A novel two-step mechanism for removal of a mitochondrial signal sequence involves the mAAA complex and the putative rhomboid protease Pcp1, *J. Mol. Biol.*, **323**, 835-843.
7. Ha, Y., Akiyama, Y., and Xue, Y. (2013) Structure and mechanism of rhomboid protease, *J. Biol. Chem.*, **22**, 15430-15436.
8. Herlan, M., Bornhovd, C., Hell, K., Neupert, W., and Reichert, A. S. (2004) Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor, *J. Cell Biol.*, **2**, 167-173.
9. Van der Bliek, A. M., Shen, Q., and Kawajiri, S. (2013) Mechanisms of mitochondrial fission and fusion, *Cold Spring Harb. Perspect. Biol.*, **5**, a011072.
10. Guan, K., Farh, L., Marshall, T. K., and Deschenes, R. J. (1993) Normal mitochondrial structure and genome maintenance in yeast requires the dynamin-like product of the *MGM1* gene, *Curr. Genet.*, **24**, 141-148.
11. Meeusen, S., DeVay, R., Block, J., Cassidy-Stone, A., Wayson, S., McCaffery, J. M., and Nunnari, J. (2006) Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1, *Cell*, **127**, 383-395.
12. Alexander, C., Votruba, M., Pesch, U. E., Thiselton, D. L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G., Bhattacharya, S. S., and Wissinger, B. (2000) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28, *Nat. Genet.*, **26**, 211-215.
13. Delettre, C., Lenaers, G., Griffoin, J. M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E., Astarie-Dequeker, C., Lasquellec, L., Arnaud, B., Ducommun, B., Kaplan, J., and Hamel, C. P. (2000) Nuclear gene *OPA1*, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy, *Nat. Genet.*, **26**, 207-210.
14. Cipolat, S., Rudka, T., Hartmann, D., Costa, V., Serneels, L., Craessaerts, K., Metzger, K., Frezza, C., Annaert, W., D'Adamio, L., Derks, C., Dejaegere, T., Pellegrini, L., D'Hooge, R., Scorrano, L., and De Strooper, B. (2006) Mitochondrial rhomboid PARL regulates cytochrome *c* release during apoptosis via OPA1-dependent cristae remodeling, *Cell*, **126**, 163-175.
15. Griparic, L., Kanazawa, T., and Van der Bliek, A. M. (2007) Regulation of the mitochondrial dynamin-like pro-

tein Opa1 by proteolytic cleavage, *J. Cell Biol.*, **178**, 757-764.
16. Olichon, A., Elachouri, G., Baricault, L., Delettre, C., Belenguer, P., and Lenaers, G. (2007) OPA1 alternate splicing uncouples an evolutionary conserved function in mitochondrial fusion from a vertebrate restricted function in apoptosis, *Cell Death Differ.*, **4**, 682-692.
17. Song, Z., Chen, H., Fiket, M., Alexander, C., and Chan, D. C. (2007) OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L, *J. Cell Biol.*, **178**, 749-755.
18. Baricault, L., Segui, B., Guegand, L., Olichon, A., Valette, A., Larminat, F., and Lenaers, G. (2007) OPA1 cleavage depends on decreased mitochondrial ATP level and bivalent metals, *Exp. Cell Res.*, **313**, 3800-3808.
19. Merkwirth, C., Dargazanli, S., Tatsuta, T., Geimer, S., Lower, B., Wunderlich, F. T., von Kleist-Retzow, J. C., Waisman, A., Westermann, B., and Langer, T. (2008) Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria, *Genes Dev.*, **22**, 476-488.
20. Ehses, S., Raschke, I., Mancuso, G., Bernacchia, A., Geimer, S., Tondera, D., Martinou, J. C., Westermann, B., Rugarli, E. I., and Langer, T. (2009) Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1, *J. Cell Biol.*, **187**, 1023-1036.
21. Head, B., Griparic, L., Amiri, M., Gandre-Babbe, S., and Van der Bliek, A. M. (2009) Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells, *J. Cell Biol.*, **187**, 959-966.
22. Griparic, L., Van der Wel, N. N., Orozco, I. J., Peters, P. J., and Van der Bliek, A. M. (2004) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria, *J. Biol. Chem.*, **279**, 18792-18798.
