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Mitochondrial dynamics and cell death in heart failure

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Abstract The highly regulated processes of mitochondrial fusion (joining), fission (division) and trafficking, collectively called mitochondrial dynamics, determine cell-type specific morphology, intracellular distribution and activity of these critical organelles. Mitochondria are critical for cardiac function, while their structural and functional abnormalities contribute to several common cardiovascular diseases, including heart failure (HF). The tightly balanced mitochondrial fusion and fission determine number, morphology and activity of these multifunctional organelles. Although the intracellular architecture of mature cardiomyocytes greatly restricts mitochondrial dynamics, this process occurs in the adult human heart. Fusion and fission modulate multiple mitochondrial functions, ranging from energy and reactive oxygen species production to Ca^{2+} homeostasis and cell death, allowing the heart to respond properly to body demands. Tightly controlled balance between fusion and fission is of utmost importance in the high energy-demanding cardiomyocytes. A shift toward fission leads to mitochondrial fragmentation, while a shift toward fusion results in the formation of enlarged mitochondria and in the fusion of damaged mitochondria with healthy organelles. Mfn1, Mfn2 and OPA1 constitute the core machinery promoting mitochondrial fusion, whereas Drp1, Fis1, Mff and MiD49/51 are the core components of fission machinery. Growing evidence suggests that fusion/fission factors in adult cardiomyocytes play essential noncanonical roles in cardiac development, Ca^{2+} signaling, mitochondrial quality control and cell death. Impairment of this complex circuit causes cardiomyocyte dysfunction and death contributing to heart injury culminating in HF. Pharmacological targeting of components of this intricate network may be a novel therapeutic modality for HF treatment.

Keywords Mitochondrial fusion and fission · Mfn1 · Mfn2 · OPA1 · Drp1 · Mitophagy · Cell death · Cardiomyopathy · Heart failure

Introduction

In mammals, the relentlessly beating heart is one of the most mitochondria-enriched organs. Mitochondrial oxidative phosphorylation (OXPHOS) generates up to 90 % of ATP, required for constant contraction of cardiomyocytes, and the organelles occupy almost 30 % of their volume [1–3]. Over the past two decades, mitochondria have emerged not only as cellular powerhouses, but also as critical integrators of fundamental cellular processes, ranging from the generation of reactive oxygen species (ROS) and signal transduction to the maintenance of Ca^{2+} homeostasis, stress responses and cell death [4–6]. Comprehensive studies of cardiac mitochondria have convincingly demonstrated that their dysfunction is implicated in the pathogenesis of common cardiovascular diseases (CVD), such as dysrhythmias, myocardial ischemia, cardiomyopathies culminating in end-stage heart failure (HF) [7–10].

Mitochondria are dynamic organelles—the two opposing highly regulated processes, fusion (joining) and fission (division)—determine cell-type specific mitochondrial morphology, intracellular distribution and activity (Fig. 1). Furthermore, mitochondria can move along the cytoskeleton interacting with various intracellular organelles and

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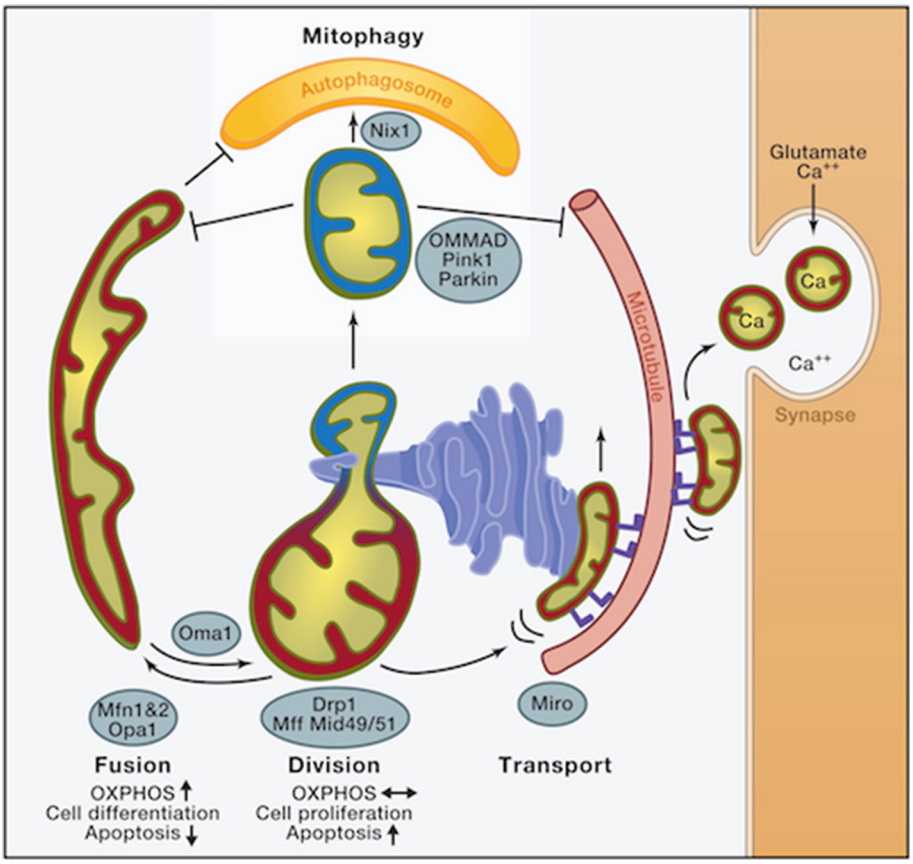

