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REVIEW

Fusion, Fragmentation, and Fission of Mitochondria

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Abstract—Individual mitochondria which form the chondriom of eucaryotic cells are highly dynamic systems capable of fusion and fragmentation. These two processes do not exclude one another and can occur concurrently. However, fragmentation and fusion of mitochondria regularly alternate in the cell cycle of some unicellular and multicellular organisms. Mitochondrial shapes are also described which are interpreted as intermediates of their "equational" division, or fission. Unlike the fragmentation, the division of mitochondria, especially synchronous division, is also accompanied by segregation of mitochondrial genomes and production of specific "dumbbell-shaped" intermediates. This review considers molecular components and possible mechanisms of fusion, fragmentation, and fission of mitochondria, and the biological significance of these processes is discussed.

Key words: mitochondria, chondriom, fragmentation, fusion, fission, dynamins

Mitochondria are universal organelles which are present in the vast majority of eucaryotic cells. Their main functions include synthesis of adenosine triphosphate (ATP), regulation of ionic homeostasis and lipid metabolism, and involvement in the apoptotic death of cells [1-7].

Although greatly varied in shape and size, mitochondria have the same principle of their structural organization: they consist of two highly specialized membranes separated by the intermembrane space and of the internal matrix. The external membrane of normal (orthodox) mitochondria has a smooth surface, whereas the internal membrane has many invaginations (cristae) which significantly increase the total area of its surface. Both membranes can have local regions of close contacts which, according to some data, are sites of translocation into the matrix of cytoplasmic proteins and/or lipids [8-10].

Mitochondria occupy a specific "semiautonomous" place in the complicated hierarchy of intracellular structures because they have their own genome and molecular machines for replication of DNA and synthesis of RNA and proteins. On cytological preparations stained with DNA-binding fluorochromes, mitochondrial genomes are visualized as small bodies of different size which are usually called nucleoids [11]. As in bacteria, mitochondrial nucleoids are structurally related to the internal membrane, and this relation is very significant for the replication, transcription, and segregation of mitochondrial genomes [12]. In total, the population of mitochondria of an individual cell, or in other terms, the chondriom, is characterized by the following features: 1) the shape, size, and location of mitochondria depend on functional conditions of the cell or (in unicellular organisms) on the stage of the life cycle [13, 14]; 2) increase or decrease in the total mass of the chondriom determined morphometrically or biochemically is strictly correlated with changes in the volume of the cytoplasmic compartment [15, 16]; and 3) the number of mitochondria in the cells of the same organism is not constant but varies widely, from units to some hundreds or even thousands [17]. The totality of these data means that mitochondria are dynamic structures capable of growth, fusion, and fission [18]. The phenomenology of these processes has long been described in detail by cytologists; nevertheless, many aspects of behavior of mitochondria are disputable [19]. Thus, in the current literature in English all cases of fragmentation of mitochondria are more often called "fission", which means division. But there are arguments suggesting that fragmentation and division of mitochondria are independent processes which have quite different consequences, although their final results are apparently similar. There is also another intriguing and not quite clear question: what are the structural and molecular mechanisms of fusion and fragmentation of mitochondrial membranes and how are these processes controlled by the cell?

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TIME COURSE OF FUSION AND FRAGMENTATION OF MITOCHONDRIA

The first convincing data in favor of fusion and fragmentation of mitochondria were obtained with the electron microscope by combined ultrastructural and morphometric analysis of the chondriom behavior during the life cycle of unicellular organisms.

During the interphase, cells of many protists have gigantic reticular mitochondria (the so-called basket-complexes) which occupy the periphery of the cytoplasm. With cell growth, the extent of these complexes increases and oval or rod-shaped mitochondria appear in them. Immediately before division the chondriom breaks into a multiplicity of small fragments which are randomly distributed during the division in the daughter cells and gradually united into new reticular complexes [20].

Similar behavior of the chondriom has been described for the life cycle of a representative of yeast, *Saccharomyces cerevisiae*. During the exponential growth phase the majority of the *S. cerevisiae* cells contain several mitochondria (about ten), and one of them, as a rule, the largest one is about a half of the chondriom volume and has a complicated reticular structure. In some cells, the chondriom consists of only a gigantic reticular mitochondrion. During the stationary phase the chondriom is fragmented, which increases the total number of mitochondria to 30-50 per cell. The time course of fusion and fragmentation of mitochondria in yeast cells was followed in detail by video microscopy, and the fusion or fragmentation acts occurred every 2 min, but as a whole the two processes were balanced and the more or less stationary state of the chondriom was maintained in yeast cells [21-23].

In higher eucaryotes the large-scale fusion or fragmentation of mitochondria are more often observed during cell differentiation.

The formation of large mitochondrial supercomplexes has been observed in spermatocytes of insects and spiders. These complexes are round-shaped in drosophila [24] or ring-shaped in scorpion [25] and consist of several mitochondria stuck together. Just another situation, a large-scale fragmentation of the chondriom, occurs in growing oocytes of the clawed frog *Xenopus laevis* [17] and the sea urchin *Paracentrotus lividus* [26]. A large-scale transition of mitochondria from "thread-like" shapes to small "granulated" ones has been described for cells which have switched on the program of apoptosis [27, 28]. It is important that the fragmentation of the chondriom induced by ROS (hydrogen peroxide) is not a consequence of cell death because it precedes the deenergization of mitochondria and the release of cytochrome *c* into the cytosol [28].

Association of the chondriom fragmentation with the cell cycle was followed in proliferating cells of a cell culture where mitochondria were fragmented during the S-phase and seemed to be stimulated by preparation of the cells for the mitotic division [29].