23. Cipolat, S., Martins de Brito, O., Dal Zilio, B., and Scorrano, L. (2004) OPA1 requires mitofusin 1 to promote mitochondrial fusion, *Proc. Natl. Acad. Sci. USA*, **45**, 15927-15932.
24. Hales, K. G., and Fuller, M. T. (1997) Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase, *Cell*, **90**, 121-129.
25. Rapaport, D., Brunner, M., Neupert, W., and Westermann, B. (1998) Fzo1p is a mitochondrial outer membrane protein essential for the biogenesis of functional mitochondria in *Saccharomyces cerevisiae*, *J. Biol. Chem.*, **273**, 20150-20155.
26. Hermann, G. J., Thatcher, J. W., Mills, J. P., Hales, K. G., Fuller, M. T., Nunnari, J., and Shaw, J. M. (1998) Mitochondrial fusion in yeast requires the transmembrane GTPase Fzo1p, *J. Cell Biol.*, **143**, 359-373.
27. Kanazawa, T., Zappaterra, M. D., Hasegawa, A., Wright, A. P., Newman-Smith, E. D., Buttle, K. F., McDonald, K., Mannella, C. A., and Van der Bliek, A. M. (2008) The *C. elegans* Opa1 homologue EAT-3 is essential for resistance to free radicals, *PLoS Genet.*, **2**, e1000022.
28. Fritz, S., Rapaport, D., Klanner, E., Neupert, W., and Westermann, B. (2001) Connection of the mitochondrial outer and inner membranes by Fzo1 is critical for organellar fusion, *J. Cell Biol.*, **4**, 683-692.
29. Rojo, M., Legros, F., Chateau, D., and Lombes, A. (2002) Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo, *J. Cell. Sci.*, **115**, 1663-1674.
30. Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., and Chan, D. C. (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development, *J. Cell Biol.*, **2**, 189-200.
31. Wong, E. D., Wagner, J. A., Gorsich, S. W., McCaffery, J. M., Shaw, J. M., and Nunnari, J. (2000) The dynamin-related GTPase, Mgm1p, is an intermembrane space protein required for maintenance of fusion competent mitochondria, *J. Cell Biol.*, **2**, 341-352.
32. Meeusen, S., McCaffery, J. M., and Nunnari, J. (2004) Mitochondrial fusion intermediates revealed *in vitro*, *Science*, **305**, 1747-1752.
33. Olichon, A., Baricault, L., Gas, N., Guillou, E., Valette, A., Belenguer, P., and Lenaers, G. (2003) Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome *c* release and apoptosis, *J. Biol. Chem.*, **10**, 7743-7746.
34. Ramonet, D., Perier, C., Recasens, A., Dehay, B., Bove, J., Costa, V., Scorrano, L., and Vila, M. (2013) Optic atrophy 1 mediates mitochondria remodeling and dopaminergic neurodegeneration linked to complex I deficiency, *Cell Death Differ.*, **1**, 77-85.
35. Chen, H., Chomyn, A., and Chan, D. C. (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction, *J. Biol. Chem.*, **28**, 26185-26192.
36. Frey, T. G., and Mannella, C. A. (2000) The internal structure of mitochondria, *Trends Biochem. Sci.*, **7**, 319-324.
37. Tomasello, M. F., Guarino, F., Reina, S., Messina, A., and De Pinto, V. (2013) The voltage-dependent anion selective channel 1 (VDAC1) topography in the mitochondrial outer membrane as detected in intact cell, *PLoS One*, **12**, e81522.
38. An, H. J., Cho, G., Lee, J. O., Paik, S. G., Kim, Y. S., and Lee, H. (2013) Higd-1a interacts with Opa1 and is required for the morphological and functional integrity of mitochondria, *Proc. Natl. Acad. Sci. USA*, **32**, 13014-13019.
39. Otera, H., Ishihara, N., and Mihara, K. (2013) New insights into the function and regulation of mitochondrial fission, *Biochim. Biophys. Acta*, **5**, 1256-1268.
40. Mattenberger, Y., James, D. I., and Martinou, J. C. (2003) Fusion of mitochondria in mammalian cells is dependent on the mitochondrial inner membrane potential and independent of microtubules or actin, *FEBS Lett.*, **3**, 53-59.