Fig. 1 Roles of mitochondrial dynamics. *Red* Mitochondria with high membrane potential, with high oxidative phosphorylation (OXPHOS) activity. *Blue* Mitochondria with low membrane potential. Mitofusin 1 or 2 (Mfn1, Mfn2) mediates mitochondrial outer-membrane fusion in a tissue-specific manner, and optic atrophy gene1 (OPA1) mediates inner-membrane fusion. The zinc metalloprotease OMA, also known as "Overlapping with the m-AAA protease 1 homolog," is an essential enzyme in mitochondrial maintenance that proteolytically cleaves OPA1 under low membrane potential conditions, promoting fission. Mitochondrial dynamics factors 49 and 51 or mitochondrial fission factor (Mff) recruit dynamin-related protein 1 (DRP1) onto mitochondria at sites marked by endoplasmic reticulum tubules (ER), and DRP1 mediates mitochondrial division. In cultured cells, upon a decrease in mitochondrial membrane potential, PINK1 kinase recruits Parkin, a ubiquitin E3 ligase, which ubiquitinates several mitochondrial targets, including MFN1 and mitochondria Rho (Miro), to facilitate the degradation of mitochondria via mitophagy. Parkin-mediated ubiquitination triggers outer mitochondrial membrane-associated degradation (OMMAD)—a proteosomal pathway that degrades ubiquitinated OM proteins in a CDC48-dependent manner. OMMAD is probably cell type dependent and may also function in quality control. In erythrocytes, mitophagy receptor Nix1 is involved in autophagosome recruitment. ER forms close contacts with mitochondria, essential for calcium regulation in cellular microcompartments. Miro (*blue feet*) is a mitochondrial receptor for kinesin via Milton that facilitates the transport of mitochondria on microtubules in a Ca^{2+}-regulated manner. Upon synaptic activity in neurons, influx of glutamate and Ca^{2+} halts mitochondrial transport via Miro to position them at sites of synaptic activity that require Ca^{2+} uptake and ATP. From Nunnari and Suomalainen [6] with permission of Elsevier

ensuring region-specific cellular requirements. These finely tuned processes, which have been termed mitochondrial dynamics, modulate mitochondrial shape and function allowing living cells to respond properly to frequently changing environmental conditions [11–14].

Mitochondrial fusion produces interconnected mitochondrial network and is essential for the maintenance and inheritance of mitochondrial DNA (mtDNA), the transmission of membrane potential and Ca^{2+} signaling along the mitochondrial network [12, 15, 16]. The opposing process, mitochondrial fission, leads to smaller, more discrete organelles and plays important roles in mitochondrial partitioning during mitosis, cytoskeleton-mediated trafficking to energy-demanding intracellular compartments and in selective autophagic removal of damaged mitochondria by the process called *mitophagy* [12, 15–17]. Moreover, elongation of mitochondrial tubules has been shown upon differentiation of progenitor cells into cardiomyocytes, while mitochondrial fragmentation can contribute to cytochrome *c* release leading eventually to apoptosis [18–21]. Alterations in the fine-tuned balance between mitochondrial fusion and fission are implicated in the pathogenesis of cancer, neurodegenerative, metabolic and cardiac disorders [15, 20, 22–25].

The mechanisms of mitochondrial dynamics have been studied mainly in cell types other than cardiomyocytes. The high energy need to fuel excitation–contraction coupling determines not only the great density of mitochondria, but also their specific arrangement within the cardiomyocytes. In mature cardiomyocytes, mitochondria are tightly packed between the sarcomere myofibrils or between the myofibrils and the plasma membrane, or clustered nearby the nucleus [1]. Furthermore, mitochondria in cardiomyocytes are closely associated with the sarcoplasmic reticulum (SR), the major compartment for Ca^{2+} storage and release, required for cardiac contraction [26, 27]. Such mitochondrial localization provides close contact with sarcomeres and efficient SR-mitochondrial crosstalk, linking high Ca^{2+} microdomains and energy generation organelles during excitation–contraction coupling [28]. However, this unique arrangement significantly restricts mitochondrial dynamics in adult cardiomyocytes compared to other cell types (e.g., neurons, fibroblasts or liver cells). Notably, the major proteins, which mediate mitochondrial dynamics, are highly expressed in the mammalian myocardium and their cardiomyocyte-specific genetic ablation is lethal [29–33]. Increasing evidence suggests that fusion/fission factors in adult cardiomyocytes play essential noncanonical roles in cardiac development, Ca^{2+} signaling, mitochondrial quality control and cell death.

In this review, we will provide an overview of the general mechanisms of mitochondrial fusion and fission, and the core proteins that mediate these complex processes. Then, we will discuss recent progress in our understanding of the role of mitochondrial dynamics in the pathogenesis of HF, focusing on noncanonical functions of fusion/fission proteins in the mitophagic removal of damaged mitochondria and in the initiation of cell death. The potential of therapeutic targeting of mitochondrial dynamics proteins will also be discussed.

Mechanisms of mitochondrial dynamics

Evolutionary conserved large GTPases, related to the dynamin superfamily, along with a number of binding partners promote both mitochondrial fusion and fission (Table 1) [20, 34–36]. Importantly, these dynamin family GTPases are highly expressed in the human adult heart.

Mitochondrial fusion

In mammals, the dynamin family GTPases—two mitofusin isoforms, Mfn1 (Mgm1 in yeast) and Mfn2, and optic atrophy protein 1 (OPA1; Fzo1 in yeast)—are the core components of the mitochondrial fusion machinery [20, 37].