Cases are also known when the chondriom structure is modified by exogenous factors influencing the functional state of mitochondria. Thus, "thread-like" mitochondria were totally fragmented in the presence of diazepam which inhibited the benzodiazepine receptor in the external membrane of mitochondria [30] or after incubation of the cells with rotenone which inhibited the respiratory complex I [28].

Fine details of space and time changes during the fusion and fragmentation of mitochondria can be observed during the lifetime with a phase-contrast microscope. Usually these changes are observed in tissue culture cells spread on a substrate surface. The cultured cells have a peculiar chondriom which consists of elongated, branched, or "reticular" mitochondria (from Latin *reticulum*, network) and a small number of little organelles (Fig. 1).

The fusion of mitochondria occurs in some stages [20]. At first mitochondria several times contact one another with their ends, or the end of one mitochondrion comes in contact with the lateral surface of the other organelle, and then they combine structurally. The fusion itself takes a very short time, its duration is about 3-10 sec; therefore, it is virtually impossible to follow it on the level of ultrastructure.

During fragmentation, fragments of different size which, as a rule, maintain the shape of the "parental" organelle, are separated from individual mitochondria or from the mitochondrial reticulum [20]. The separated fragment usually remains close to the zone of tearing off

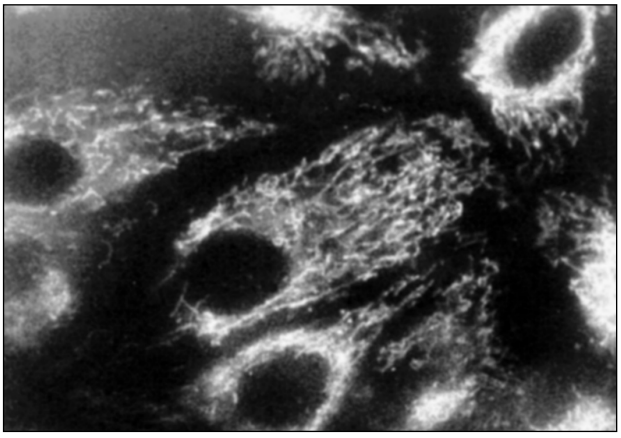

Fig. 1. Life-time photo of a tissue culture cell stained with the fluorescent dye Rhodamine 123. Multiple thread-like and "branching" mitochondria are visible in the dark cytoplasm. Photo by D. B. Zorov.

and can fuse again with the general mitochondrial network. The time of the fragment separation is about 3-10 sec.

Both fusion and fragmentation can occur concurrently and independently. It has been calculated that in tissue culture cells which contain some hundred mitochondria about 40 acts of fusion and fragmentation occur in 1 h (one event every 1.5 min) [20].

Still now, nearly nothing is known about the behavior of the genetic matter during the fusion and fragmentation of mitochondria. It has been shown by vital staining with a fluorescent dye Pico green that nucleoids are located without order in the chondrioms of cultured cells: mitochondria with multiple nucleoids occur more frequently, and some mitochondria contain unit nucleoids. The number of nucleoids in individual mitochondria varies from one to fifteen [31, 32]. Nucleoids can fuse or divide independently of fusion or fragmentation of the organelles themselves in both the mitochondrial reticulum and individual mitochondria. However, in some cell lines with a relatively frequent fragmentation, from 6 to 60% of mitochondria have no DNA [32]. That means that the fragmentation observed in tissue culture cells can result in genetically imperfect mitochondria.

MOLECULAR MECHANISMS OF FUSION AND FRAGMENTATION OF MITOCHONDRIA

Advances in comprehension of the molecular mechanisms which control the fusion and fragmentation of mitochondrial membranes are associated with identification of dynamins, proteins of a large family of GTP-binding proteins. Initially dynamins were isolated from the cells of calf brain and identified as molecular motors associated with microtubules [33]. Later dynamins were shown to play an important role during the final stage of endocytosis, namely, in the separation or "splitting off" of endocytosis vesicles from the plasma membrane [34]. In this connection, dynamins are considered now as molecular machines where the energy of GTP is used to cut biological membranes [35]. In an *in vitro* system, dynamins convert spherical liposomes into long over-wound tubules which are transformed to small vesicles of standard diameter on addition of GTP [36]. It is unknown how dynamins function *in vivo*. All proteins of the dynamin family have highly conservative motifs which determine their main functions [37]. These are the N-terminal GTP-binding domain with an unknown function; the domain homologous to the protein plextrine (PH-domain); the spiral domain which is involved in the binding to membranes (GED-domain); and the C-terminal domain enriched with proline (PRD-domain).

A dynamin-like protein which controls the fusion of mitochondria was first identified in mutant *Drosophila* clones with deficient development of sperm [38]. During the final stages of spermatogenesis in wild type *Drosophila* mitochondria migrated into the central region of the cell and there fused into two gigantic complexes, which relocated to the base of the flagellum. In the mutants, mitochondria aggregated but failed to fuse, and this resulted in a structure of many closely associated mitochondria, the so-called "fuzzy onion". This phenomenon needed the activity of the gene encoding the mitochondrial GTP-binding protein which is expressed in spermatids before the fusion of mitochondria. This protein is conservative because its homologs are synthesized in various tissues of mammals.

A homologous protein called Fzo1 was found in yeast [39-41]. A deletion in the gene encoding Fzo1 completely inhibited the fusion of mitochondria in conjugating cells. *FZO1* mutations resulted in anomalously high fragmentation of the chondriom and, as a consequence, in appearance in the proliferating population of cells deprived of mitochondrial DNA. Immunohistochemically Fzo1 was located on the external membrane of mitochondria with its GTP-binding domain facing the cytosol. Both terminal domains of Fzo1 (NH_2 and COOH) were recently shown to be exposed in the cytosol, and a short spiral domain is located in the intermembrane space and can associate with the internal mitochondrial membrane [42]. It was suggested that the terminal domains of the protein are responsible for the close association of the fusing mitochondria, and its subsequent conformational rearrangements promote the successive fusion of the external and internal membranes (Fig. 2).