41. Malka, F., Guillery, O., Cifuentes-Diaz, C., Guillou, E., Belenguer, P., Lombes, A., and Rojo, M. (2005) Separate fusion of outer and inner mitochondrial membranes, *EMBO Rep.*, **9**, 853-859.
42. Westermann, B. (2010) Mitochondrial fusion and fission in cell life and death, *Nat. Rev. Mol. Cell Biol.*, **12**, 872-884.
43. Ingerman, E., Perkins, E. M., Marino, M., Mears, J. A., McCaffery, J. M., Hinshaw, J. E., and Nunnari, J. (2005) Dnm1 forms spirals that are structurally tailored to fit mitochondria, *J. Cell Biol.*, **7**, 1021-1027.
44. Lackner, L. L., Horner, J. S., and Nunnari, J. (2009) Mechanistic analysis of a dynamin effector, *Science*, **5942**, 874-877.
45. Mears, J. A., Lackner, L. L., Fang, S., Ingerman, E., Nunnari, J., and Hinshaw, J. E. (2011) Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission, *Nat. Struct. Mol. Biol.*, **1**, 20-26.

46. Otsuga, D., Keegan, B. R., Brisch, E., Thatcher, J. W., Hermann, G. J., Bleazard, W., and Shaw, J. M. (1998) The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast, *J. Cell Biol.*, **2**, 333-349.
47. Manczak, M., Calkins, M. J., and Reddy, P. H. (2011) Impaired mitochondrial dynamics and abnormal interaction of amyloid β with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage, *Hum. Mol. Genet.*, **13**, 2495-2509.
48. Tieu, Q., and Nunnari, J. (2000) Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, Dnm1p, to trigger mitochondrial division, *J. Cell Biol.*, **2**, 353-366.
49. Westermann, B. (2014) Mitochondrial inheritance in yeast, *Biochim. Biophys. Acta*, **7**, 1039-1046.
50. Schauss, A. C., and McBride, H. M. (2007) Mitochondrial fission: a non-nuclear role for Num1p, *Curr. Biol.*, **12**, 467-470.
51. Otera, H., Wang, C., Cleland, M. M., Setoguchi, K., Yokota, S., Youle, R. J., and Mihara, K. (2010) Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells, *J. Cell. Biol.*, **6**, 1141-1158.
52. Messerschmitt, M., Jakobs, S., Vogel, F., Fritz, S., Dimmer, K. S., Neupert, W., and Westermann, B. (2003) The inner membrane protein Mdm33 controls mitochondrial morphology in yeast, *J. Cell. Biol.*, **4**, 553-564.
53. Tondera, D., Czauderna, F., Paulick, K., Schwarzer, R., Kaufmann, J., and Santel, A. (2005) The mitochondrial protein MTP18 contributes to mitochondrial fission in mammalian cells, *J. Cell Sci.*, **14**, 3049-3059.
54. Mitra, K., Wunder, C., Roysam, B., Lin, G., and Lippincott-Schwartz, J. (2009) A hyperfused mitochondrial state achieved at G1-S regulates cyclin E buildup and entry into S-phase, *Proc. Natl. Acad. Sci. USA*, **29**, 11960-11965.
55. Taguchi, N., Ishihara, N., Jofuku, A., Oka, T., and Mihara, K. (2007) Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission, *J. Biol. Chem.*, **15**, 11521-11529.
56. Qian, W., Choi, S., Gibson, G. A., Watkins, S. C., Bakkenist, C. J., and Van Houten, B. (2012) Mitochondrial hyperfusion induced by loss of the fission protein Drp1 causes ATM-dependent G2/M arrest and aneuploidy through DNA replication stress, *J. Cell Sci.*, **23**, 5745-5757.
57. Ishihara, N., Nomura, M., Jofuku, A., Kato, H., Suzuki, S. O., Masuda, K., Otera, H., Nakanishi, Y., Nonaka, I., Goto, Y., Taguchi, N., Morinaga, H., Maeda, M., Takayanagi, R., Yokota, S., and Mihara, K. (2009) Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice, *Nat. Cell Biol.*, **8**, 958-966.
58. Wakabayashi, J., Zhang, Z., Wakabayashi, N., Tamura, Y., Fukaya, M., Kensler, T. W., Iijima, M., and Sesaki, H. (2009) The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice, *J. Cell Biol.*, **6**, 805-816.
