Apoptosis
DOI 10.1007/s10495-016-1295-5

REVIEW

Mitochondrial dynamics during cell cycling

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Abstract Mitochondria are the cell's power plant that must be in a proper functional state in order to produce the energy necessary for basic cellular functions, such as proliferation. Mitochondria are 'dynamic' in that they are constantly undergoing fission and fusion to remain in a functional state throughout the cell cycle, as well as during other vital processes such as energy supply, cellular respiration and programmed cell death. The mitochondrial fission/fusion machinery is involved in generating young mitochondria, while eliminating old, damaged and non-repairable ones. As a result, the organelles change in shape, size and number throughout the cell cycle. Such precise and accurate balance is maintained by the cytoskeletal transporting system via microtubules, which deliver the mitochondrion from one location to another. During the gap phases G_1 and G_2, mitochondria form an interconnected network, whereas in mitosis and S-phase fragmentation of the mitochondrial network will take place. However, such balance is lost during neoplastic transformation and autoimmune disorders. Several proteins, such as Drp1, Fis1, Kif-family proteins, Opa1, Bax and mitofusins change in activity and might link the mitochondrial fission/fusion events with processes such as alteration of mitochondrial membrane potential, apoptosis, necrosis, cell cycle arrest, and malignant growth. All this indicates how vital proper functioning of mitochondria is in maintaining cell integrity and preventing carcinogenesis.

Keywords Mitochondrial fission and fusion · Cell cycle arrest · Drp1 · Mitochondria · Apoptosis · Mitophagy

Relationship of cytoskeletal proteins and mitochondria

Mitochondria are dynamic organelles that constantly change their arrangement and shape in correlation with the need of the cell [1]. The fusion/fission ratio and localization of the mitochondrial network changes depending on the stage the cell has entered. Positioning of the cytoskeletal proteins is vital in cell division as well as for distribution of mitochondria. Mitochondria are constantly in contact with other organelles, in particular endoplasmic reticulum (EPR) and plasma membrane, which helps the cell to maintain balance of the mitochondria/cell mass index [2, 3]. Mitochondria constantly migrate and their movements can be saltatory, back and forth, and strongly depend on the cellular "rails" consisting of the protein, actin, and microtubules. Migrations on the cytoskeletal rails cause mitochondrial osculation and fusion, which is necessary for mitochondrial movement [4]. An inactivation of main fission-driving proteins can lead to an increase in mitochondrial connectivity and highly depends on being transported by the microtubules. Thus, such movement does not always contribute to mitochondrial fusion, even though it can be followed by a change in shape and form of the organelle [10, 11]. In yeast, mitochondrial transport mostly depends on actin, whereas in mammalian cells it depends on microtubular transport via kinesins and dyneins [12, 13]. A blockage of fission in yeasts

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Published online: 23 September 2016

is not always lethal, since mitochondria can still divide during cytokinesis, meiosis and sporulation in diploid yeasts [14], whereas in mammalian cells it is strongly linked with cell death [10]. Neurons are one of the most energy requiring cells and, so far, are the only known eukaryotic cells to transport mitochondria via actin transition, but not microtubules [11]. Due to interaction with cortical cables budding yeast cells have a unique localization of mitochondria. A junction-protein called Num1 connects mitochondria to both plasma membrane and EPR [3]. Num1 is vital for normal division of mitochondria and supports Dnm1 (a Drp-1 homologue) in mitochondrial fission in yeast [12]. Num1 is a cortical anchor protein involved in recruiting Dnm1 to the mitochondrial surface so that it localizes for a further formation of a ring consisting of Dnm1 proteins, however its analogues yet not known for human cells [13].

As a summary, cytoskeletal proteins serve as a railway for building the mitochondrial network (actin in some simpler single cell organisms, and β-tubulin in mammalian cells). The network is in constant interaction with other organelles, such as the plasma membrane and the EPR to ensure the cell is supported with the energy required for proper functioning.