Dynamin-like proteins have now been found that are antagonists to the proteins "fuzzy onion" and Fzo1. Functions of one of these proteins, Drp1 (dynamin-related protein), have been studied in detail in the cells of human tissue culture transfected with the appropriate mutant gene [43]. Normally the chondriom of these cells is represented by elongated tubular mitochondria which are more or less equally distributed in the cytoplasm. The synthesis of the mutant protein results in dramatic changes in the chondriom organization: the mitochondria are "balling", become very different in size, and are accumulated in the perinuclear region. Note, that the expression of the mutant gene does not affect the "canonical" functions of dynamins: secretion and endocytosis. Therefore, mammalian cells are suggested to have different classes of dynamins with strictly specific functions.

A homolog of Drp1, the Dnm1 dynamin, has been found in yeast [37, 41, 44, 45]. In the wild type cells, Dnm1 is located in the cytosol and in strangulation zones of the fragmenting mitochondria. Mutation in the gene encoding Dnm1 disturbs the balance between of the fusion and fragmentation acts in the mitochondria due to inhibition of the fragmentation. In the cells with *DNM1* deletion, the mitochondria completely lose their ability for fragmentation that results in transformation of the

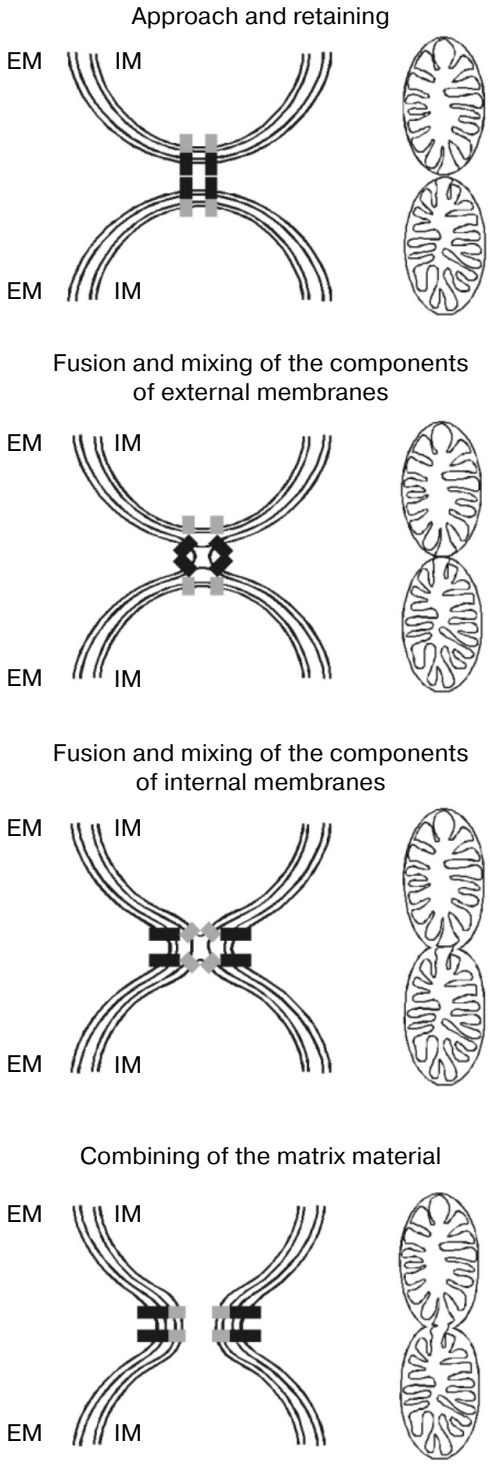

Fig. 2. A hypothetical scheme presenting the fusion of mitochondria with involvement of proteins located in the regions of mitochondrial contacts. The fusion of mitochondria is realized by mechanisms which need the coordinated interaction of the external and internal membranes (EM and IM, respectively). Protein complexes located in the external membranes are shown by black rectangles; protein complexes of the internal membranes are shown by gray rectangles. Modified after [42].

chondriom of the mutant cells into a complicated web-like structure.

Functions of Drp1 have been studied more carefully in muscle cells of the nematode *Caenorhabditis elegans* [46]. Normally these cells have long tubular mitochondria. Superexpression of the gene encoding Drp1 (the gene of the "wild" type) results in the total fragmentation of the chondriom. Morphologically this process looks like local invagination of both membranes that ends in the complete division of long mitochondria to shorter fragments. By data of immunofluorescent analysis, Drp1 fused with the GFP protein associates with zones of strangulations only during the final stages of the fragmentation of mitochondria. Thus, "mitochondrial" dynamins seem to control not the invagination of the mitochondrial membranes, but most likely their "cutting" during final stages of the fragmentation. By this feature, they are identical to "canonical" dynamins involved in endocytosis.

Two new genes have recently been identified which encode proteins also involved in the fragmentation of mitochondria. The first gene encodes a cytosolic protein with features of dynamin. This protein was independently identified in several laboratories and, therefore, has different names: Mdv1 (for mitochondrial division) [47], Net2 [48], Fis2 (for fission) [49], and Gag3 (for glycerol adapted growth) [50]. By immunocytochemistry, this protein was found either in the cytosol or in association with the external mitochondrial membrane. On the mitochondrial surface Mdv1 is co-located with Dnm1 as discrete clusters, mainly in the region where strangulations are produced. The proteins Dnm1 and Mdv1 are thought to be a part of the complicated apparatus responsible for the fragmentation of the mitochondrial external membrane. The location of the complex on the surface of mitochondria seems to be determined by Dnm1 because deletion in the gene encoding Mdv1 fails to disturb the distribution of Dnm1, whereas deletion in the gene encoding the Dnm1 protein results in significant changes in the location of Mdv1.