59. Kasahara, A., Cipolat, S., Chen, Y., Dorn, G. W., 2nd, and Scorrano, L. (2013) Mitochondrial fusion directs cardiomyocyte differentiation via calcineurin and Notch signaling, *Science*, **6159**, 734-737.
60. Shutt, T., Geoffrion, M., Milne, R., and McBride, H. M. (2012) The intracellular redox state is a core determinant of mitochondrial fusion, *EMBO Rep.*, **10**, 909-915.
61. Tondera, D., Grandemange, S., Jourdain, A., Karbowski, M., Mattenberger, Y., Herzig, S., Da Cruz, S., Clerc, P., Raschke, I., Merkwirth, C., Ehses, S., Krause, F., Chan, D. C., Alexander, C., Bauer, C., Youle, R., Langer, T., and Martinou, J. C. (2009) SLP-2 is required for stress-induced mitochondrial hyperfusion, *EMBO J.*, **11**, 1589-1600.
62. Karbowski, M., Norris, K. L., Cleland, M. M., Jeong, S. Y., and Youle, R. J. (2006) Role of Bax and Bak in mitochondrial morphogenesis, *Nature*, **7112**, 658-662.
63. Hoppins, S., Edlich, F., Cleland, M. M., Banerjee, S., McCaffery, J. M., Youle, R. J., and Nunnari, J. (2011) The soluble form of Bax regulates mitochondrial fusion via MFN2 homotypic complexes, *Mol. Cell*, **2**, 150-160.
64. Cassidy-Stone, A., Chipuk, J. E., Ingerman, E., Song, C., Yoo, C., Kuwana, T., Kurth, M. J., Shaw, J. T., Hinshaw, J. E., Green, D. R., and Nunnari, J. (2008) Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization, *Dev. Cell*, **2**, 193-204.
65. Westphal, D., Kluck, R. M., and Dewson, G. (2014) Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis, *Cell Death Differ.*, **2**, 196-205.
66. Chang, C. R., and Blackstone, C. (2010) Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1, *Ann. N. Y. Acad. Sci.*, **1201**, 34-39.
67. Chou, C. H., Lin, C. C., Yang, M. C., Wei, C. C., Liao, H. D., Lin, R. C., Tu, W. Y., Kao, T. C., Hsu, C. M., Cheng, J. T., Chou, A. K., Lee, C. I., Loh, J. K., Howng, S. L., and Hong, Y. R. (2012) GSK3β-mediated Drp1 phosphorylation induced elongated mitochondrial morphology against oxidative stress, *PLoS One*, **11**, e49112.
68. Karbowski, M., Lee, Y. J., Gaume, B., Jeong, S. Y., Frank, S., Nechushtan, A., Santel, A., Fuller, M., Smith, C. L., and Youle, R. J. (2002) Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis, *J. Cell Biol.*, **6**, 931-938.
69. Dietrich, M. O., Liu, Z. W., and Horvath, T. L. (2013) Mitochondrial dynamics controlled by mitofusins regulate Agrp neuronal activity and diet-induced obesity, *Cell*, **1**, 188-199.
70. Rambold, A. S., Kostelecky, B., Elia, N., and Lippincott-Schwartz, J. (2011) Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation, *Proc. Natl. Acad. Sci. USA*, **25**, 10190-10195.
71. Kim, B., Kim, J. S., Yoon, Y., Santiago, M. C., Brown, M. D., and Park, J. Y. (2013) Inhibition of Drp1-dependent mitochondrial division impairs myogenic differentiation, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **8**, 927-938.
72. Gomes, L. C., Di Benedetto, G., and Scorrano, L. (2011) Essential amino acids and glutamine regulate induction of mitochondrial elongation during autophagy, *Cell Cycle*, **16**, 2635-2639.
73. Samant, S. A., Zhang, H. J., Hong, Z., Pillai, V. B., Sundaresan, N. R., Wolfgeher, D., Archer, S. L., Chan, D. C., and Gupta, M. P. (2014) SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress, *Mol. Cell. Biol.*, **5**, 807-819.
74. Lawson, V. H., Graham, B. V., and Flanigan, K. M. (2005) Clinical and electrophysiologic features of CMT2A with mutations in the *mitofusin 2* gene, *Neurology*, **2**, 197-204.