Mfn1 and Mfn2 share a similar molecular architecture: the N-terminal GTPase domain, heptad-repeat domain 1 (HR1), two transmembrane (TM) domains, which anchor the proteins in the outer mitochondrial membrane (OMM), a short loop exposed in the intermembrane space (IMS) and the C-terminal heptad-repeat domain 2 (HR2) (Fig. 2a) [38]. The TM domains of Mfn1 and Mfn2 are embedded in the OMM, while their HR1 and HR2 protrude into the cytosol, where HR2 mediates interaction with their counterparts in adjacent mitochondria [38–40]. Mfn2, but not Mfn1, is also localized in the endoplasmic reticulum (ER)/SR and is involved in tethering of mitochondria with these organelles [41].

OPA1 is also the dynamin-related GTPase composed of an N-terminal mitochondrial targeting sequence, cleaved by matrix-processing peptidase (MPP), TM domain, HR domain, GTPase domain, middle domain and a C-terminal GTPase effector domain (Fig. 2b) [42]. OPA1 is localized in the inner mitochondrial membrane (IMM) and the intermembrane space [43].

OPA1 is regulated at both mRNA and protein levels. Differential splicing generates eight distinct mRNA OPA1 splice forms depending on the tissue [44]. Multiple OPA1 isoforms result from processing at two sites between the N-terminal TM region and HR [20, 36]. In mammals, several proteases, such as presenilin-associated rhomboid-like protease (PARL), *i*-AAA metalloprotease (Ymel), *m*-AAA metalloprotease (paraplegin) and zinc metalloprotease OMA1, catalyze OPA1 cleavage [45–52]. As a result, long OPA1 isoforms (L-OPA1), containing the TM domain, and short isoforms (S-OPA1), lacking the TM domain, can be generated (Fig. 2b). L-OPA1 is anchored to the IMM by its TM domain, while S-OPA, which lacks the TM, is targeted to the IMM via its association with the IMM-anchored L-OPA1 [46, 47]. The loss of mitochondrial membrane potential induces L-OPA1 cleavage leading to accumulation of S-OPA1 isoforms and inhibition of fusion and targeting mitochondria to mitophagy [48, 53]. Furthermore, proapoptotic stimuli induce OMA1-mediated OPA1 cleavage resulting in the formation of fusion inactive S-OPA1 isoforms [50].

According to current paradigm, mitochondrial fusion is a 3-step process, which includes OMM tethering followed by a highly coordinated OMM and IMM fusion. First, the HR2 of mitofusins of adjacent mitochondria interacts to form homodimers (Mfn1–Mfn1 or Mfn2–Mfn2) or more potent heterodimers (Mfn1–Mfn2) to tether approaching mitochondria [40]. This initial docking step brings the OMM of two mitochondria close together to initiate the OMM fusion. After fusion of OMM, OPA1 mediates IMM fusion [54]. GTP hydrolysis catalyzed by the GTPase activities of Mfn1/Mfn2 and OPA1 provides energy for these processes [36]. In yeast, an additional OMM protein

Table 1 Core components of the human mitochondrial fusion and fission machineries

Protein	Location	Function	Disorder
Fusion proteins			
Mitofusin 1 (Mfn1)	OMM	Dynamin family GTPase; GTP-dependent OMM fusion	Unknown
Mitofusin 2 (Mfn2)	OMM	Dynamin family GTPase; GTP-dependent OMM fusion	Charcot–Marie–Tooth type 2A, pulmonary arterial hypertension, arterial restenosis
Optic atrophy protein 1 (OPA1)	IMM (L-OPA1) IMM and IMS (S-OPA1)	Dynamin family GTPase; GTP-dependent IMM fusion	Optic atrophy type 1, hypertension
Fission proteins			
Dynamin-related protein 1 (Drp1, also known as Dlp1)	Cytosol and OMM through interaction with Mff, Fis1 and MiD49/51	Dynamin family GTPase; forms oligomers; GTP-dependent fission	Huntington's disease, Parkinson's disease, pulmonary arterial hypertension, congenital microcephaly
Fission protein 1 (Fis1)	OMM	Putative factor for Drp1 recruitment to the OMM	Unknown
Mitochondrial fission factor (Mff)	OMM	Putative factor for Drp1 recruitment to the OMM	Unknown
Mitochondrial dynamics proteins of 49/51 kDa (Mid49/51)	OMM	Putative factor for Drp1 recruitment to the OMM	Unknown

IMM mitochondrial inner membrane, *IMS* intermembrane space, *OMM* mitochondrial outer membrane, *L- and S-OPA1* long and short OPA1 isoforms, respectively

Ugo1 coordinates the OMM and IMM fusion [55, 56]. In higher eukaryotes, no structural or functional Ugo1 equivalents have been found; hence, the precise mechanism underlying the coupling of the OMM and IMM fusion remains to be determined. It has been hypothesized that interactions between mitofusins and OPA1 may be a part of such coordination mechanism [57].

Mitochondrial fission

In mammals, mitochondrial fission is under control of a 80-kDa dynamin-related protein 1 (Drp1; Dnm1 in yeast), also known as dynamin-like protein 1 (Dlp1) [17, 20, 37, 58]. Drp1 is the dynamin family GTPase composed of an N-terminal GTPase domain, middle domain and a C-terminal GTPase effector domain essential for self-assembly (Fig. 2b) [59]. Drp1 is mainly a soluble cytosolic protein, but its subpool colocalizes with mitochondria at sites of future fission [60, 61]. It forms dimers/tetramers in the cytosol and higher-order structures upon interaction with membranes [62, 63]. Cytosolic Drp1 is recruited to mitochondria by interaction with OMM proteins, such as Fis1, Mff and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51, respectively) [64–67].