The other gene encodes a transmembrane protein of the external mitochondrial membrane. It is called Mdv2 or Fis1 [47, 49]. The greater moiety of the molecule of this protein is exposed in the cytosol. Unlike Mdv1, which is always located in the strangulation region, the Mdv2 protein is uniformly distributed over the mitochondrial surface. This protein is suggested to be required for immobilization of Mdv1, but it is not involved in the fragmentation of mitochondrial membranes.

A generalizing model of topological interactions of proteins involved in the fragmentation of the external mitochondrial membrane based on data of mutational analysis and immunocytochemical location is presented in Fig. 3.

A fundamental question is whether this apparatus can control the fragmentation not only of the external but also of the internal mitochondrial membrane. This prob-

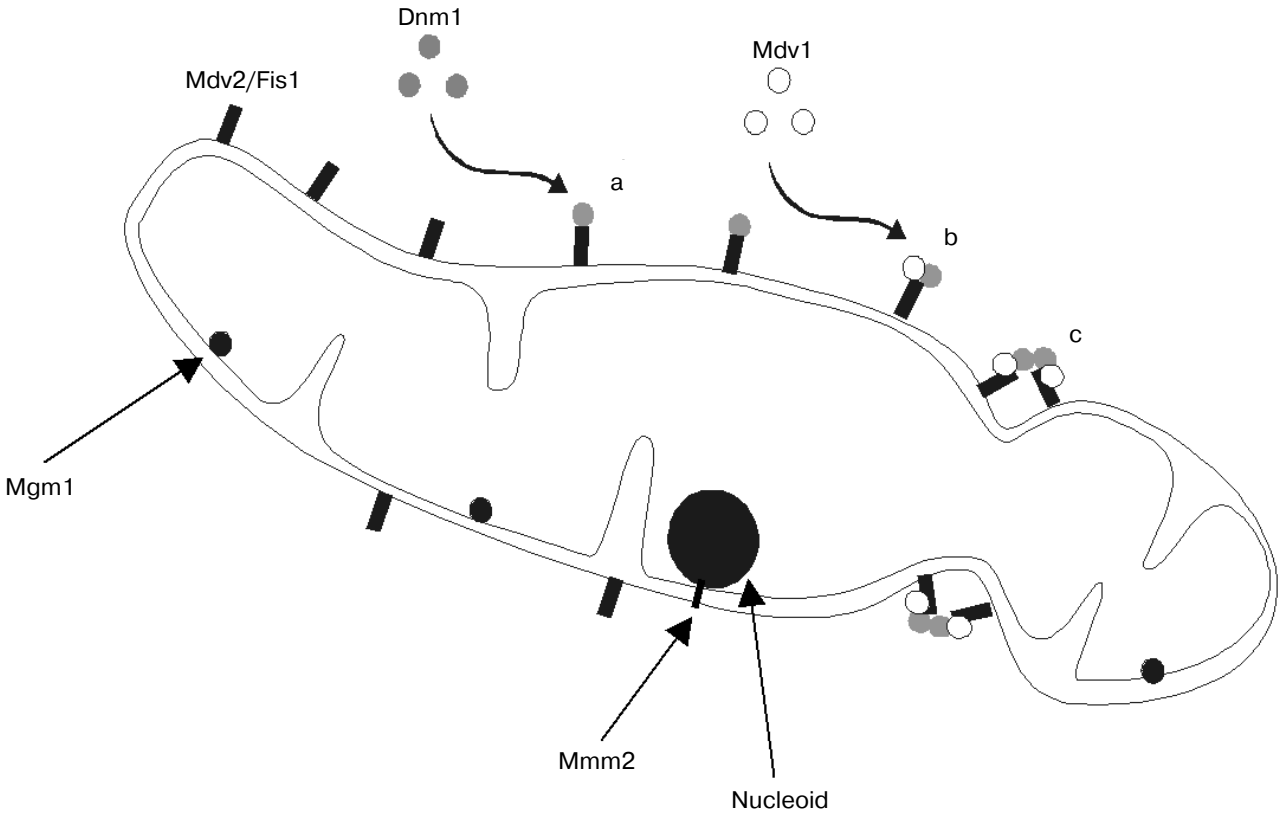

Fig. 3. A hypothetical scheme of successive stages in the fragmentation of the external mitochondrial membranes with involvement of dynamins. In the first stage (a) the integral protein Mdv2 (Fis1) of the external mitochondrial membrane recruits Dnm1; in the second stage (b) the protein Mdv1 is joined to the complex Mdv2(Fis1)/Dnm1. In the third stage (c) a "definitive" apparatus of the fragmentation is produced, possibly with the inclusion of some unknown additional protein factors. Modified after [37].

lem has a long history. It was shown even early by electron microscopy that various stress factors, such as thermal shock or injections of riboflavin or herbimycin, caused progressing invagination of the internal mitochondrial membrane resulting in division of the matrix to isolated compartments [15, 51, 52]. Based on these works, the hypothesis on the autonomous invagination of the internal membrane as the initial stage of mitochondrial fission became widely believed [51].