Mitophagy, apoptosis and Kif-proteins

Mitophagy is a form of autophagy that is initiated in order to selectively eliminate defective mitochondria, whereas apoptosis is a form of selective self-elimination of potentially harmful or aged cells. Mitochondria are not just cell's power plant, but they can also trigger death-promoting signaling cascades. Thus, mitophagy and apoptosis are two interlinked processes, whose connection is perfectly described elsewhere [14, 15]. A change in the intracellular environment, including interaction of mitochondria with EPR, may force mitochondria to release pro-death factors which will interrupt the ATP supply for the cell and so lead to programmed cell death [16].

In neurons, the cells most critical to ATP supply, the distribution of mitochondria involves dynein, Kif1B (molecular motor kinesine-1) and Kif5B (molecular motor kinesine-5) proteins [25, 26]. Kif1B is a motor for anterograde transport of mitochondria [19]. It interacts in a calcium-dependent manner with CHP1 (Calcineurin B homologous protein 1), a multifunctional protein essential for EPR-mitochondria interaction [20], and with KPB (KIF1-binding protein), a protein required in mitochondrial transport and in axonal microtubule interacting with the cytoskeleton. However, the role of KPB in mitochondrial transport is still to be confirmed [30]. Kif5B is a microtubule-dependent motor required for normal distribution of mitochondria and lysosomes [31]. Mitochondria and Kif5B are linked through a protein called syntabulin, a part of a kinesin motor-adapter complex that is critical for the anterograde axonal transport. However, syntabulin isoforms 3, 4 and 5 are expressed in the HeLa cell line, but not the isoform 2, part of a kinesin motor-adapter complex critical in presynaptic assembling at early development of neurons [33, 34]. KIF5 also binds with Miro proteins in a Ca^{2+}-dependent manner [35, 36]. LC3 (Microtubule-associated proteins 1A/1B light chain 3A) interacts with FUNDC1 (FUN14 domain-containing protein 1), an integral mitochondrial outer-membrane protein, and is likely involved in mitochondrial transport. A knockdown of FUNDC1 significantly prevents hypoxia-induced mitophagy [37, 38], a form of autophagy in which damaged mitochondria were selectively eliminated to prevent further passing of genetic material.

Yeast is probably the most studied model when it comes to mitochondrial fission/fusion balance, with most molecular partners described being yeast proteins. A deletion of Uth1 (Youth protein 1), a mitochondrial biogenesis regulator localized on the mitochondrial outer membrane, inhibits mitochondrial fission and is involved in mitophagy [31]. However, mitophagy is associated with failure in mitochondrial functioning, rather than in its fragmentation. The balance of fission and fusion of mitochondria during apoptosis is critical, as a failure in one affects the other process, and mitophagy is frequently part of programmed cell death. Muscle cells form highly interconnected mitochondrial tubules, which transport energy across the cell, whereas the majority of mitochondria in pancreatic β-cells exist as "independent" [32].

Regulation of mitochondrial dynamics in cell cycling

Mitochondrial transportation and motility are important features of mitochondrial dynamics that help the mitochondrial network to supply the cell with energy. Mitochondria play a crucial role in cell cycle regulation and a balance of fission and fusion regulates down- or upstreaming of the cell cycle. Total mitochondrial number is increased during the growth phase compared to the stationary phase [33]. Thus, the total number of mitochondria throughout the cell cycle and the overall volume occupied by the mitochondria is mostly constant during the whole cell cycle [34]. Some proteins regulate both fission/fusion machinery and transport of mitochondria via microtubule activity. As an example, overexpression of tau, a microtubule-associated protein, causes aberrant distribution of mitochondria via the inhibition of kinesin-dependent movement of the organelles and causes impairment in trafficking and localization of organelles. This helps to regulate both increases and decreases in mitochondrial activity at certain stages of the cell cycle [45, 46]. Drp1 was shown to interact with amyloid beta protein and phosphorylated protein tau, both involved in Alzheimer pathology. It is suggested that Drp1 may result

in excessive mitochondrial fragmentation and deficiencies, it also decreases mitochondrial motility and shortens the length of the mitochondrion [47, 48]. Another study suggested that tau is responsible for a mislocalization of Drp1, and so an disproportionate separation of the organelle [39].

