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SPOTLIGHT REVIEW

Mitochondrial morphology and cardiovascular disease

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Mitochondria are dynamic and are able to interchange their morphology between elongated interconnected mitochondrial networks and a fragmented disconnected arrangement by the processes of mitochondrial fusion and fission, respectively. Changes in mitochondrial morphology are regulated by the mitochondrial fusion proteins (mitofusins 1 and 2, and optic atrophy 1) and the mitochondrial fission proteins (dynamin-related peptide 1 and mitochondrial fission protein 1) and have been implicated in a variety of biological processes including embryonic development, metabolism, apoptosis, and autophagy, although the majority of studies have been largely confined to non-cardiac cells. Despite the unique arrangement of mitochondria in the adult heart, emerging data suggest that changes in mitochondrial morphology may be relevant to various aspects of cardiovascular biology—these include cardiac development, the response to ischaemia–reperfusion injury, heart failure, diabetes mellitus, and apoptosis. Interestingly, the machinery required for altering mitochondrial shape in terms of the mitochondrial fusion and fission proteins are all present in the adult heart, but their physiological function remains unclear. In this article, we review the current developments in this exciting new field of mitochondrial biology, the implications for cardiovascular physiology, and the potential for discovering novel therapeutic strategies for treating cardiovascular disease.

Keywords Mitochondrial morphology • Mitochondrial dynamics • Ischaemia–reperfusion injury • Mitochondrial fusion • Mitochondrial fission

This article is part of the Review Focus on: Mitochondria in Cardiac Disease: Emerging Concepts and Novel Therapeutic Targets

1. Introduction

Mitochondria are dynamic organelles which are able to interchange their morphology between two distinct arrangements by undergoing the processes of mitochondrial fusion and fission to generate either an elongated interconnected mitochondrial network or a fragmented discrete phenotype, respectively.[1,2] Developments in this field of mitochondrial biology has been rapid since the ability to visualize directly mitochondrial dynamics and changes in mitochondrial morphology using fluorescence confocal microscopy was achieved in the 1990s. However, it is interesting to note that the origins of the word 'mitochondrion', first coined in 1898 from the Greek words 'mitos' ('*thread*') and 'chondron' ('*grain*'), had already alluded to the heterogeneity of mitochondrial morphology as visualized by light microscopy.[3] The ability of mitochondria to move and fuse is required by the cell to distribute ATP and mitochondrial DNA to all parts of the cell, whereas mitochondrial division allows the correct redistribution of mitochondrial DNA during cell division. Alterations in mitochondrial morphology have been implicated in embryonic development, metabolism, apoptosis, and autophagy.

Changes in mitochondrial morphology are orchestrated by a group of mitochondrial fusion and fission proteins (listed in *Table 1*), the majority of which were first identified in yeast or *Drosophila* in studies using non-cardiac cells. Of these, mitofusin 2 (Mfn2) and optic atrophy 1 (OPA1) have been reported to have pleiotropic effects which are independent of their pro-fusion properties. For general reviews of mitochondrial morphology, the reader is directed to several recent articles.[2,4] Given the rigid well-defined arrangement of mitochondria in the adult heart, it may be difficult to appreciate the relevance of changes in mitochondrial morphology to this organ. Of note, this characteristic arrangement of mitochondria arises during cardiac growth, as in neonatal cardiomyocytes, the mitochondria are arranged in filamentous networks as in many other non-cardiac cells. Emerging data suggest that changes in mitochondrial morphology may actually be relevant to various aspects of cardiovascular biology: these include cardiac development, the response to ischaemia–reperfusion injury (IRI), heart failure, diabetes mellitus, and apoptosis. In this article, we review how changes in mitochondrial morphology can impact on the heart, thereby providing potential novel therapeutic targets for treating cardiovascular disease.

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Table 1 List of the major mitochondrial-shaping proteins

Yeast orthologue	*Drosophila* orthologue	Human homologue	Location	Function	Notes
Mgm1	OPA1	Opa1	IMM	IMM fusion	Pleiotropic effects include cristae remodelling
Fzo1p	Fzo	Mfn1	OMM	OMM fusion	
Fzo1p	Fzo	Mfn2	OMM	OMM fusion	Pleiotropic effects on metabolism, apoptosis, proliferation, ER tethering
Ugo1	?	?	OMM	OMM fusion	
Dnm1p	DRP1	Drp1 or DNM1L	Cytosol and OMM	OM fission	
Fis1p	FIS1	Fis1	OMM	OM fission	
?	?	Mtp18	IMM	IMM fission	Thought to be the IMM equivalent of OPA1
Mdv1p and Caf4p	?	?	OMM	OMM fission	Adaptor proteins which aid Drp1 docking to hFis1
Mdm33	?	?	IMM	IMM fission	

OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; ER, endoplasmic reticulum.

2. The mitochondrial fission and fusion proteins

2.1 Dynamin-related protein 1

In 1998, several independent groups first identified dynamin-related peptide 1 (Drp1), a mammalian homologue of yeast and *Drosophila*, as a protein capable of inducing mitochondrial division in mammalian cells,[5,6] an action which is related to the yeast orthologue Dnm1p.[7] Drp1 is mainly cytosolic and translocates to scission sites on the outer mitochondrial membrane (OMM) to induce fission (or division) of mitochondria. Structurally, Drp1 is made up of a GTPase, the central domain, and the GTPase effector domain (GED) or assembly domain[5] (*Figure 1*). On activation, the Drp1 oligomerizes and constricts the mitochondrial scission site (*Figure 1*), a process which requires GTPase. Mitochondrial fission is prevented in cells expressing the K38A mutation of Drp1 or siRNA to Drp1.[8,9] The GED domain is required for the regulation of GTPase activity and for mitochondrial targeting.[9] Because Drp1 does not have a transmembrane domain, for it to bind to the OMM, it either binds directly to mitochondrial fission protein 1 (Fis1)[10] or induces fission independently of Fis1. Alternatively, in a similar manner to its yeast homologue, Dnm1p, adaptor proteins such as Mdv1 and Caf4 mediate its binding to Fis1, although the mammalian homologous proteins have yet to be identified.[11,12]