A gene called *MGM1* (mitochondrial genome maintenance) has relatively recently been found in yeast, and mutation of this gene results in changes in the morphology of mitochondria and in the loss of mitochondrial DNA by the cells [53, 54]. This gene encodes a dynamin-like protein Mgm1 which has a signal sequence responsible for penetration into the intermembrane compartment of mitochondria [55]. Mutational analysis shows that Mgm1 is functionally coordinated with the Dnm1 protein of the external mitochondrial membrane: a deletion in the *MGM1* gene stimulates the Dnm1-dependent fragmentation of mitochondrial membranes.

A striking phenomenon has been found in muscle cells of *C. elegans* which express the mutant Drp1. In this case, the external mitochondrial membrane remains intact, whereas the internal membrane is fragmented and divides the matrix of individual mitochondria to structurally isolated compartments [46]. Consequently, Drp1 is involved in the cutting only of the external mitochondrial membrane. In total, the above-presented observations suggest that the fragmentation of mitochondria should be controlled by various molecular machines specific for the external and internal membranes.

FISSION OF MITOCHONDRIA

The fragmentation of mitochondria described in tissue culture and yeast cells is characterized by two fea-

tures: the high rate of the process and unregulated distribution of the genetic matter. However, quite another type of fragmentation of mitochondria is described in the literature, which is always associated with certain stages of ontogenesis, is large-scale, takes significantly more time, and is necessarily accompanied by division and segregation of mitochondrial genomes.

Just such a fragmentation has been found in the growing plasmodium of *Physarum polycephalum* [56, 57]. Mitochondria of this fungus are nearly ball-shaped, and their DNA is clearly visible as compact structures (nucleoids). At certain stages of the mycelium development, the mitochondria synchronously enter the division. At first the nucleoids extend with determination of the future division axis of the organelles; the local invagination of both mitochondrial membranes starts concurrently strictly above the middle of the nucleoid. As a result, the mitochondria become dumbbell-shaped. In the final stages of the division, the membranes close and the daughter mitochondria become separated. As a result, each daughter mitochondrion gets its own portion of DNA. The whole fragmentation from the beginning of the membrane invagination to the separation of daughter mitochondria takes about 1.5 h.

Mitochondria of the sea urchin *Paracentrotus lividus* fragmented similarly during early stages of embryogenesis [58]. Mature oocytes of this echinoderm contain about 300,000 small ball- and rod-shaped mitochondria. Fifteen minutes after fertilization "dumbbell-shaped" mitochondria appear in the cell (Fig. 4) and the fraction of ball-shaped mitochondria starts to increase. At the stage of two blastomeres (about 1.5 h after fertilization), the number of ball-shaped mitochondria is sharply increased, and the total number of mitochondria is increased twofold up to 600,000. During all subsequent stages of the cleavage up to the late gastrula the number of mitochondria of the embryo does not change (table).

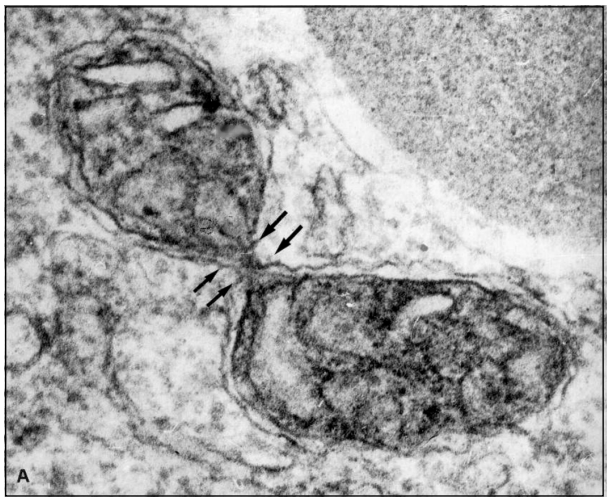

Fig. 4. Electron microscope photograph of a dividing mitochondrion in the fertilized oocyte of the sea urchin *Paracentrotus lividus*. The mitochondrion has a specific dumbbell-like shape; such mitochondria never occur in unfertilized oocytes. Photo by M. Yu. Soukhomlinova.

Time course of proliferation of the chondriom during the cleavage of oocytes of the sea urchin *Paracentrotus lividus* normally and after treatment with cytochalasin D and colcemid

Developmental stage	Number of mitochondria ($\times 10^3$)			% dumbbell-shaped mitochondria		
	control	cytochalasin D	colcemid	control	cytochalasin D	colcemid
Oocyte	340 ± 40			0	0	
15 min	420 ± 42	290 ± 40		5.3	0	
50 min	550 ± 50	335 ± 45		4.1	0	
100 min	590 ± 60	305 ± 40			(4)*	
		(607 ± 60)*	576 ± 50	2.5	0	2.5
Four blastomeres	600 ± 60			1.8		
Blastula	560 ± 55	cleavage is inhibited		0	cleavage is inhibited	
Gastrula	530 ± 55			0		

Note: Inhibitors of the cytoskeleton were added to suspension of unfertilized eggs 5 min before the fertilization. In some experiments cytochalasin or colcemid was added 15 min after the fertilization (*).

It is fundamentally important that during early stages of the cleavage, sea urchin mitochondrial DNA is not synthesized at all [59]. This suggests that the initial mitochondria in the unfertilized oocytes had several nucleoids or that their nucleoids were "polyploid", i.e., contained at least two identical genomes before the cleavage. This suggestion is indirectly supported by the finding of nucleoids with anomalously high content of DNA in "resting" mitochondria in tissue culture cells [32] and in small dividing rod-shaped mitochondria of the fungus *Physarum polycephalum* [31].

The fragmentation of mitochondria by the local invagination of membranes has been convincingly shown on unicellular red algae *Cyanidium caldarium* and *Cyanidioschyson merolae* [60]. Vegetative cells of these protists have a single mitochondrion which starts fragmentation not long before cell division. In the region of the membrane invagination appearance a ring structure is generated which embraces the whole section of the mitochondrion.