Mitosis

Mitosis is considered as the most active stage of the cell cycle to have an unorganized mitochondrial network. It was initially reported that a fusion of smaller mitochondria takes place just before cell division in order for the cell to be prepared to separate the total mitochondria pool equally between the two daughter cells [40]. In mitosis, positioning and redistribution of mitochondria between two newly formed cells is vital in order to proceed to cytokinesis [41, 51]. However, most of the fusion processes of mitochondria occur in the late telophase, and are controlled by mitofusins [43, 44]. A chance exists of lacking of up to 40% of the mitochondrial DNA (mtDNA) due to an inappropriate segregation of the mtDNA to daughter cells during mitochondrial fission or fusion. However, the loss is lower if the mitochondria are interconnected [52, 53]. After the cell entered interphase, mitochondria are observed as a tubular network and get clustered around the nucleus and on the cell periphery; however, the mitochondrial network gets disorganized in metaphase. During much of mitosis, mitochondria stay separated (punctuated) [46]. This could result from the Num1/Dnm1 complex helping the mother cell to retain the mitochondria for itself, or in the case of an absence of the complex, one newly formed cell keeps the entire mitochondrial mass for itself. However, it also has been shown that up to 20% of this mitochondrial mass is in a highly fused state [13]. During this stage, the whole process gets stochastic and it is hard to predict the outcome.

Interphase

Most changes of the mitochondrial network occur throughout the G_1-G_2 interphase period. It is known that the cell cycle can be arrested by different substance interference during the OXPHOS, ATP production, and cellular respiration as well as by starvation at certain stages of the cell cycle. The energy requirement differs significantly throughout the interphase because of the changes in the morphology of the mitochondrial network. This slows down mitochondrial activity, and favors networking of mitochondria and saving energy [47–49].

G_1/S transition

In the G_1/S transition period, mitochondrial shape and function can be affected [50]. Cyclin E, active throughout G_1- and S-phases, plays a significant role in regulating fusion/fission through the entire cell cycle. An overexpression of cyclin E relieves G_1/S cell cycle arrest [51], which correlates with formation of a single dynamic giant tubular network that constantly undergoes mitochondrial fission and fusion. Mitraa et al. [52] showed that hyperfusion of mitochondria into large networks is linked with increased cyclin E levels in the G_1/S transition, which is associated with the entrance into S-phase. Most likely this shift is caused by the need to produce more ATP for the cell to have the pool of energy required to re-enter the cell cycle. A shift from fragmented mitochondrial network in G_1 to tubular one can be seen in G_1/S. The oxidative capacity of mitochondria is greater at late G_1 than in early G_1, and the average oxygen consumption of the cell increases from early G_1 to late G_1 stage [53]. However, this state reverts in S and G_2/M to fragmented mitochondria [52]. The G_1/S transition is considered to be a stage of mitochondrial reorganization that represents a point of no return for the cell to re-enter the cell cycle [54]. It is hypothesized that hyperfused mitochondria force G_0 cells to enter/re-enter the cell cycle. Furthermore, cells in such states appear after they get relieved from serum starvation in G_0. Cells expressing Drp1 or held at G_1/S also exhibit a rapid membrane depolarization [52]. This transition might contribute to cell cycle re-entrance and the formation of giant mitochondrial networks.