2.1.1 Regulation of Drp1 activity

Recent studies have suggested that Drp1 activation and translocation to the mitochondria may be post-translationally regulated by sumoylation, ubiquitination, *S*-nitrosylation, or phosphorylation of serine residues within the GED. Recent studies have suggested that mitochondrial E3 ubiquitin ligase, MARCHV, can promote mitochondrial fission by facilitating Drp1 translocation to the mitochondria without affecting its stability.[13] The small ubiquitin-like modifier (SUMO) proteins are also involved in regulating DRP1 activity.[14] Sumoylation alters subcellular localization of protein substrates or protects proteins from ubiquitination. SUMO-1 and its conjugating enzyme Ubc9 are believed to induce mitochondrial fission by stabilizing Drp1.[14] In contrast, the sentrin/SUMO-specific protease, SENP5, reduces Drp1 levels, thereby preventing mitochondrial fission.[15] It has been suggested that Drp1 may also be subject to *S*-nitrosylation, a post-translational modification which enhances its pro-fission activity by inducing dimerization and GTPase activity,[16] although these findings have been recently contested with the finding that nitric oxide induces mitochondrial fission by phosphorylating Ser616 on Drp1.[17]

With respect to the regulation of Drp1 activity by phosphorylation, the data can be rather confusing, given the differing Drp1 isoforms used in the studies. Taguchi *et al.*[18] demonstrated that cyclic-dependent kinase 1 (Cdk1/cyclin B) phosphorylated Ser585 on rat Drp1 (Ser616 on human Drp1 splice variant 1) within the GED during mitosis in HeLa cells, thereby allowing transient mitochondrial fission and the distribution of mitochondria into daughter cells.[18] Bossy *et al.*[17] have recently implicated the phosphorylation of Drp1 at Ser616 as the mechanism for mitochondrial fission induced by nitric oxide.

In contrast, the phosphorylation of another serine residue, Ser637 (on human Drp1 splice variant 1)[19] or Ser656 (on rat Drp1 splice variant 1),[20] which are located at the NH_2-terminal end of the GED, by protein kinase A (PKA), inhibited Drp1-induced mitochondrial fission and apoptotic cell death. Most interestingly, PKA activation using the β-adrenergic agonist, isoproterenol, or enforced exercise (swimming) also increased Drp1 phosphorylation at Ser656 in adult murine hearts,[20] suggesting Drp1 regulation by phosphorylation in cardiac tissue. However, the effect of Drp1 phosphorylation at Ser656 on mitochondrial morphology in cardiac tissue was not investigated. The phosphorylation at Ser637 was thought to inhibit GTPase activity by decreasing the intramolecular interactions that normally drive GTP hydrolysis,[19] although no difference in GTPase activity was observed on phosphorylation of Ser656, the rat equivalent of Ser637.[20] Dephosphorylation of Ser637 or Ser656 by the phosphatase, calcineurin, resulted in Drp1 translocation to the mitochondria and fission.[20,21]

In contrast, a recent report has shown that the phosphorylation of Ser600 of Drp1 isoform 3 (equivalent to Ser637 on human Drp1 splice variant 1) by the Ca^{2+}/calmodulin-dependent protein kinase Iα (CaMKIα) resulted in mitochondrial fission, possibly due to an increased affinity for hFis1.[22] This effect could be reproduced by high potassium extracellular levels, which induced calcium influx

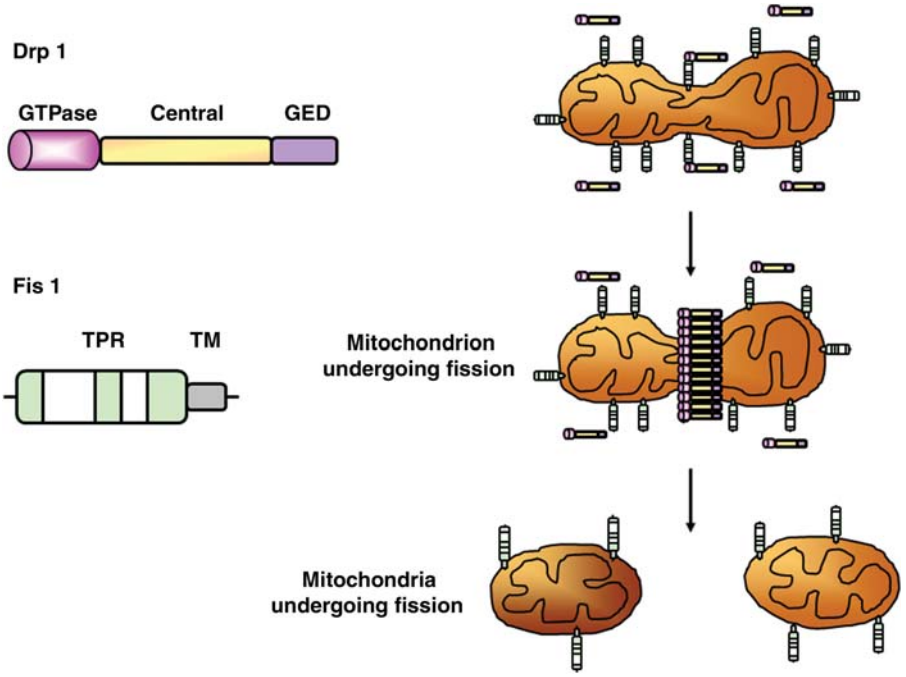

Figure 1 Mitochondrial fission. The mitochondrial fission protein, Drp1, is located mainly in the cytosol and comprises a GTPase, a central region, and a GTPase effector domain (GED) or assembly domain. Fis1 is localized in the OMM with most of the protein facing into the cytosol, acting as a docking station for Drp1. On activation, Drp1 translocates to the mitochondria (a process which is regulated by phosphorylation and sumoylation), oligomerizes, and constricts the mitochondrial scission site, a process which requires GTPase, thereby resulting in mitochondrial division.

through voltage-dependent calcium channels.[22] Therefore, it appears that the same serine residue (Ser637 on human Drp1 splice variant 1 and Ser600 on Drp1 isoform 3) resulted in mitochondrial fission if phosphorylated by CaMKIα, but mitochondrial fusion if phosphorylated by PKA. Finally, a recent study in developing skeletal muscle suggests that nitric oxide–cGMP–PKG may result in phosphorylation and inhibition in Drp1 pro-fission activity, thereby allowing the development of elongated mitochondrial networks required in the change from myoblast to myotube; however, in this study, the site of Drp1 phosphorylation was not determined.[23]

2.2 Human mitochondrial fission protein 1

Mammalian Fis1 was first identified in 2001 by its homology with Fis1p, the yeast orthologue.[10,24] It is a small protein of 17 kDa and 152 amino acids whose primary function is to promote fission of mitochondria and peroxisomes (*Figure 1*). It is ubiquitously expressed and is detected throughout the mitochondrial network where it is inserted into the OMM. It inserts into the OMM via its COOH-terminal part, which contains an α-helix, a transmembrane domain, and a COOH-terminal tail exposed to the intermembrane space. The NH_2-terminal part of the protein contains four distinct regions with five α-helices,[25,26] with the first α-helix of rat Fis1 critical for oligomerization and for its fission activity.[27] The next four α-helices make up two tetratrico-peptide repeat peptides (TPR1 and TPR2), which are involved in protein–protein interactions required for fission but are not required for Fis1 oligomerization.[27]