Complicated changes in the chondriom accompanied by fusion and fragmentation of mitochondria are found in ameboid cells of *Physarum polycephalum* during their conjugation [61]. In the zygote which was produced immediately after the fusion of the cells and nuclei, rod-shaped mitochondria fused together and their nucleoids produced a ring with the diameter comparable to the diameter of the nucleus. Then, before the first post-conjugational division, the gigantic ring-shaped mitochondrion was fragmented again, and every new mitochondrion acquired its own nucleoid. Cycles of the fusion and fragmentation of mitochondria took approximately the same time (about 2 h).

A similar process can be observed during the conjugation of some yeasts. The fusion and fragmentation of mitochondria during meiosis and sporulation of yeast cells finally result in formation of four mitochondria which surround the nuclei of the spores produced [62].

In total, the above-described scheme of fragmentation of mitochondria is remotely reminiscent of the mitotic division of the cell. A key role in the division of eucaryotic cells is played by two special cytoskeletal structures, the mitotic spindle, which consists of microtubules, and the contractible ring of actin and myosin filaments. If a dividing cell is treated with specific substances inhibiting the polymerization of tubulin or actin, the chromosome disjunction and cytokinesis are suppressed completely. Unfortunately, there are very few studies on the effect of cytoskeleton inhibitors on the division of mitochondria. However, even these separate works suggest that agents depolarizing microtubules have no influence on the division of mitochondria [58, 60].

Findings obtained with inhibitors of polymerization of actin microfilaments are more interesting. Depolymerization of actin in yeast cells in the presence of a specific inhibitor latruncullin A resulted in a large-scale fragmentation of the mitochondria [63]. It is fundamentally important that this type of the fragmentation depended on the presence of the protein Dnm1: in mutant Dmn1-deficient clones, the effect of latruncullin A was prevented and mitochondria did not fragment.

Another inhibitor of polymerization of actin, cytochalasin, completely prevented the division of mitochondria in mycelium of the fungus *Physarum polycephalum* and in cells of the alga *Cyanidium caldarium* [60]. Based on these data, the authors proposed a hypothesis that polymeric actin should produce contractile ring structures on the external membrane and in the intermembrane space of the dividing mitochondria, but by immunocytochemistry no actin was detected in the invagination region of the mitochondrial membrane of the alga *Cyanidium caldarium* where the presence of contractile rings seemed most likely [64, 65].

It is very interesting that the effect of cytochalasin strongly depended on what stage of the mitochondrial division the polymerization of microfilaments was inhibited. In sea urchin eggs, the large-scale synchronous division of mitochondria was induced 15 min after the fertilization and occurred for about 1.5 h. The addition of cytochalasin before the fertilization completely inhibited the division of mitochondria. The addition of cytochalasin 15 min after the fertilization, when the mitochondria entered the phase of active division, resulted in "uncoupling" of the coordinated invagination of the internal and external membranes in some mitochondria: the external membrane remained intact, whereas the internal membrane formed a septum which separated the mitochondrial matrix into two isolated compartments [58] (Fig. 5; table).

Thus, in eucaryotic cells two types of fragmentation of mitochondria coexist that result in quite different consequences for the cell. The first type specifies a stationary state of the chondriom and is accompanied by the fusion of mitochondria. And the fragmentation and fusion occur, as a rule, concurrently and in a balanced ratio, although in some cases the balance can be shifted to one or side or the other. The second type occurs on certain stages of ontogenesis and results in a dramatic increase in the number of mitochondria accompanied by segregation of nucleoids. Based on these features, this type of the fragmentation is suggested to be a special life phase of the chondriom and is called "equational" division by analogy with the mitotic division of the cell.

Based on analysis of the division of mitochondria during the early stages of the sea urchin development, some ideas are suggested concerning the possible mechanism of the "equational" division of mitochondria.

Obviously, the division of mitochondria requires the presence of specific factors which induce the fragmentation of mitochondrial membranes ("mitokinesis") and provide for the segregation of nucleoids ("nucleokinesis"). As stated above, mitochondrial membranes are