Fis1 is a small ($\sim$17 kDa) single-pass TM protein with a C-tail anchored in the OMM [64, 68, 69]. Its N-terminal multiple tetratricopeptide repeat motif, facing the cytosol, is thought to be involved in the recruitment of Drp1 to mitochondria (Fig. 2b) [70–73]. Mff contains N-terminal heptad repeats, coiled-coil domain and a C-terminal TM tail, which anchor it to the OMM (Fig. 2b) [74]. In contrast to the uniform Fis1 localization in the OMM, Mff mainly colocalizes with the Drp1 foci on the OMM during fission.

However, the specific roles of Fis1, Mff and MiD49/51 in the recruitment of Drp1 to mitochondria and Drp1-mediated fission are uncertain [17, 58]. Yeast Fis1 is essential for the recruitment of Dnm1 (yeast orthologue of mammalian Drp1) [75], in mammals; however, Fis1 appears to be dispensable for Drp1 recruitment [65]. A recent study has shown that Fis1, Mff and MiD49/51 contribute to Drp1 recruitment; however, they can serve as Drp1 receptors on the OMM independently of each other [67]. Furthermore, both Fis1 and Mff, beyond their role in Drp1 recruiting, appear to facilitate Drp1 assembly into spirals on the OMM during fission [67].

According to current paradigm, mitochondrial fission is coupled to the inhibition of the mitochondrial fusion machinery. Cytosolic Drp1 as small oligomers is recruited to mitochondria through interactions with several OMM proteins, including Fis1, Mff and MiD49/51. Drp1 oligomers polymerize into spiral structures around the mitochondria and form fission foci, which constrict and divide mitochondria in a GTP-dependent manner [17, 58]. Recently, it has been shown that MiD51 can stimulate the GTPase activity of Drp1 and therefore assist Drp1-mediated constriction [76, 77]. Several additional proteins, such

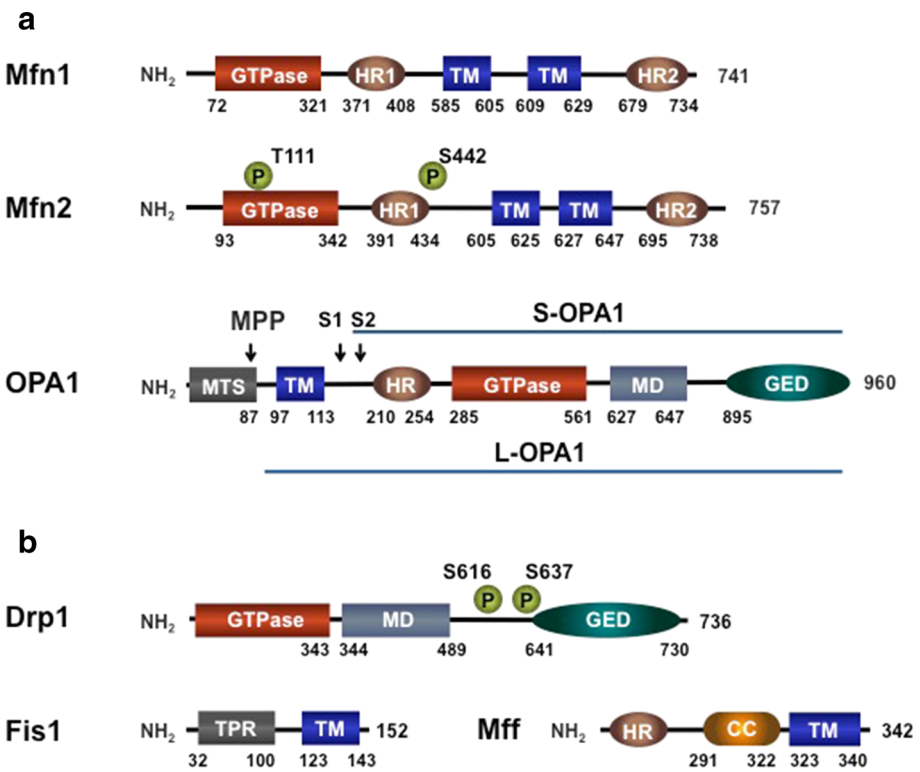

Fig. 2 Molecular structure of core mitochondrial fusion and fission proteins. **a** Fusion proteins. Mitofusins, Mfn1, Mfn2, located in the outer mitochondrial membrane (OMM), contain a GTPase domain (GTPase), two heptad-repeat regions (HR1 and HR2) and two transmembrane (TM) domains. Phosphorylation sites of Mfn2 are also shown. OPA1 is located in the inner mitochondrial membrane (IMM) and contains mitochondrial targeting sequence (MTS), TM domain, heptad-repeat region (HR), GTPase domain, middle domain (MD) and GTPase effector domain (GED). Proteolytic cleavage within MTS by matrix-processing peptidase (MPP) and at the S1 and S2 sites by metalloproteases, which produce long OPA1 isoforms (L-OPA1) and short isoforms (S-OPA1), is shown by *arrows*. **b** Fission proteins. Cytoplasmic dynamin-related protein 1 (Drp1) contains a GTPase domain, middle domain (MD), GTPase effector domain (GED) and heptad-repeat region (HR). Phosphorylation sites of Drp1 are also depicted. Fission protein 1 (Fis1) and mitochondrial fission factor (Mff) are anchored by their TM domains to the OMM. In addition, Fis1 contains tetratricopeptide repeat (TPR), while Mff contains heptad-repeat region (HR) and coiled-coil region (CC)

as mitochondrial protein of 18 kDa (MTP18), ganglioside-induced differentiation-associated protein 1 (GDAP1), endophilin B1 (Endo B1) and leucine-rich repeat kinase 2 (LRRK2), may contribute to mitochondrial fission; however, their roles in the process remain to be determined [17, 58].