Over-expression of Fis1 in cells induces mitochondrial fragmentation which can result in Drp1-dependent cytochrome *c* release and apoptosis.[28] In contrast, inhibiting Fis1 can protect against apoptotic cell death.[28] Fis1 can also induce apoptotic cell death independent of Drp1 and can promote mitochondrial fragmentation without inducing apoptotic cell death.[29] Finally, Fis1 is required to induce mitochondrial fission in senescent long mitochondria to facilitate their removal by mitophagy.[30]

2.3 Mitofusins 1 and 2

Mitofusin 1 (Mfn1) and Mfn2, which were first discovered by Santel and Fuller[31] in 2001, are transmembrane GTPases located in the OMM whose primary function is to induce fusion of this membrane. They are the mammalian homologues of *Drosophila* Fuzzy onions protein (Fzo1p), a transmembrane GTPase first discovered in 1997, which is required for the formation of the giant mitochondrial derivative during spermatogenesis.[32] Independent to this discovery, Mfn2 has also been identified as mitochondrial assembly regulatory factor in muscle from obese Zucker rats[33] and hyperplasia suppressor gene (HSG) in vascular smooth muscle cells (VSMCs),[34] which in part may explain some of the observed non-fusion-related effects of Mfn2.

Structural studies of Mfn1 (743 residues) and Mfn2 (757 residues) reveal that the COOH-terminal part of the proteins contains a transmembrane domain and a coiled-coil domain 2 (also called heptad repeat domain, HR2), whereas the NH_2-terminal part of the proteins contains the GTP-binding domain and another coiled-coil domain (HR1) (*Figure 2A*).[35] The GTPase activity is required for both Mfn1- and Mfn2-mediated mitochondrial fusion.[31] The mitofusins mediate the first step of mitochondrial fusion, which comprises the fusion of the OMM. The HR2 domains tether the mitofusin proteins of two

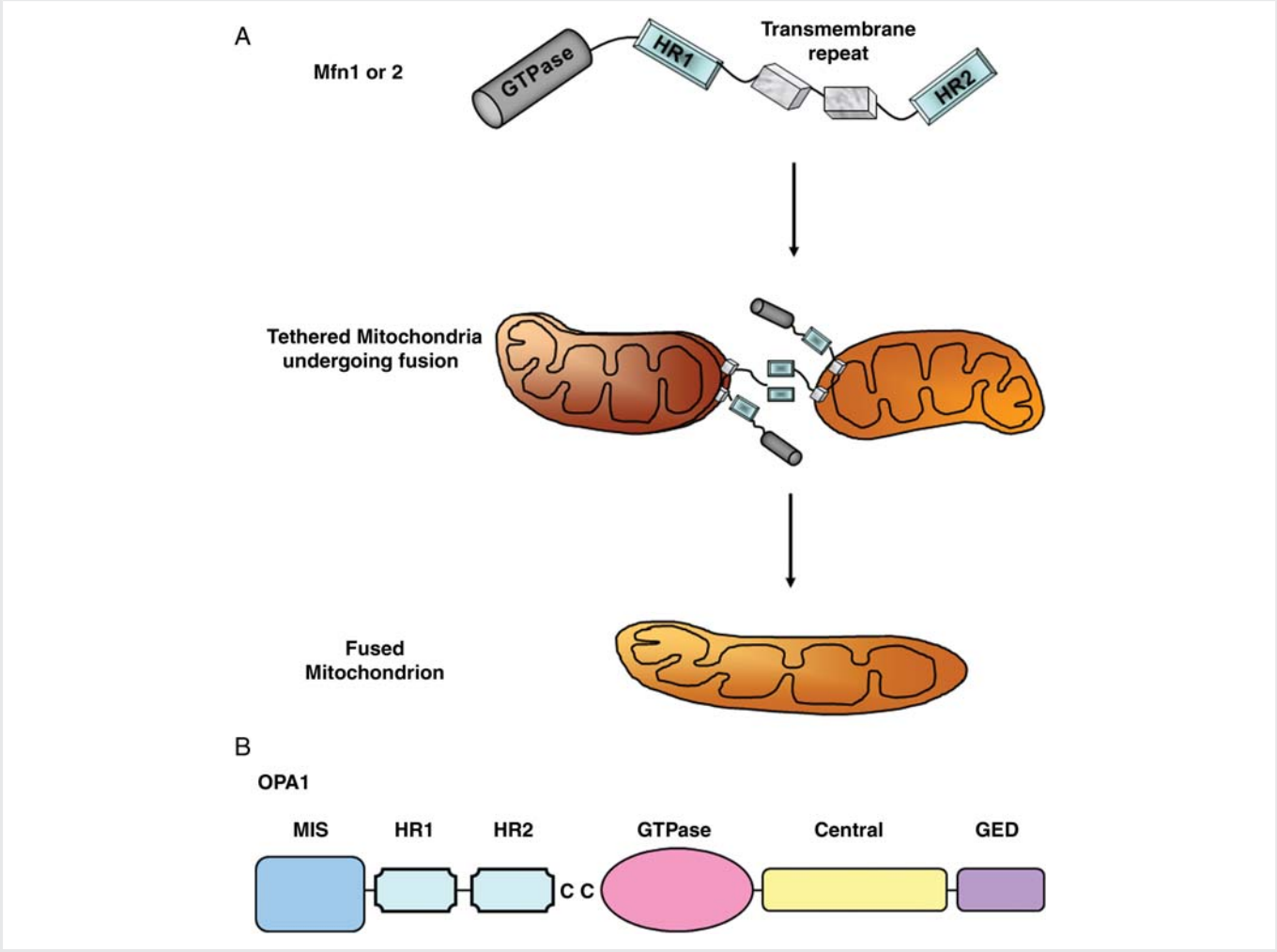

Figure 2 (*A*) Mitochondrial fusion. The mitochondrial fusion proteins Mfn1 and Mfn2 are located on the OMM with a cytosolic GTPase domain and two hydrophobic heptad repeat (HR) regions separated by a transmembrane repeat. The COOH-terminal HR region (HR2) mediates oligomerization between Mfn molecules on adjacent mitochondria allowing the membranes to fuse. GTP hydrolysis facilitates the fusion process. (*B*) OPA1 structure. The mitochondrial fusion protein, OPA1, comprises an N-terminal mitochondrial import sequence (MIS), hydrophobic heptad repeat (HR) segments, coiled-coil domain (C C), GTPase domain, a central domain, and a GTPase effector domain (GED) at the C terminus. OPA1 mediates the fusion of the inner mitochondrial membranes.

adjacent mitochondria, forming homotypic (Mfn1–Mfn1) or heterotypic (Mfn1–Mfn2) dimers (*Figure 2A*).