In the G_0 stage of the cell cycle, both hyperfused (dominating) and fragmented mitochondria are present [52]. During G_1 stage, mitochondria are highly fused, whereas in G_2, as the cell heads towards mitosis, an opposite effect is observed. It can be assumed that a shift from mitochondrial fusion towards fission takes place in the G_1-G_2 period [57, 61]. It is also shown that starvation reduces the number of mitochondria by forcing cell cycle arrest, self-elimination, and mitochondrial fusion [56]. Other studies indicate that starvation causes cell cycle arrest and affects mitochondrial fission response to PKA-dependent Drp1 phosphorylation of Drp1 Ser637 [24, 58]. Drp1 is inactivated by phosphorylation at Ser637 within the GTPase effector domain and activated by phosphorylation of the Ser618. The activation is made by the Cdk1/cyclin B complex and results in mitochondrial fragmentation [55]. This leads to elongation of mitochondria and an increase in density of cristae, which affects the efficiency of ATP production [22, 24]. Such a reduction in ATP production arrests cells in the G_1/S transition stage in Drosophila flies [51]. However, starvation can also protect mitochondria from autophagosomal degradation [22, 24]. During the G_1/S gap mitochondria form a powerful network with and increased ATP output greater than at any other stage of the cell cycle [52]. This might be proof that Drp1 is linked to cell cycle regulation, and switching from cell cycle arrest to progression, and *vice versa*, is done *via* regulating mitochondrial activity. Mitochondrial fusion that

is associated with absence of growth factor and cell cycle arrest is followed by Drp1 inhibition [58]. During G_1/S transition, mitochondria form a giant tubular mitochondrial network, whereas in S and G_2/M the network becomes fragmented [63]. It was discovered that mitochondrial deficiency blocks cells in G_1/S transition. This intermission of the cell cycle strongly depends on cyclin E levels. A drop in ATP production forces the cell to arrest and keeps ATP levels enough for survival. A loss of cytochrome c oxidase increases AMP level, activates AMPK and p53, and triggers cyclin E degradation which results in G_1/S cell cycle arrest [7]. Cyclin E overexpression relieves the block, so the cell can enter S-phase [58, 60], which also contributes to initiation of DNA replication in S-phase [51]. Interestingly, that cyclin E activity correlates with mitochondrial fusion, as well as increase in Drp1 levels and formation of giant tubular mitochondrial networking [58].

The cell cycle regulator Cdk5 (cyclin-dependent kinase 5) is required for mitochondrial movement [61]. Since Cdk5 is involved in T-cell activation, a dysregulation of Cdk5 can lead to Alzheimer disease and multiple sclerosis [62]. Cdk5 is highly active in postmitotic neurons and in many cancers, allowing tumors like medulloblastoma to evade immune elimination; interferon-γ–induced PD-L1 up-regulation on medulloblastoma requires Cdk5, and disruption of Cdk5 expression results in potent CD4+ T cell–mediated mouse tumor rejection [63]. Cdk5 is also required during autophagy and a Cdk5-mediated phosphorylation of endophilin B1 takes place in Parkinson's disease [64]. Since Cdk5 reduces caldesmon activity, an actin regulatory protein, it has been implicated in invasive cancer appearance [65]. Cdk5 was shown to keep an actin filament reorganization regulator WAVE1 (Wiskott-Aldrich syndrome protein family member 1) phosphorylated, so that it stays inactive [66]. Activation of N-methyl-D-aspartic acid (NMDA) receptors is linked with downregulation of p35, a subunit of Cdk5. Cdk5 also recruits mitochondria to dendritic spines [72]. Expression of prohibitin, a protein interacting with Stoml2 and a possible chaperone-like protein for respiration chain proteins and mitochondrial morphology and function regulator, increases threefold upon entry into G_1 phase compared to other phases of the cell cycle and inhibits DNA synthesis [73].