Regulation of mitochondrial dynamics

Opposing processes of mitochondrial fusion and fission are tightly regulated to maintain mitochondrial morphology and function in response to changing conditions [17, 58]. The core components of the mammalian machineries that promote mitochondrial dynamics represent main targets for complex regulatory mechanisms operating at multiple levels.

Complex regulation of OPA1 by mRNA and proteolytic processing has already been described (Fig. 2b). Mfn2 concentration is regulated at the transcriptional level. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), one of the critical regulators of mitochondrial biogenesis, upregulates Mfn2 expression in response to increased metabolic demand [78]. Importantly, downregulation of PGC-1α associated with reduced Mfn2 expression has been found in rats and in patients with pulmonary arterial hypertension that can contribute to the development of HF [79, 80].

Furthermore, several E3 ubiquitin-protein ligases, including anaphase-promoting complex (APC/C)^{CDH1}, Huwe1 and Parkin, catalyze the ubiquitination of both Mfn1 and Mfn2 inhibiting their fusion activity as well as targeting them to degradation [81–87]. Stress-induced mitochondrial depolarization—the sign of mitochondrial damage—targets PTEN-induced putative kinase 1 (PINK1) and/or JNK to the OMM [82]. PINK1 selectively accumulated on dysfunctional mitochondria phosphorylates Mfn2 on Thr^{111} and Ser^{442} recruits and activates cytosolic E3 ubiquitin ligase Parkin to ubiquitinate Mfn2 directing thereby damaged mitochondria to mitophagy [85, 88–91]. PINK1/Parkin mediates the ubiquitination of Mfn1 and

Mfn2 as well as other mitochondrial proteins, such as voltage-dependent anion channel 1 (VDAC1) and p62 (also known as sequestosome 1), targeting them to proteosomal degradation [85, 92, 93].

Overexpression of fission protein Drp1 does not induce mitochondrial fission suggesting that post-translational modifications, which affect its mitochondrial recruitment, GTPase activity or self-assembly ability, play an essential regulatory role [17, 58]. Phosphorylation of Drp1 at Ser^{616} and Ser^{637} has been extensively studied. Cyclin B-dependent kinase (CDK1) phosphorylates Drp1 at Ser^{616} to target Drp1 to the mitochondria and stimulates mitochondrial fission ensuring inheritance of mitochondria by daughter cells during mitosis [94, 95]. In this process another mitotic kinase Aurora A, the small Ras-like GTPase RALA, its effector RALBP1 and Mff, but not Fis1, appear to be involved [94, 96]. As the adult human heart has low mitotic potential, this regulatory event occurs infrequently in this organ. Oxidative stress induces protein kinase Cδ[delta]-mediated phosphorylation of human Drp1 at Ser^{616} resulting in aberrant mitochondrial fission associated with hypertension-induced brain damage [97].

Both CDK1 and cAMP-dependent protein kinase A (PKA) phosphorylate human Drp1 at Ser^{637} near the GED domain interfering with the interaction between GED and GTPase domains. This results in the inhibition of Drp1 GTPase activity and its recruitment to mitochondria and eventually in attenuation of mitochondrial fission [98, 99]. The PKA-mediated Drp1 phosphorylation protects mitochondria from autophagosomal degradation and enhances cell viability during nutrient starvation [100, 101]. Conversely, the phosphatase calcineurin dephosphorylates Drp1-Ser^{637} in a Ca^{2+}-dependent manner targeting Drp1 to mitochondria and inducing mitochondrial fission [99, 102]. Moreover, the dephosphorylation of Drp1-Ser^{637} is involved in apoptotic and programmed necrotic death pathways [98, 99, 103, 104].

Drp1 is also subject of O-linked-*N*-acetyl-glucosamine glycosylation (O-GlcNAcylation) and S-nitrosylation [105, 106]. In cardiomyocytes, the O-GlcNAcylation of Drp1 at Thr^{585} and Thr^{586} has led to Drp1-Ser^{637} dephosphorylation associated with mitochondrial fragmentation and loss of membrane potential [106, 107]. The O-GlcNAcylation of OPA1 linked to mitochondrial fragmentation has also been demonstrated in neonatal cardiomyocytes [106].

Intriguingly, the phosphorylation of the same residue by calcium/calmodulin-dependent protein kinase 1α (CaMKIα [alpha]) and the Rho-associated coiled-coil-containing protein kinase1 (ROCK1) induces Drp1 recruitment to mitochondria and enhances mitochondrial fission [108, 109]. Although the reason of this seemingly different consequence of Drp1-Ser^{637} phosphorylation is yet unclear, it might be linked to tissue-specific response and/or phosphorylation of additional factors, which may be involved in Drp1-promoted fission [17].

Mitochondrial dynamics, mitophagy and cell death in heart failure

In contrast to other cell types and neonatal cardiomyocytes, in adult human cardiomyocytes, mitochondrial fusion and fission are very rare events due to specific intracellular mitochondrial arrangements [110]. Growing evidence suggests that the cardiomyocyte mitochondrial dynamics machinery performs additional noncanonical functions governing Ca^{2+} handling, mitophagy, mitochondrial quality control and cell death pathways. Nevertheless, recent conditional cardiac-specific ablation of both Mfn1 and Mfn2 has provided clear evidence that mitochondrial fusion occurs in adult mammalian cardiomyocytes, albeit at extremely slow rate [29]. Moreover, perinatal cardiac-specific deletion of both mitofusins caused early lethality due to severe mitochondrial abnormalities associated with cardiomyopathy [30]. The conditional Mfn1/Mfn2 double knockout in the adult mouse heart has led to mitochondrial fragmentation associated with severe cardiomyocyte respiratory defects culminating in HF within 6–8 weeks [29]. These findings highlight the critical role that mitofusins play in cardiac development and homeostasis.