Mfn1 and Mfn2 mRNA tissue levels vary—Mfn1 is present throughout the body but dramatically elevated in the heart, whereas Mfn2 levels are elevated in the heart and muscle tissue but at low levels in other tissues.[35] The two mitofusins also differ in their ability to induce mitochondrial fusion with Mfn1-tagged mitochondria having greater GTPase activity and better tethering ability, when compared with Mfn2.[36]

The physiological non-fusion function of Mfn1 in adult mammalian tissues is unknown, although it is believed to be required during embryonic development for correct placental formation.[37] In contrast, Mfn2 has been demonstrated to exert a variety of pleiotropic effects, many of which are independent of its ability to promote mitochondrial fusion.[38] Most recently, Mfn2 has been reported to be present in endoplasmic reticulum (ER), tethering mitochondria to the ER,[39] and allowing the communication of calcium between these two organelles. Mfn2 ablation in mice is embryonic lethal but mutations in humans causes Charcot–Marie–Tooth type IIa, a peripheral sensorimotor neuropathy.[40]

2.3.1 Pleiotropic actions of Mfn2

2.3.1.1 Cardiac hypertrophy and VSMC proliferation

In 2004, Chen *et al.*[34] were the first to implicate the mitochondrial fusion protein, rat Mfn2, as a novel HSG, capable of inhibiting VSMC proliferation in a variety of vascular proliferative conditions. Interestingly, these effects of Mfn2 were independent of its ability to promote mitochondrial fusion. These authors found that the expression of HSG was reduced in VSMC harvested from spontaneously hypertensive rat (SHR) arteries and angioplasty balloon-induced endothelial injury in arteries from Wistar Kyoto rats and in murine arteries from *ApoE*-knockout mice.[34] The over-expression of HSG reduced VSMC proliferation induced by serum, blocked angioplasty balloon-induced neointimal VSMC proliferation, and inhibited restenosis in rat carotid arteries, effects which were found to be dependent on the binding of Mfn2 to Ras and the subsequent

inhibition of Ras-Raf-MAPK-Erk1/2 signalling, one of the major growth factor responsive signalling pathways in mammalian cell proliferation.[34] In a subsequent study, the same authors have demonstrated that the over-expression of Mfn2 inhibits VSMC proliferation triggered by oxidative LDL and also reduced atherosclerotic formation, beneficial effects which were dependent on the down-regulation of the MEK1/2–Erk1/2 and P3IK–Akt pathways.[41] With respect to a potential mechanism for this anti-proliferative effect of Mfn2, the same authors have gone onto show that over-expressing Mfn2 promotes apoptotic cell death in VSMC.[42] The pro-apoptotic effect of Mfn2, which was again found to be independent of its action on mitochondrial morphology, was reported to be mediated through the down-regulation of the PI3K-Akt pathway and executed through the mitochondrial pathway of apoptosis.[42] Most recently, the same researchers have implicated PKA in the regulation of Mfn2 in its anti-proliferative actions on VSMCs in culture, and neointimal hyperplasia and restenosis in a rat carotid artery balloon injury model, in a manner which was found to be independent on mitochondrial morphology.[43] These authors demonstrated that after mutating a PKA-specific phosphorylation site (Ser442) on Mfn2, the anti-proliferative effects in VSMC could be regulated such that constitutive phosphorylation at this site inhibited the suppressive effects on VSMC proliferation and preventing the phosphorylation of this site actually enhanced this anti-proliferative effect.[43]

Whether Mfn2 has a similar role to play in cardiomyocytes has been investigated by Fang *et al.*,[44] who have examined the role of Mfn2 in several different *in vitro* and *in vivo* models of cardiac hypertrophy and demonstrated reduced mRNA expression of Mfn2 associated with increased Erk1/2 expression in phenylephrine-treated neonatal rat cardiomyocytes, SHR hearts, mice with transverse aortic constriction (TAC), and β2-TG mice. Interestingly, Mfn2 was not found to be down-regulated in hypertrophied hearts with 15 weeks of TAC, nor in hypertrophied non-infarcted myocardium post-myocardial infarction (MI).[44] However, protein levels of Mfn2 were not assessed and the significance of these findings remains undetermined.

2.3.1.2 Apoptotic cell death

Studies suggest that Mfn2 is able to form a functional unit with Drp1 and BAX at OMM scission sites to mediate apoptotic cell death and this effect is independent of its pro-fusion effect.[45] Shen *et al.*[46] have shown that Mfn2 may actually mediate oxidative stress-induced apoptotic cell death in neonatal cardiomyocytes. In this study, the authors demonstrated that hydrogen peroxide, hypoxia, simulated ischaemia, and serum starvation all induce an increase in mRNA expression in neonatal cardiomyocytes after 4 h which peaked at 8 h and lasted for 24 h.[45] Over-expressing Mfn2 was sufficient to induce apoptotic cell death which was mediated by the mitochondrial pathway as it was prevented by Bcl-xL, PI3K-Akt activation, and caspase 9 inhibition.[45] Finally, siRNA antagonism of Mfn2 prevented oxidative stress-induced apoptotic cell death in the H9C2 cardiac cell line.[45] Interestingly, this apparent pro-apoptotic role of Mfn2 appears contrary to its protective effect discussed in later sections,[47] which may reflect differences between the pleiotropic and pro-fusion effects of Mfn2.

In contrast, there are studies which suggest that Mfn2 can protect cerebellar neurons against apoptotic cell death induced by DNA damage, oxidative stress, or potassium deprivation.[48] Again this neuroprotective effect appeared to be mediated, independent of it pro-fusion effects.

2.3.1.3 ER tethering to mitochondria

It has recently been demonstrated that Mfn2 present on the ER forms complexes with either mitochondrial Mfn1 or Mfn2, thereby tethering the ER to mitochondria.[39] The close juxtaposition of ER to mitochondria has been demonstrated to be required for IP3 signalling and calcium-induced apoptotic signalling—whether this is relevant to the heart remains to be determined.