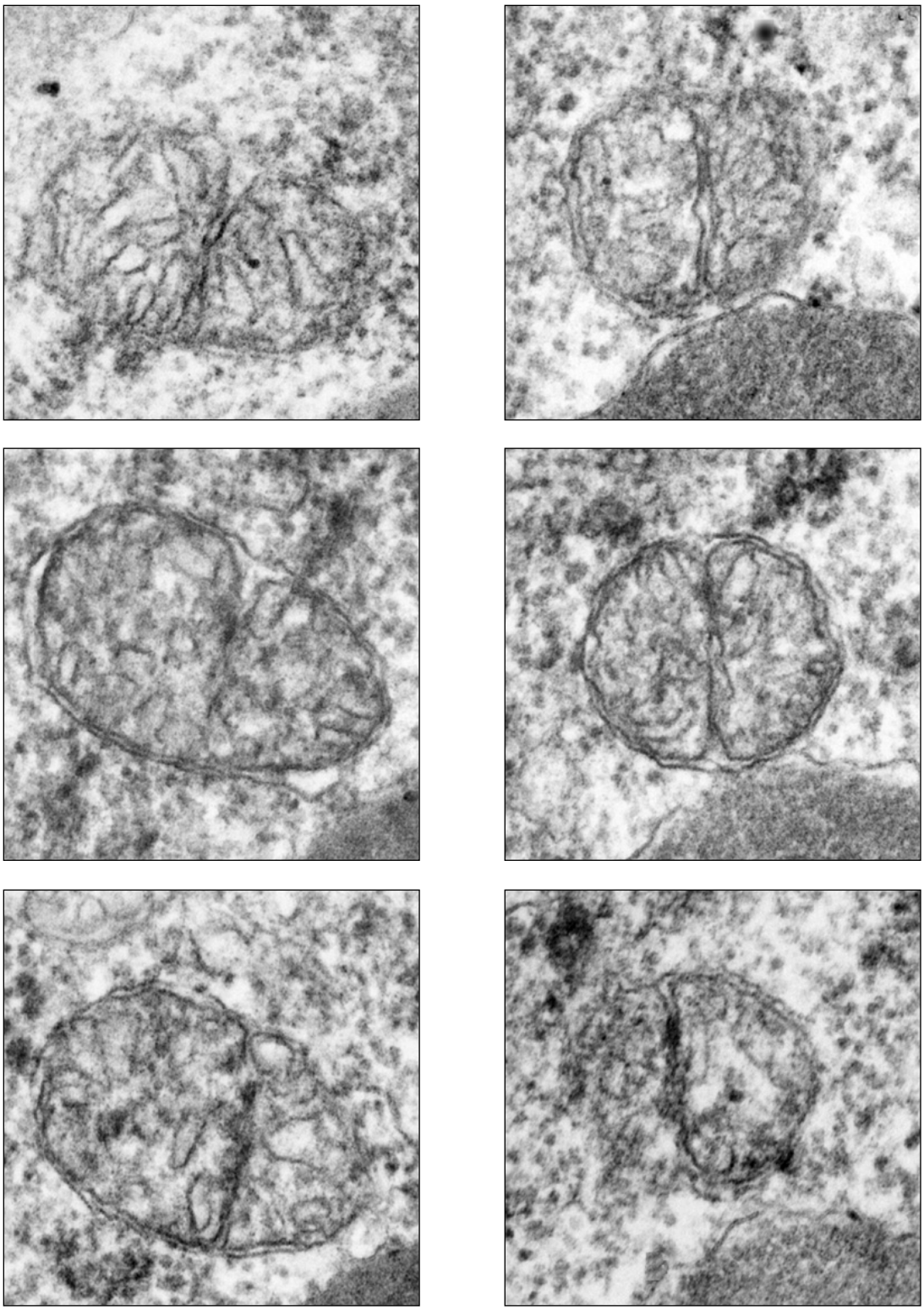

Fig. 5. Electron microscope photographs of successive serial sections of a mitochondrion in the fertilized oocyte of the sea urchin *Paracentrotus lividus* treated with cytochalasin, an inhibitor of actin polymerization. Cytochalasin was added 15 min after the fertilization, when mitochondria started the division. The "septum" is well visible, which was produced from the internal membrane and separated the mitochondrion into two isolated compartments. Photo by M. Yu. Soukhomlinova.

fragmented by dynamins, which in sea urchin eggs are accumulated in cortical granules and enter the cytoplasm immediately after fertilization [66]. These proteins can produce a pool of dynamins which are not integral components of the mitochondrial membranes but are associated with the mitochondria only during their division. It is supposed that in unfertilized eggs of sea urchin (as in cells of other organisms) mitochondria should contain only such dynamins that have sequences addressed for penetration into the intermembrane space and proteins which control the invagination of the external membranes should be transported to the mitochondria along actin microfilaments. The structural and functional relation of dynamins with the actin cytoskeleton has been shown experimentally: inhibition of the actin polymerization causes significant disorders in formation of endocytotic vesicles [35, 67, 68]. These data make clear why cytochalasin prevents the normal division of mitochondria or causes the "uncoupling" of invagination of the external and internal membranes. It seems that in this case the transport of dynamins to the external mitochondrial membranes is inhibited and dynamins which control the fragmentation of the internal membranes continue their functioning. Thus, the formation of the internal septum separating the mitochondrial matrix into isolated compartments is more likely not the initial stage of the division but a pathology. The appearance in the population of mitochondria of organelles with septa suggests that the chondriom has received a signal for fragmentation or division but is unable to adequately realize it because of lack (or inhibition) of the due factors on the external surface of the mitochondria. It is conceivable that the existence of mitochondria "competent" or "incompetent" for fragmentation is determined by the cell differentiation. This idea is in agreement with data that the induction of apoptosis in tissue culture cells results in a complete fragmentation of the chondriom [27, 28], whereas cardiomyocytes respond to apoptotic stimuli by generation of mitochondria which consist of two structurally different mitoplasts surrounded by the same external membrane [69].

Polymeric actin can also provide for the segregation of nucleoids. The presence of immobilized actin-binding proteins on the surface of mitochondria has been shown in some works [70-72]. A protein (Mmm1) has recently been found in *S. cerevisiae* cells that seems to be responsible for the structural association of the external and internal mitochondrial membranes and for the concurrent fixation of the nucleoid location in the mitochondrial matrix [73]. *MMM1* mutation results in disorders in the normal segregation of nucleoids and dramatic changes in the structure of the internal mitochondrial membrane. It was suggested that Mmm1 should be able to directly or indirectly bind to actin and function similarly to kinetochores of mitotic chromosomes. The protein Mdv1 can function similarly [74].

However, the polymerization of actin is necessary but insufficient for the division of mitochondria. Experiments with the artificial activation of *Paracentrotus lividus* eggs with the calcium ionophore A23187 show that the entrance of Ca^{2+} into the oocyte results in formation of the fertilization envelope and full-value actin cytoskeleton, but mitochondria fail to enter the division [58]. This observation indirectly suggests the necessity of a specific signal to induce the division of mitochondria, but the character of this signal remains unknown.