G_2/M transition

Unlike during G_1/S transition, mitochondria in G_2/M transition form a highly fragmented network [63]. During cell cycle arrest in G_2/M phase after vinblastine treatment, a G_2/M-specific anticancer agent, mitochondria of murine lymphoma cells have been shown to remain functional, even the significantly enlarged ones. Rhodamine-123 test has shown a correlation between cell cycle arrest, increased mitochondrial activity and cellular respiration. However, no clear pattern of an organized mitochondrial network was observed [47]. A loss of Drp1 induces mitochondrial hyperfusion and causes ATM-dependent G_2/M arrest and aneuploidy, which are hallmarks of the giant cell formation [71]. Depletion of a Drp1 binding protein Fis1 is involved in cell cycle arrest at the G_2/M checkpoint, meaning that Drp1 failed to attach to the mitochondrion and didn't form a septum on the mitochondrion. Furthermore, a reconstitution of the Cdk1/cyclin B complex failed to restore mitotic entry in hFis1-depleted cells [72]. Another study has shown that in the G_2/M checkpoint intermission after the mitochondrial network becomes hyperfused, cell undergoes caspase-dependent cell death [73]. The giant cancer cell formation and significantly increased mitochondrial activity might be a result of drug treatment, or exposure to radiation [55, 78].

In overall, the organized in G_2-phase mitochondrial network becomes more fragmented as it nears the G_2/M checkpoint and will stay in this state until end of mitosis. An organized interconnected mitochondrial network is observed in both Gap phases and G_1/S checkpoint, whereas in the S-phase, G_2/M checkpoint and mitosis it becomes more fragmented. The main processes in mitochondrial dynamics are briefly summarized in Fig. 1.

Drp1 and cell cycle regulation

Since the discovery of Drp1, a mitochondrial fission protein, our understanding of the role of mitochondria in mitochondrial dynamics during cell cycling have changes drastically [76]. By forming a complex with Fis1, Drp1 separates the mitochondrion into two new mitochondria [77, 78]. The discovery of the Drp1/Fis1 complex was a huge breakthrough in understanding how mitochondrial fission/fusion works and how it is linked with the cell cycle and cytoskeleton. Drp1 is essential in Fis1 activation, which forms a complex of great importance in cell death activation and mitochondrial fission. Vice versa, where levels of mitofusins (Mfn1 and Mfn2) decrease, both proteins are linked with apoptosis via a mitochondrial protein Bax [79]. These examples show a connection between apoptosis and mitophagy, and a link to microtubular involvement in mitochondrial distribution, biogenesis and mitophagy, since the cytoskeleton is tightly connected with the mitochondria, as it transports, supports, and distributes it in accordance with the needs of the cell [80].

Early mitosis was shown to be vital in determining mitochondrial morphology and networking. F-actin can block the translocation of Drp1 and so mitochondrial fission [81]. Throughout this period, Drp1 is linked with mitochondrial networking and cell proliferation. Under oxidative stress conditions phosphorylation of Ser579 in human Drp1 isoform 3 is mediated by protein kinase Cδ [82]. In early mitosis Cdk1/cyclin B complex, that is active throughout the period