2.4 Optic atrophy 1

Mutations in the OPA1 gene were first identified in 2000 to be associated with the human neurogenerative condition, autosomal dominant optic atrophy.[49] The OPA1 protein is a GTPase of the dynamin family, which is present in the inner mitochondrial membrane. Its main function is to promote mitochondrial fusion of the inner mitochondrial membranes,[49] although it can also prevent apoptotic cell death with its role in cristae remodelling.[50] It is the human homologue of the yeast mitochondrial-shaping protein Mgm1 first discovered in yeast in 1992.[51] OPA1 is expressed throughout the body, but is present in largest quantities in the retina, brain, testis, liver, heart, skeletal muscle, and pancreas.[52] Structurally, it comprises an NH_2-terminal mitochondrial import sequence, hydrophobic segments, coiled-coil domain, GTPase domain, a middle domain, and GED at the COOH terminus (*Figure 2B*). The hydrophobic segments constitute the transmembrane domain that has been predicted to anchor or associate OPA1 with the mitochondrial membranes. The GTPase domain is required for mitochondrial fusion.[53] Alternative splicing of OPA1 can produce eight different human isoforms of OPA1 (four in mice) with differing actions depending on the exon they contain. The OPA1 isoform 1 (containing exon 4) may mediate mitochondrial fusion and maintain mitochondrial membrane potential, whereas alternative isoforms containing exons 4b and 5b may control cytochrome *c* release.[52] Proteolytic cleavage of OPA1 at two discovered cleavage sites add further complexity to its regulation with longer isoforms in general mediating mitochondrial fusion. One protease is presenilin-associated rhomboid-like protease, which generates a small soluble form of OPA1 which locates in the intermembrane space and is responsible for keeping cytochrome *c* contained within cristae, thereby preventing apoptosis.[50,54] A role for OPA1 in 'tagging' depolarized fragmented mitochondria for removal by autophagy has recently been proposed.[55] In addition, OPA1 has been reported to regulate cristae junctions preventing the cristae remodelling required for the cytosolic release of cytochrome *c* during apoptosis, an action which is independent of its effect on mitochondrial morphology.[50]

3. Mitochondrial morphology and apoptosis

Changes in mitochondrial morphology have been linked to apoptotic cell death.[56] In response to an apoptotic stimulus, it has been demonstrated that cells undergo mitochondrial fission generating fragmented mitochondria. However, the presence of mitochondrial fragmentation does not necessarily indicate that the cell is undergoing apoptosis. Frank *et al.*[8] first demonstrated that the mitochondria of COS cells changed from a reticulo-tubular interconnected network to a fragmented discrete punctiform phenotype in response to the apoptotic inducer, staurosporine, a process which was dependent on the activation and mitochondrial translocation of Drp1. Crucially, transgenic

over-expression of $Drp1_{K38A}$, a dominant negative form of Drp1, prevented mitochondrial cytochrome *c* release and apoptotic cell death.[8] Subsequently, it has been demonstrated that the process of OMM rupture, a key step in mitochondrial-induced apoptosis, is actually mediated by a complex interplay between Drp1, the pro-apoptotic protein BAX, and Mfn2 which form foci on the OMM.[45] It has been suggested that the formation of Drp1-induced mitochondrial fission sites are required for BAX translocation to the mitochondria, as the over-expression of the anti-apoptotic protein BCL-xL prevented apoptosis but not mitochondrial fragmentation.[57] Under normal conditions, it has been suggested that 20% of Drp1 is associated with mitochondria, and the presence of BAX mediates an increase in mitochondrial Drp1 translocation prior to mitochondrial cytochrome *c* release. The translocation of Drp1 is also regulated by phosphorylation and sumoylation (see Section 2.1.1). In addition, it has been suggested that accumulation of Mfn2 at these foci mediates BAX-induced OMM permeabilization.[45] There is the intriguing possibility that these three proteins may form functional units at sites where ER tethers to mitochondria, thereby introducing calcium from the ER into the equation.[39] However, it must be appreciated that this pro-apoptotic effect of Mfn2 appears to be independent of its ability to induce mitochondrial fusion (see Section 2.3.1). Finally, although Drp1 may dock with hFis1 to induce mitochondrial fission, the latter induces apoptotic cell death independent of both Drp1 and BAX, and does so via the ER apoptosis pathway.[24,29]

4. Mitochondrial morphology and autophagy

It is accepted that the process of mitochondrial fusion allows for the transfer of soluble and membranous components including mitochondrial DNA between mitochondria, thereby providing a mechanism for renewing function in damaged mitochondria. Emerging studies in non-cardiac cells suggest that changes in mitochondrial morphology may be essential in selecting damaged depolarized mitochondria for removal by autophagosomes (termed mitophagy).[55,58] Selective mitochondrial fusion between two polarized mitochondria is synchronized to mitochondrial fission with the creation of uneven daughter mitochondria, one of which is depolarized and the other is polarized—the depolarized mitochondria is then removed by mitophagy,[55] a process which appears to be dependent on proteolytic cleavage of OPA1.[55] It has been said that the frequency and selectivity of fusion are required to maintain healthy mitochondrial function and allow mitophagy to take place.[58] Some studies have suggested that mitochondrial depolarization induced by the opening of the mitochondrial permeability transition pore (mPTP) may stimulate removal by autophagy.[59,60] The physiological role for the mPTP as a signal for autophagy has been recently confirmed in cardiomyocytes lacking cyclophilin D (an essential component of the mPTP), which appeared to be resistant to starvation-induced autophagy.[61] Whether changes in mitochondrial morphology are able to influence the process of mitophagy in the cardiovascular system remains to be determined.

5. Mitochondrial morphology and metabolism

The concept that changes in mitochondrial morphology may impact on mitochondrial metabolism was first introduced in 1988 before the identification of the first mitochondrial-shaping proteins. Amchenkova *et al.*[62] noted that long mitochondrial filaments in fibroblasts and mitochondrial clusters in cardiomyocytes were electrically interconnected allowing the movement of energy from the cell to the periphery. The mitochondrial fusion proteins, Mfn2 and OPA1, play an important role in mitochondrial metabolism. Knock-down of Mfn2 has been demonstrated to reduce mitochondrial membrane potential, lower oxygen consumption, and reduce substrate oxidation, resulting in less oxidative phosphorylation and increased dependency on anaerobic glycolysis with a higher rate of glucose uptake and a lower rate of glycogen synthesis.[33,63] These effects appear to be mediated through the reduced expression and activity of various subunits of the electron transport carriers, resulting in down-regulation of oxidative phosphorylation.[63] In addition, the lack of Mfn2 may reduce calcium-dependent increases in mitochondrial respiration due to a loss of tethering between the ER and mitochondria.[39] The over-expression of Mfn2 activates mitochondrial metabolism and increases the expression of subunits of the oxidative phosphorylation system in a manner which appears to be independent of mitochondrial fusion.[63] Knock-down of OPA1 results in similar effects with lower mitochondrial membrane potential and decreased respiration rates via decreased activity of complexes I, II, and III of the electron transport chain.[64,65] However, the over-expression of OPA1 did not improve mitochondrial respiration.[64] Interestingly, knock-down of the mitochondrial fission protein, Drp1, has similar effects on mitochondrial metabolism,[66] suggesting that any alteration in the mitochondrial network appears to impact on metabolism in a negative way. Whether the effects on mitochondrial metabolism are due to pleiotropic actions of the mitochondrial fusion or fission protein or are the result of changes in mitochondrial morphology is not thoroughly understood.