Thus, the above-presented examples show that the fusion, fragmentation, and fission of mitochondria are specific features of the chondrioms in the overwhelming majority of eucaryotic cells. What is the biological significance of these processes?

The final result of the mitochondrial fusion is formation of functional complexes which combine dozens and sometimes hundreds of mitochondria. It seems that regulation of such megacomplexes especially in large cells of protists should have certain advantages. Thus, mitochondria are shown to play the role of "cables" transmitting energy over various intracellular compartments [75]. Some types of cells which during evolution decided that to "draw cables" is more effective than to transport energetic setups successfully realize this mechanism [76-78]. And, finally, one more possible significance of the fusion is the recombination of mitochondrial DNA. Well-studied fusion and fission of mitochondria in the *Physarum polycephalum* zygote are interpreted as analogs of the mitochondrial sexual process accompanied by the reducing division of nucleoids [61].

The fragmentation increases the number of mitochondria and decreases their size. The functional significance of the fragmentation and fusion of mitochondria in the interphase cells of tissue culture or yeast remains unknown. In any case, this is not the way for the cell to provide for the "homogenization" of the chondriom that is required for the correct segregation of mitochondria during mitosis because the rates of fusion and fragmentation acts are balanced. Moreover, because of a significant fraction of "genome-free" fragments, this type of the fragmentation cannot be considered as the final stage in the exchange of the genetic material between individual mitochondria. An original hypothesis on the role of fragmentation of mitochondria in the cell metabolism was proposed in the works of V. P. Skulachev [75] and V. P. Skulachev et al. [28]. Based on numerous experimental data of these authors, it was suggested that the change in the chondriom (or of its part) from the mainly fibrillar structure to the granular one should be used by the cell as a mechanism for changing elongated reticular mitochondria to transportable shape or for isolation of damaged fragments of the chondriom from intact mitochondria.

This hypothesis allows us to understand the significance of the large-scale fragmentation of the chondri-

om timed to the start of mitosis: in this way the cell ensures the correct inheritance of mitochondria in the set of generations. Except for some exotic cases, there are no data that the segregation of mitochondria during mitosis is ordered similarly, for example, to the distribution of mitotic chromosomes. Like other membrane organelles of cells, mitochondria during mitosis are redistributed to the daughter cells at random (stochastically). Such segregation requires the obligatory presence of genomes in every mitochondrion, especially if their number in the cell is relatively low. The equational division is the only way to ensure the repeatedly dividing cells with genetically full-value mitochondria. The association of the large-scale fragmentation of mitochondria with mitosis suggests that the division of cells and mitochondria should be regulated by common signal mechanisms.

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ADDENDUM

After this paper had been prepared for press, works were published which seem to be a significant addition to the data presented in the review.

A methodical screening of mutants of *Saccharomyces cerevisiae* yeast with aberrant morphology of mitochondria was performed in work [1]. The authors showed that the general morphology of mitochondria is controlled by 15 genes which encode proteins with different functions, including the proteins of the respiratory complex.

Factors involved in the fragmentation and fusion of mitochondrial membranes in higher eucaryotes in normal and different pathological situations were analyzed in detail in review [2]. The authors present data that some inherited diseases associated with mutations in mitochondrial DNA directly or indirectly (via defects in the synthesis of mitochondrial proteins) affect the morphology of mitochondria, their topology in the cell, and the proliferation of the chondriom. Mutations of nuclear genes encoding the mitochondrial proteins are displayed similarly. Note that some of these proteins are extremely important for normal functions of the body. Thus, mutation of the nuclear gene *OPA1*, which encodes a dynamin-like protein Mgm1 and is accompanied by intense fragmentation of mitochondria, causes a severe pathology of vision.

The morphology of mitochondria is known to change with changes in the functional state of the cell, including early stages of apoptosis [28]. In work [3] the topology was analyzed of a proapoptotic agent Bax and proteins Drp1 and mitofusin Mfn2 in mitochondria of cells treated with staurosporin (STS), a specific inducer of apoptosis. As stated above, the function of Drp1, which is a homolog of the yeast protein Dnm1, is associated with its involvement in the fragmentation of mitochondria. Mitofusin (Mfn), a homolog of the yeast protein Fzo, has recently been identified and characterized in detail in human and mouse cells [4]. It has a transmembrane domain interacting with the external mitochondrial membrane, and its C- and N-terminal domains are exposed in the cytoplasm. The superexpression of Mfn results in clustering of mitochondria in the perinuclear region of the cell, changes in their morphology, and a close association of the external mitochondrial membranes. The latter data are especially interesting in connection with earlier studies with electron microscopy

which described special structures, intermitochondrial contacts [13], and also in connection with the hypothesis on the possible role of these structures in the energy metabolism of the cell [68].

By immunofluorescence the proapoptotic factor Bax was shown to be located on the ends of tubular mitochondria or on their lateral surfaces [3]. A similar distribution of Bax is retained within the first minutes after the induction of apoptosis independently of the absence or presence of a specific inhibitor of caspases, zVAD. Therefore, the authors conclude that formation of Bax clusters should belong to early events in apoptosis preceding the activation of caspases. After 60-129 min from addition of STS to cell cultures when the fragmentation of mitochondria is induced, Bax clusters are found in the strangulation region of the dividing organelles. An inhibitor of apoptosis Bcl-X_L, which prevents the fragmentation of mitochondria, inhibits the STS-dependent distribution of Bax. The spatial distribution of mitofusin and the Drp1 protein suggests that in apoptotic cells the label corresponding to these proteins is co-located with Bax clusters. Overall, the results of this work show that Bax together with dynamin-like proteins Drp1 and mitofusin is involved in the apparatus of segregation of mitochondria induced by apoptosis.

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