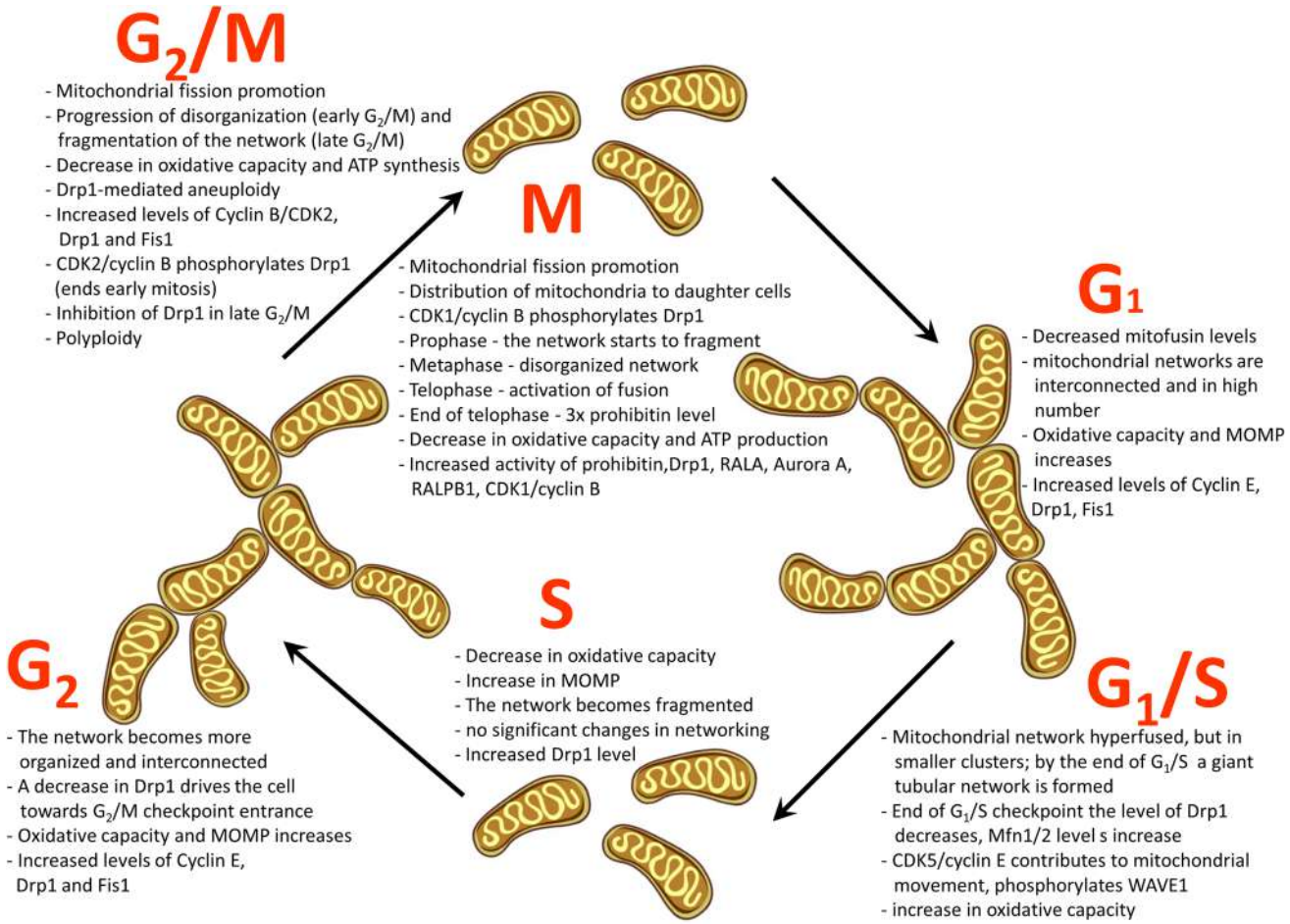

Fig. 1 Cell cycle regulation by fission/fusion machinery. Drp1 was shown to be vital in organizing the mitochondrial network during cell cycle arresting and reentering the cell cycle. Most mitochondria in mitosis are both interconnected and fragmented, whereas during longer cell cycle arrest mitochondria form organized giant tubular networks. At stationary phase mitochondria are shorter and rounder, whereas a tubular form can be observed during their logarithmic growth