6. Mitochondrial morphology and the adult heart

The phenomenon of mitochondrial morphology has recently been investigated in the cardiovascular system using cultured cardiovascular cell lines, in which the mitochondria are arranged in a filamentous network and in which the mitochondria are highly dynamic and are constantly undergoing fusion and fission. A similar mitochondrial network is present in primary vascular endothelial cells, VSMCs, cardiac cell lines, and neonatal cardiomyocytes, making them amenable to the exploration of mitochondrial morphology. The HL-1 cardiac cell line can be readily used to assess mitochondrial morphology (*Figure 3A* and *B*).

However, in the adult cardiomyocyte, there are three distinct populations of mitochondria: (i) peri-nuclear mitochondria, (ii) subsarcolemmal (SSC) mitochondria, and (iii) interfibrillar (IF) mitochondria (*Figure 4*). Whether the unique spatial organization of IF mitochondria in adult cardiomyocytes into a regular crystal-like lattice arrangement[67] restricts their movements and prevents them from undergoing fusion or fission is unclear. Of course, the processes may actually occur in the adult heart but at a far slower rate. Certainly, the machinery required for controlling mitochondrial morphology, in terms of the mitochondrial and fission proteins, are abundantly present in the adult heart and would have been active during cardiomyocyte differentiation to ensure the unique spatial organization of the three different subpopulations of cardiac mitochondria. However, the role

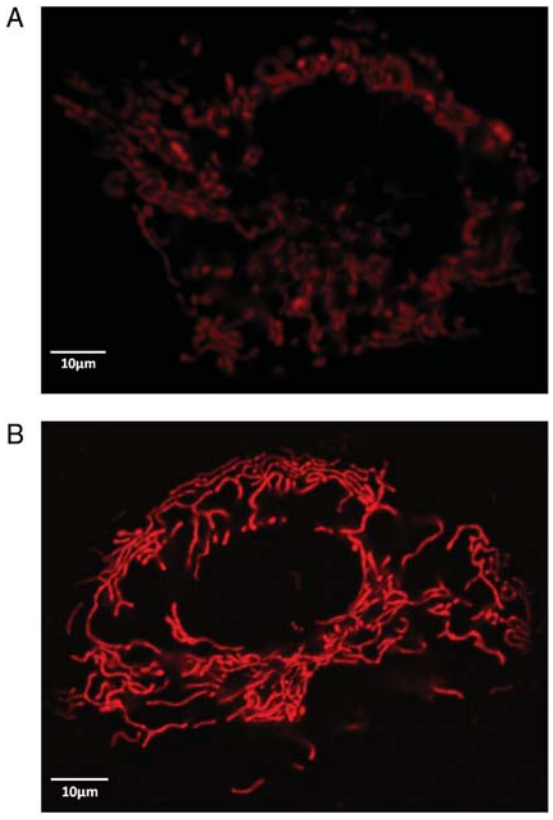

Figure 3 Representative confocal images of HL-1 cardiac cells transfected with mitochondrial red fluorescent protein depicting: (*A*) a cell displaying relatively fragmented mitochondria and (*B*) a cell displaying relatively elongated mitochondria.

of the mitochondrial fusion and fission proteins in the adult heart is presently unclear.

The conventional belief is that adult IF cardiac mitochondria are confined to the limits of a single sarcomere, with one or two such mitochondria lying alongside an adjacent sarcomere. This arrangement of adult cardiac mitochondria would of course be expected to restrict their movements. It has been recently demonstrated that IF mitochondria in adult rat cardiomyocytes are dynamic structures which are capable of undergoing rapid low-amplitude fluctuations, although they appear isolated with respect to electrical activity.[68] However, electron microscopy of adult rat hearts has provided evidence of elongated mitochondria, extending to more than two to three sarcomeres in length,[69,70] and as far back as 1969, Sun *et al.*[71] demonstrated 'mitochondrial fusion' occurring in isolated perfused adult rat hearts in response to short periods of hypoxia (3–7 min), which resulted in the formation of elongated mitochondria ranging from three to seven sarcomeres in length. This raises the interesting, although at this point speculative, possibility that short non-lethal periods of hypoxia may have induced mitochondrial fusion as an endogenous protective mechanism from further hypoxic or ischaemic insult. In this regard, a recently published study suggests that mitochondrial fusion may be an initial response to a low level of stress, which provides a protective response against a future insult.[72] Examples of elongated mitochondria extending over two sarcomeres in length in untreated non-diseased adult murine hearts are shown in *Figure 5A* and *B*.

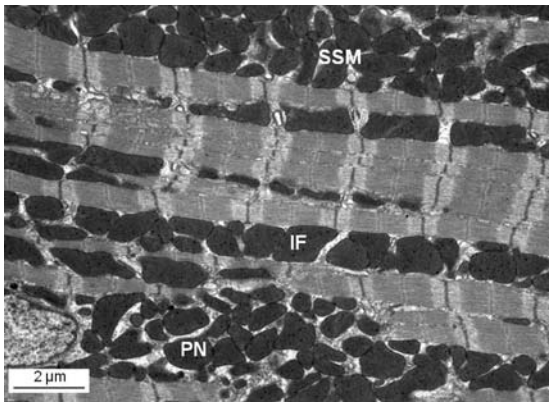

Figure 4 Representative electron micrograph of adult murine heart depicting the three subpopulations of mitochondria: perinuclear (PN) mitochondria which are freely arranged in an area adjacent to the nucleus; interfibrillar (IF) mitochondria which are arranged along the myofibrils alongside the sarcomere; subsarcolemmal mitochondria (SSM) which are freely arranged in an area located just beneath the subsarcolemma.