Importantly, single knockout of Mfn1 and Mfn2 in mice has displayed distinct phenotypes. Mfn1 deficiency resulted in fragmented mitochondria and elevated apoptosis in neonatal rat cardiac myocytes, and these defects can be rescued by Mfn1 overexpression [111]. However, although mature mouse $Mfn1^{-/-}$ cardiomyocytes have also accumulated fragmented mitochondria, they have paradoxically been more resistant to stress-induced mitochondrial permeability transition pore (MPTP) opening and apoptosis [112].

Unlike Mfn1 deficiency, ablation of Mfn2 in mouse heart has not impaired mitochondrial fusion as evidenced by increased mitochondrial size. However, $Mfn2^{-/-}$ cardiac mitochondria displayed dissipation of mitochondrial membrane potential and elevated ROS production [113, 114]. Importantly, Mfn2-deficient mice developed cardiac hypertrophy and ventricular dysfunction with age [91]. Furthermore, cardiac-specific deletion of Mfn2, but not Mfn1, has impaired tethering of mitochondria to SR and disrupted Ca^{2+} handling that is critical for cardiac function [113, 114].

Another fusion protein OPA1 also plays an important role in heart physiology. OPA1 deficiency caused mitochondrial fragmentation and abnormal cristae remodeling [115]. Heterozygous $OPA1^{+/-}$ mice exhibited mitochondrial dysfunction, mtDNA instability and elevated ROS

production and developed cardiomyopathy [116, 117]. Consistently, cardiomyocytes derived from these animals have characterized abnormal Ca^{2+} handling, contractility and high susceptibility to ischemia reperfusion injury (IRI) [116]. Importantly, reduced OPA1 levels associated with accumulation of fragmented mitochondria have been reported in human failing hearts [118].

Mitochondrial fusion has traditionally been envisioned as a prosurvival antiapoptotic mechanism. Indeed, in some cell types silencing of Mfn1 or Mfn2 has enhanced cellular susceptibility to apoptotic stimuli, whereas overexpression of Mfn2 or OPA1 has attenuated apoptosis [115, 119, 120]. However, recent studies on mature cardiomyocytes unexpected effects of inhibiting mitochondrial fusion have been reported (see also above). Ablation of both mitofusins in the adult heart has led to no significant change in MPTP sensitivity, a key event in the initiation of apoptosis [29]. Similarly, although cardiac mitochondria of heterozygous $Opa1^{+/-}$ mice displayed disorganized mitochondrial cristae, unexpectedly they exhibited higher Ca^{2+} retention capacity and delayed MPTP opening under Ca^{2+} stimulation [117]. In addition, it has been demonstrated that mitochondrial fusion can be harmful to the cell when damaged mitochondria are fused with functional organelles due to attenuated mitophagy [121].

Prolonged and/or high-level stress can eventually lead to mitochondrial damage and dysfunction. Mitophagy induced by cardiac stress removes damaged dysfunctional mitochondria preventing thereby oxidative damage, which can otherwise initiate apoptosis and ultimately HF [122, 123]. Increasing evidence suggests that Mfn2 plays a complex role in cardiac physiology and pathophysiology orchestrating mitochondrial fusion, mitochondrial-SR Ca^{2+} signaling, mitochondrial quality control and cell death [28, 124, 125]. Its localization in the OMM and the ER/SR may facilitate organelles tethering and autophagosome formation and maturation during mitophagy [126]. It has also been suggested that regulation of mitophagy rather than mitochondrial remodeling per se is a primary role of Mfn2 in the adult human heart [21].

Mitofusins on the damaged mitochondria are rapidly ubiquitinated by the PINK1/Parkin complex (critical mediator of mitophagy), degraded by the proteosome, preventing fusion of dysfunctional mitochondria with the healthy mitochondrial network [127, 128]. Stabilization and accumulation of mitochondrial kinase PINK1 in damaged mitochondria is the initiating signal for translocation of the cytosolic Parkin E3 ubiquitin ligase to damaged organelles [129, 130]. Although crosstalk between PINK1 and Parkin is yet poorly understood, PINK1-mediated phosphorylation of Mfn2 is essential for Parkin recruitment to damaged mitochondria [91]. Parkin promotes ubiquitination of multiple OMM proteins in dysfunctional mitochondria, including both Mfn 1 and 2, attracting autophagosomes and initiating thereby mitophagy. It is well established that loss-of-function mutations in the *PINK1* and *Parkin* genes cause early-onset autosomal recessive Parkinson's disease [131, 132]. Recently, it has been shown that impairment in PINK1/Parkin-promoted mitophagy has also led to cardiac dysfunction. Indeed, $Pink1^{-/-}$ mice exhibited abnormal cardiac mitochondrial function and elevated oxidative stress [133], whereas deletion of *Parkin* resulted in accumulation of abnormal mitochondria associated with heart damaged with age [122, 123]. Importantly, $Mfn2^{-/-}$ mice displayed reduced PINK1/Parkin-mediated mitophagy associated with severe cardiac dysfunction leading to HF by 30 weeks of age [134].

Reduced OPA1 levels in the IMM of depolarized mitochondria have also contributed to the prevention of the damaged mitochondria to be fused, targeting them to mitophagy [135]. Consistently, inhibition of the autophagic processes has led to accumulation of dysfunctional mitochondria in various tissues, especially those with elevated energy demands, such as brain, heart, kidney, liver and pancreatic β [beta] cells [135–138].