of early S-phase to late metaphase of mitosis, phosphorylates Ser616 in human Drp1 isoform 1 [83]. This contributes to mitochondrial fission and impairment after Ser579 and Ser616 phosphorylation by protein kinase Cδ, and can lead to mitochondrial fission promotion, which forces the cell to distribute mitochondria to daughter cells in the case of Ser616 phosphorylation by Cdk1/cyclin B complex [86, 87]. Drp1 strongly depends upon Cdk1/cyclin B complex. Drp1 gets activated through Cdk1/cyclin B phosphorylation of Ser618. Such interaction contributes to mitochondrial fragmentation and is linked with programmed cell death [61, 88, 89]. Another study showed that Drp1 gets phosphorylated and activated by cAMP-dependent protein kinase (PKA) at Ser637. This event affects the morphology of the mitochondria, mostly by affecting the cristae. By releasing Drp1 from mitochondria, cell viability and mitochondria network formation and extension are promoted [85]. This mechanism is mediated by a mitotic kinase Aurora A, small Ras-like GTPase RALA and its effector protein RALBP1 (aka RIP1). Aurora A kinase phosphorylates Ser 194 of RALA and relocates it to the mitochondrion. Under such conditions RALBP1 and Drp1 accumulate on the mitochondrial outer membrane, so that the fission mechanism is ready to be launched. RALBP1 is associated with the Cdk1/cyclin B complex and phosphorylates Drp1 on Ser616. However, negative changes in RALA or RALBP1 activity results in loss of mitochondrial fission at early mitosis, improper mitochondrial segregation during cytokinesis and a drop in ATP activity. Mitochondria fission starts when aurora A kinase and Cdk1/cyclin B link RALA to RALBP1, which might be the turning point in Drp1 activation and, subsequently, mitochondrial fragmentation/fission. As a result an appropriate mitochondrial functioning and normal distribution of mitochondria to daughter cells can be fulfilled by the mother cell. Fragmentation of the mitochondrial network in mitosis was made possible due to Drp1 localization around the mitochondrial axis. This mechanism ensures that proper segregation of mitochondria takes place between the newly

born daughter cells [86, 87]. It was also found that such action requires preliminary phosphorylation of Ser616 on Drp1 by Cdk1/cyclin B complex [55].

Summarizing, mitochondrial networking is of a specific pattern at different stages of the cell cycle. A hyperfusion is likely associated with a higher demand in energy and most clearly is observed in G_1/S transition state, G_1- and G_2-phase. However, in most part of mitosis, G_2/M transition and S-phase it appears more fragmented. The pattern can vary due to different type of cells, neighbouring cells, pathology and other factors.

Importance of mitochondrial fission/fusion in mitochondrial membrane potential regulation throughout the cell cycle

In order to progress from one stage of the cell's cycle to another a cell needs to meet the required energy state. Mitochondrion changes constantly due to ion exchange throughout the cell cycle. A low ATP output and mitochondrial $\Delta\Psi_m$ can interrupt cell cycle progression, by driving the cell cycle toward arrest. Increased membrane potential is observed at the G_1 and S stages of the cell cycle due to increased needs of ATP as the main form of energy [52]. To enter the fusion process mitochondria need a certain mitochondrial $\Delta\Psi_m$, which is considered as an indicator of selectivity for normal mitochondria to fuse together, whereas damaged ones get eliminated by fusing with healthy or digested ones. The process of fusion is simultaneous, but not for all of the mitochondria. The whole fusion process of mitochondria can be considered as a quality control stage of the entire mitochondrial network. Fission of a mitochondrion can yield a depolarized daughter mitochondria and a hyperpolarized one, which inherits the majority of mother cell mitochondria. However, the depolarized daughter mitochondrion is less likely to undergo fusion due to reduced OPA1 level, a protein involved in cristae junctions [88]. Such mitochondria are highly probable to be autophagocytosed due to several impairments, including low mitochondrial $\Delta\Psi_m$. The regions that no longer are capable of fusing will separate and might undergo mitophagy [89]. A damaged fragment of the mitochondria can be restored and fused again to the network. However, the more it is damaged, the smaller the chance of a recovery since the fusion process is mitochondrial $\Delta\Psi_m$ dependent [86]. Depolarization of the mitochondrial membrane may restore the membrane potential to maintain the fission/fusion equilibrium. The mitochondria become more fragmented and clustered on the periphery of the cell. Depolarized or damaged mitochondria frequently undergo autophagy. However, Drp1 is required for such clearance, and, since mitochondrial fission is associated with cristae structure protein OPA1 (regulated by Oma1 protease cleavage due to proton electromagnetical gradients) and Drp1 overexpression, there is little chance for mitophagy to take place [87, 90]. For the recent data on the role of mentioned proteins in neurodegeneration see the review of Bertholet et al. [91].