Other electron microscopic studies of adult rat cardiac mitochondria have reported inter-mitochondrial contacts,[69] partitioning between mitochondrial membrane suggestive of division,[73] and evidence of membrane fusion[74] between adjacent mitochondria. In the setting of cardiac disease, more marked changes in mitochondrial morphology have been observed. Disorganized small mitochondria have been observed in a variety of cardiac conditions such as dilated cardiomyopathy[75] and myocardial hibernation.[76] Most recently, giant mitochondria arising from fusion of large and normal mitochondria have been observed using transmission and three-dimensional scanning electron microscopy in a patient with diagnosis of mitochondrial cardiomyopathy.[77]

However, the clear disadvantage of electron microscopy is that it only provides a snap-shot in time and space, and cannot visualize directly mitochondrial dynamics or mitochondrial morphology. For example, the presence of elongated mitochondria in the adult heart does not necessarily imply increased rate of mitochondrial fusion as they could have arisen from reduced mitochondrial fission. There exist several experimental methods for assessing the process of mitochondrial fusion. *In vitro*, these are based on a polyethylene glycol assay, in which the fusion of mitochondria is assessed by the combination of mitochondrial red and green fluorescent proteins.[78] More recently, an *in vitro* quantitative assay for mitochondrial fusion based on *Renilla* luciferase complementation has been described.[79] *In vivo*, the process of mitochondrial fusion can be tracked using a photo-activatable mitochondrial green fluorescent protein.[80] Whether these techniques can be applied to monitor mitochondrial fusion in the adult heart remains to be determined.

6.1 Mitochondrial morphology and adult skeletal muscle

In adult skeletal muscle, in which IF mitochondria are also arranged in a similar manner to the adult heart, several roles for mitochondrial morphology have been recently demonstrated. Chen *et al.*[81] have demonstrated that the conditional inactivation of Mfn1 or Mfn2 in

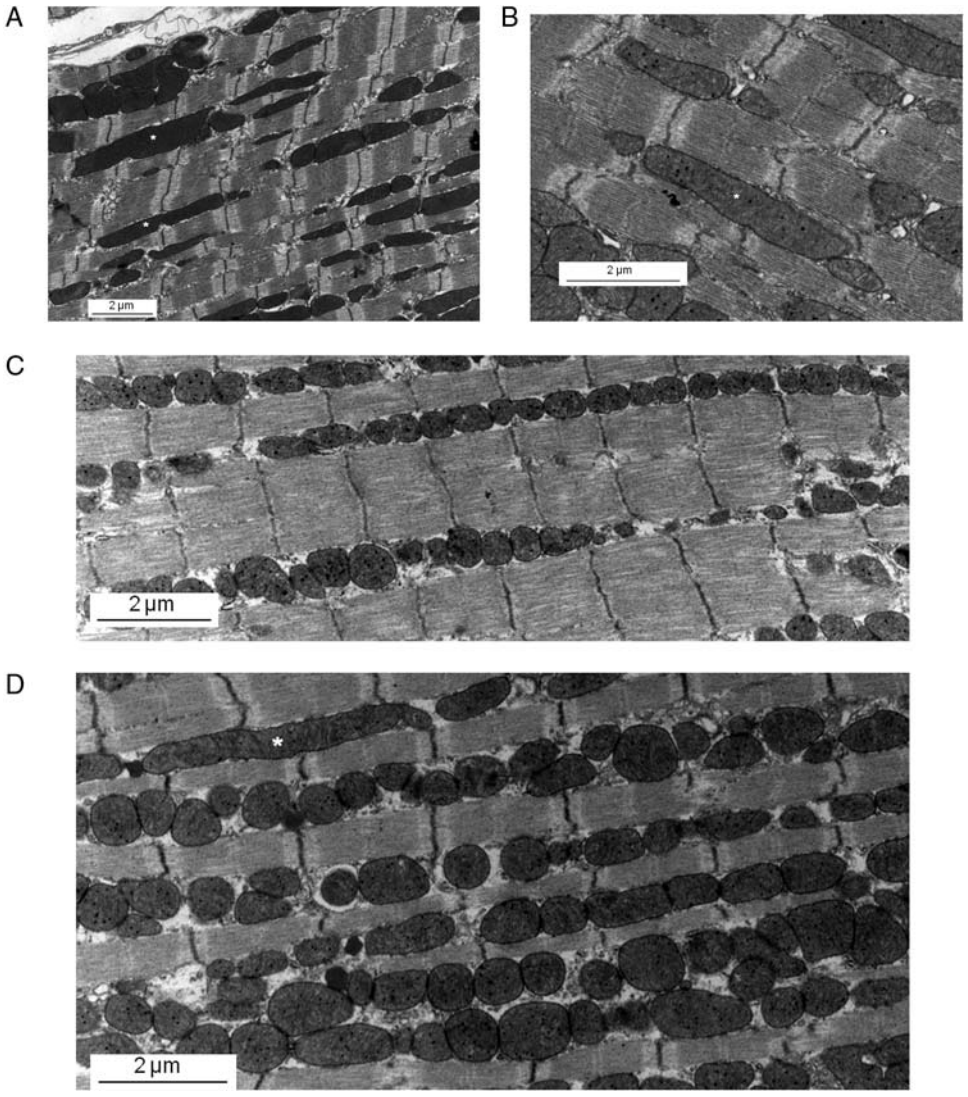

Figure 5 (*A* and *B*) Representative electron micrographs of adult murine heart depicting elongated IF mitochondria marked with an asterisk, extending two to three sarcomeres in length. (*C* and *D*) Representative electron micrographs of adult murine heart following an episode of mild ischaemia (20 min), depicting fragmented rounded IF mitochondria. Of note, there is one elongated mitochondrion present on (*D*) marked with an asterisk.

murine tibialis anterior skeletal muscle induced mitochondrial fragmentation and accumulation of both IF and SSC mitochondria. This genetic manipulation also prevented the exchange of mitochondrial DNA, resulting in the accumulation of defective DNA, confirming the essential requirement for mitochondrial fusion in mitochondrial DNA renewal.[81]

In contrast, the process of mitochondrial fission has been implicated in the pathogenesis of adult skeletal muscle atrophy, a condition which is executed by the autophagy–lysosomes and ubiquitin–proteasome systems.[82] Romanello *et al.*[82] found that the *in vivo* genetic inhibition of Drp1 and hFis1 prevented the development of skeletal muscle atrophy, whereas expression of the pro-fission machinery was reported to induce skeletal muscle wasting via AMPK activation.

Most recently, changes in mitochondrial morphology have been implicated in the development of adult skeletal muscle. Specifically, De Palma *et al.*[23] have demonstrated that the inhibition of mitochondrial fission is required during myogenic differentiation to allow the short mitochondria in myoblasts to change into the elongated mitochondrial networks of myotubes. These authors have found that nitric oxide–cGMP produced by muscle-specific neuronal synthase is responsible for inhibiting Drp1-mediated mitochondrial fission, thereby allowing the development of mitochondrial networks required for myogenic differentiation.[23]

These findings suggest that changes in mitochondrial morphology are relevant to adult skeletal muscle. Whether similar processes occur in adult cardiac muscle, in which the mitochondrial arrangement is similar to that in adult skeletal muscle, remains to be determined.