Mitochondrial fission leading to organelle fragmentation is a prerequisite for mitophagy. Drp1 recruitment and Fis1 recruitment to mitochondria are the early events of the process in various cells, including cardiomyocytes [135, 139–142]. Cardiac-specific $Drp1^{-/-}$ mice exhibited accumulation of dysfunctional mitochondria due to suppressed mitophagy, developed left ventricular dysfunction and died within 13 weeks. Furthermore, cardiac-specific heterozygous $Drp1^{+/-}$ mice exhibit significantly greater infarct size after ischemia/reperfusion than control animals [143]. Other proteins implicated in mitochondrial dynamics are also active players in these processes forming the complex mitochondrial dynamics-mitophagy-cell death interactome [21, 144, 145].

Apoptotic stimuli have triggered mitochondrial hyperfusion followed by mitochondrial fragmentation concomitantly with OMM permeabilization and cytochrome *c* release [146, 147]. Consistently, fission protein Drp1 is implicated in this process and its suppression has not only resulted in reduced mitochondrial fission but also prevented cytochrome *c* release and subsequent apoptosis [148–151]. Of note, Drp1 depletion has not completely attenuated mitochondrial fission suggesting that additional factors contribute to this process during apoptosis [152, 153]. Drp1 collaborates with the proapoptotic Bcl-2 family proteins Bax and Bak by enhancing Bax oligomerization during apoptosis [154–156]. Apoptotic Bax activation induces Bax/Bak-mediated sumoylation of Drp1 leading to Drp1 translocation from the cytosol to mitochondria to promote mitochondrial fission [157].

Another mechanism contributing to ischemia-induced mitochondrial fragmentation has recently been suggested. It has been shown that myocardial ischemia has down-regulated miR-499 leading to the activation of calcineurin [158]. Activated calcineurin dephosphorylates and activates Drp1 stimulating its recruitment to mitochondria, to promote mitochondrial fission. Usually, mdivi-1, a pharmacological Drp1 inhibitor, has prevented mitochondrial depolarization, fragmentation and ischemia-induced cell death in both HL-1 cells and adult cardiomyocytes [151, 159]. Basically, the mdivi-1 inhibition of Drp1-mediated fission has a cardioprotective effect reducing significantly myocardial infarction size after IRI [159]. Attenuation of other fission proteins, such as Fis1, Mff or MTP18, has also led to a delay in cytochrome *c* release and reduced apoptosis [74, 149, 160].

In addition to Bax/Bak proteins, two other members of Bcl-2 family, the BH3-only proteins Bnip3 and Nix (also known as Bnip3L), which are involved in post-infarction cardiac remodeling and cardiomyocyte death, also contribute to this complex process [21]. $Nix^{-/-}$ mice have developed cardiac dysfunction and hypertrophy with age, while double *Nix/Bnip3* knockout mice accumulated dysfunctional mitochondria and developed cardiac dysfunction at about twice the rate than $Nix^{-/-}$ mice [161].

Consistent with their role in mitochondrial turnover, overexpression of Bnip3 or Nix has led to activation of PINK1/Parkin-mediated mitophagy playing a protective role [142, 162]; however, upon cardiac stress these proteins can exert detrimental effects. Bnip3 mediates cardiomyocyte death in ischemia-induced HF [163, 164], whereas Nix is upregulated in hypertrophic hearts and promotes the transition from cardiac hypertrophy to HF [165–167]. Similar to Mfn2, these proteins have dual subcellular localization to mitochondria and adjacent ER/SR. Interestingly, Nix and Bnip3 localization to the mitochondria or ER/SR determines whether they mediate cardiomyocyte death, predominantly through apoptosis or necrosis, respectively [167–169].

In summary, under physiological conditions, basal levels of mitophagy are critical for maintaining the appropriate number of functional mitochondria, preserving therefore cardiac integrity and contractile function. Mitophagy also plays an essential role in the heart adaptation to mild stress. However, upon prolonged and/or high stress, mitophagy can be detrimental to the heart. Imbalanced activation or inhibition of this process can lead to excessively reduced number of functional mitochondria or accumulation of damaged organelles, respectively, resulting in cardiac dysfunction and cardiomyocyte death (via apoptosis or necrosis) and culminating in HF (Fig. 3) [170].

Discussion

Over the past decade, cardiac mitochondria have emerged as critical integrators of energy production, ROS generation, Ca^{2+} handling and multiple signaling and cell death pathways. Tightly balanced processes of mitochondrial fusion and fission contribute to the multifaceted role that mitochondria play in myocardial physiology. Great progress has been recently achieved deciphering the complex multiprotein machineries that promote mitochondrial dynamics, and growing evidence suggests that defects in the core components of these machineries can cause alteration in mitochondrial structure and function leading eventually to various human disorders, including HF.

The most established causative link between mutations in the genes encoding proteins, which mediate mitochondrial fusion and fission, and pathological conditions has been demonstrated in inherited neurological and neurodegenerative disorders, including autosomal dominant optic atrophy, Charcot–Marie–Tooth neuropathy and Wolf–Hirschhorn syndrome. Abnormalities in mitochondrial dynamics have also been associated with age-related progressive neurodegenerative disorders, such as Alzheimer's, Parkinson's and Huntington's diseases. Notably, patients with Parkinson's and Danon's diseases, which are characterized by impaired mitophagy, develop cardiomyopathy and HF [171, 172].