In HeLa cells, as well as other tumor cell lines, mitochondria can be not only of an independent morphological and functional state, but also of different mitochondrial $\Delta\Psi_m$, rate of permeability transition pore activation and Ca^{2+} localization. Eventually fusion stops or inhibits formation of pores, whereas fission activates the process of pore formation [92, 93]. Asymmetric mitochondrial division into two daughter mitochondria in normal and cancer cells is often a result of a failure in an appropriate Drp1-dependent fission [94]. After an unbalanced mitochondrial fission, one of the newly formed mitochondria has an increased electrochemical potential, whereas the other one has a low membrane potential. Hence, little is known about the viability of the weaker mitochondrion [95]. A disruption in Fcj1 functioning (core protein in MINOS/MitOS complex) is vital not only for proper inner organization of mitochondria, but to reduce mitochondrial outer membrane potential (MOMP) and import efficiency of proteins transported into/across the inner mitochondrial membrane via TIM23 complex [96]. This component of the inner mitochondrial membrane mediates translocation of transit peptide-containing proteins across its periphery [97]. All these peculiarities result in poor mitochondrial separation and may interrupt the mitochondrial network, which might lead to failure in apoptosis and initiation of carcinogenesis.

Conclusion

A normal balance in fission/fusion of mitochondria is vital in cell cycle progression. To be in a functional state a cell needs healthy young mitochondria. Mitochondria dynamics are studies in both rapidly dividing prokaryotes, such as yeast, and more complex human cells. The dynamics are regulated by two essential processes in mitochondria—fission and fusion. The cell can regulate the number of mitochondria by fusing them, separating *via* fission into daughter mitochondria and even eliminating them, when the organelle is damaged and can't be repaired. Activity, distribution, number, MOMP as well as other parameters of mitochondria largely depend on the cytoskeletal proteins, which distribute mitochondria throughout the cell cycle progression, and the type of tissue (for instance, neurons require higher mitochondrial activity comparing to other types of cells). Overall the following patterns can be observed: (1) In more active phases of the cell cycle (such as mitosis) mitochondrial networks are of an interconnected tubular form, predominantly mitochondria are of an elongated form. (2) At G_1 and G_2 mitochondria are interconnected and increased in

number. However, the tendency is a decrease of mitofusins and increase in Drp1, which shifts the network to become more fragmented before entering S-phase or mitosis. (3) Most active reorganization of the mitochondria network happens while the cell is at arrest, since it is an energy consuming period of the cell cycle (S-phase and mitosis). In cancer cells, this pattern may be changed due to inconsistent time length of stages of the cell cycle, so that no clear pattern can be observed. In cell cycle arrest, mitochondria tend to be more dynamic and can rapidly change the network from interconnected to fragmented and vice versa. However, prolonged arrest can cause increased fragmentation of the network; even in this state mitochondria still are in a fully functional state. When the cell undergoes apoptosis or mitophagy mitochondria are very active in order to prevent neoplastic transformation.

All this is possible due to the activity of the cytoskeletal proteins dyneins and kinesins. Microtubules tend to transport and reorganize mitochondrial network throughout the cell in interphase, and make sure both daughter cells have same equal amounts of mitochondria when entering cytokinesis.

Acknowledgments The authors would like to thank Prof., Dr. Darrell Mousseau from the Mousseau Lab at the University of Saskatchewan and Dr. Wilma Groening and Dr. Blaine Chartrand from Saskatchewan Polytechnic Instititute for helpful comments and analysis of the paper.

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