Although the human heart has a great density of mitochondria and is characterized by high levels of the major proteins implicated in mitochondrial dynamics, we have only begun to uncover the multifaceted role of the fusion–fission processes in cardiac physiology and pathophysiology. The simplified view that upregulation of the fusion machinery is cardioprotective, while upregulation of the fission factors inevitably lead to mitochondrial fragmentation and trigger cell death, has recently been challenged. Similarly, mitophagy, a highly complex and tightly regulated pathway, is mediated by the coordinated action of multiple proteins. As a mitochondrial quality control mechanism mitophagy plays a critical cardioprotective role by removing dysfunctional mitochondria, although when impaired it can be detrimental to the heart.

The role that fusion protein Mfn2 and other mitochondrial dynamics factors play in mitophagy and stress-induced cardiomyocyte death remains controversial and requires further investigation. Recent evidence suggests that in the human heart Mfn2 interacts with various proteins and primarily functions as a key orchestrator of mitochondrial fate and cardiac homeostasis [21, 125]. However, the precise molecular mechanisms underlying the interaction of mitochondrial dynamics proteins with

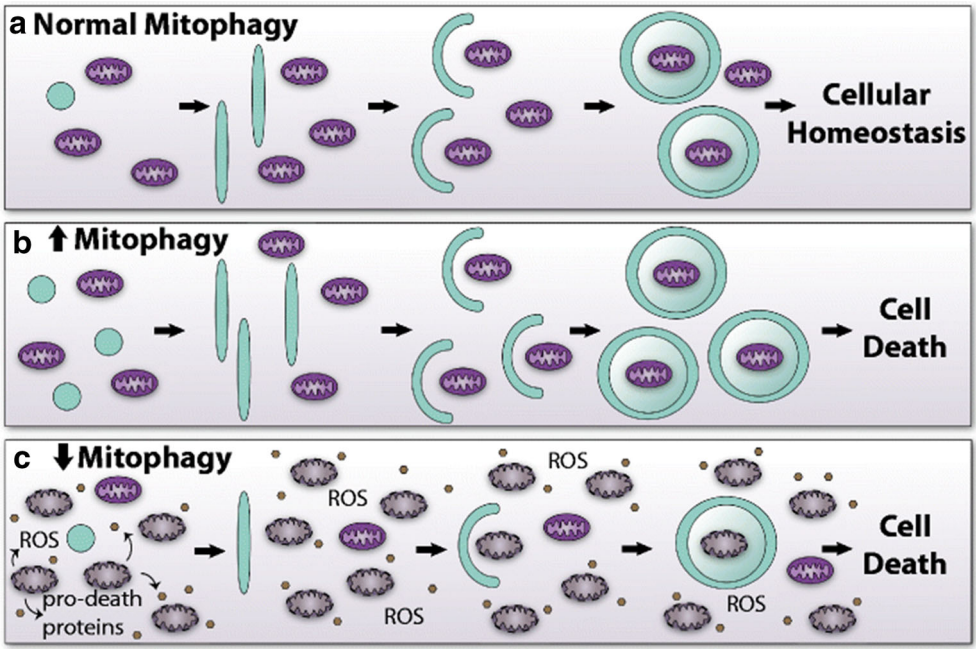

Fig. 3 Mitophagy and mitochondrial quality control. **a** Normal mitophagy begins with the initiation and elongation of a double-membraned autophagic vesicle. The vesicle then sequesters and engulfs mitochondria for degradation. Proper regulation of mitophagy leads to mitochondrial quality control and cellular homeostasis. **b** Increased mitophagy may greatly reduce the pool of functional mitochondria. With too few mitochondria, the cell loses its ability to produce sufficient energy and eventually dies. **c** Reduction in mitophagy causes accumulation of dysfunctional mitochondria. The dysfunctional mitochondria generate excessive ROS and release prodeath proteins, triggering rapid cell death. From Shires and Gustafsson [170] with permission of Springer Publishing Co.

mitophagy and cell death factors, and their involvement in the development and progression of HF remain to be determined.

Pharmacological targeting of components of the mitochondrial fusion and fission machineries that shift the balance toward normal mitochondrial numbers, morphology and function may be a novel therapeutic modality for CVD, including HF. One of the first evidence that a specific Drp1 inhibitor, mdivi-1, with direct effects on mitochondrial fission can act as a preconditioning agent, protecting the myocardium against IRI, is very promising [159]. Another recent example of beneficial targeting of mitochondrial dynamics is the generation of transgenic mice overexpressing miR-499, which exhibited protection against post-ischemic cardiomyocyte death, myocardial infarction and ventricular remodeling [158]. The emerging critical role of Mfn2 and OPA1 in the differentiation of embryonic stem cells into cardiomyocytes may also be important for the development of innovative cell-based therapy for HF [173]. Lastly, further research is needed to establish whether the targeting of mitochondrial fusion–fission and mitophagy machineries can restore the number, morphology and function of this critical organelle and whether it could be translated into clinically relevant therapy for HF.

Conclusions

- Mitochondria are able to vary their morphology through complex processes of fusion and fission. These processes also allow the transmission of signals and the exchange of metabolites within the cell.
- Mitochondrial fusion and fission are implicated in numerous biological processes including embryonic development and cell death.
- It is important to understand at which stage mitophagy is adaptive and when it is maladaptive, since excessive mitophagy may deplete the mitochondrial pool, which if falling below required level for cardiac contractile activity or maintenance of cellular integrity will lead to cardiac dysfunction and to the death of individual cardiomyocytes.
- Changes in mitochondrial morphology may contribute to cardiac development, the myocardial response to IRI, and HF.
- Failure to remove damaged mitochondria might increase cellular death from excessive ROS generated by defective mitochondria.
- Targeting the mitochondrial fusion–fission and mitophagy machineries may restore the number, morphology and function of this organelle. However, further

research is needed to translate these findings into successful therapy for HF.

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