6.2 Mitochondrial morphology and cardiomyocyte apoptosis

Several experimental studies have investigated changes in mitochondrial morphology in response to apoptotic stimuli in primary neonatal cardiomyocytes (*Table 2*). Parra *et al.*[47] found that ceramide-induced

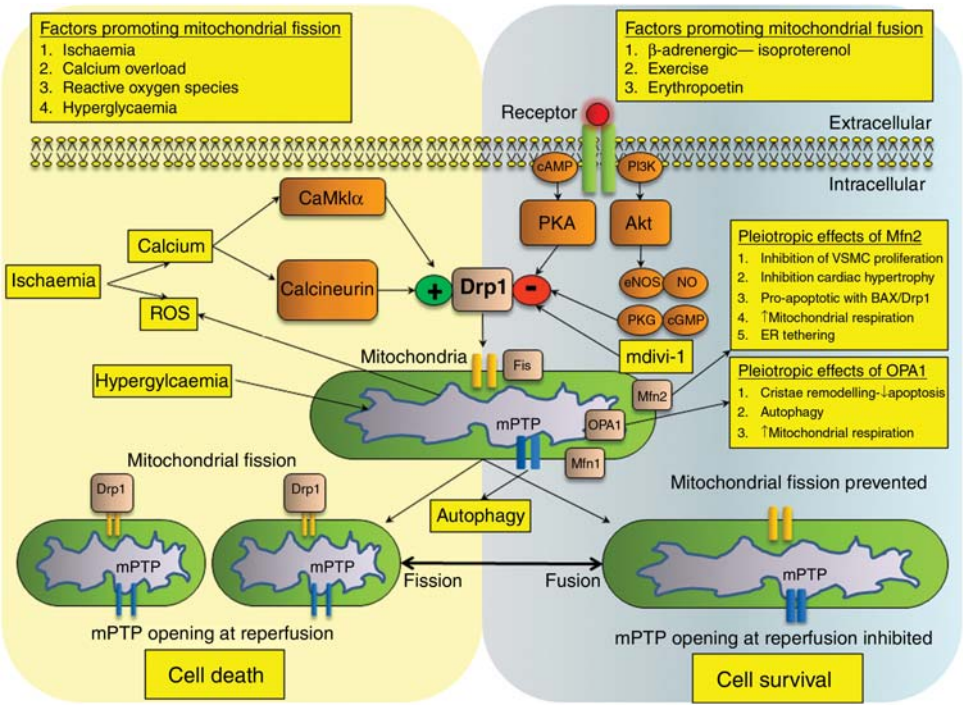

Figure 6 Hypothetical scheme highlighting the potential roles of mitochondrial morphology in cardiovascular disease with factors promoting mitochondrial fission on the left of the figure and factors promoting mitochondrial fusion on the right of the figure. Central to the control of mitochondrial fission is the protein, Drp1, whose activity is regulated by phosphorylation. Cytosolic calcium overload (such as in ischaemia) induces mitochondrial fission by two mechanisms: (i) increasing the activity of calcineurin mediated dephosphorylation of Drp1 at Ser637[21] or (2) by increasing the activity of CaMkII which phosphorylates Drp1 at Ser637.[22] In addition, in both the diabetic heart and the non-diabetic heart, hyperglycaemia induces mitochondrial fission through Drp1,[98,103] through a mechanism which is dependent on reactive oxygen species (ROS). Mitochondrial fission results in mitochondria which are more susceptible to mPTP opening, which results in cell death at the time of myocardial reperfusion.[84] The phosphorylation of Drp1 at Ser637 by PKA prevents the translocation of Drp1 to the mitochondria and inhibits the process of mitochondrial fission. PKA can be activated using cAMP analogues, or by exercise or β-adrenergic stimulation using isoproterenol.[19,20] Our recent preliminary data suggest that the cytokine erythropoietin can also induce mitochondrial fusion through the activation of Akt in cardiac cells, but the mechanism downstream of Akt remains underdetermined. However, one downstream target of Akt is the eNOS–NO–cGMP–PKG pathway which has recently been reported to phosphorylate and inhibit the pro-fission activity of Drp1.[23] The mitochondrial division inhibitor-1 (mdivi-1) is a recently described small molecule inhibitor of Drp1,[105] which has been demonstrated to limit both myocardial[84] and renal ischaemic injury.[88] In addition, the over-expression of Mfn1, Mfn2, and Drp1$_{K38A}$ can inhibit mitochondrial fission and promote survival in a cardiac cell line. However, both OPA1 and Mfn2 have a number of pleiotropic effects which appear independent of their pro-fusion effects. The inhibition of mitochondrial fission or the promotion of mitochondrial fusion prevents mPTP opening at reperfusion promoting cell survival. Recently, the mPTP has been demonstrated to play a role in signalling to the cell that a mitochondrion needs to be removed by autophagy.

mitochondrial fragmentation, elevated levels of Drp1 and hFis1, increased co-localization of Drp1 with hFis1 and apoptotic cell death in primary neonatal cardiomyocytes. In addition, doxorubicin treatment which is associated with cardiomyopathy and apoptosis also induced mitochondrial fragmentation. Importantly, knocking down Mfn2 enhanced mitochondrial fragmentation and membrane depolarization and increased cytochrome *c* release, suggesting that in this particular model, Mfn2 was protecting against ceramide-induced apoptosis by preventing mitochondrial fission.[47]

6.3 Mitochondrial morphology and cardiac differentiation

The development of undifferentiated non-beating embryonic stem cells into contracting cardiomyocytes requires a change in mitochondrial morphology from fragmented mitochondria to elongated mitochondrial filaments which are capable of mitochondrial oxidative phosphorylation to provide for the huge increase in energy demands.[83] This change in mitochondrial morphology has been reported to be associated with a reduction in Drp1 expression and an up-regulation of Mfn2.[83] As expected, disruption of mitochondrial respiration prevented cardiomyocyte differentiation.[83]

6.4 Mitochondrial morphology and IRI

Whether changes in mitochondrial morphology occur in the heart in response to IRI has been recently investigated by our research group[84] and others.[85] Brady *et al.*[85] were the first to demonstrate extensive fragmentation of mitochondria in HL-1 cells (a murine atrial-derived cardiac cell line) in response to a sustained episode of simulated ischaemia, changes which persisted into simulated reperfusion. Interestingly, in that study, the authors observed that reperfusing the ischaemic cells with SB203580, a pharmacological p38MAPK inhibitor, caused the mitochondria to re-fuse and re-gain their

Table 2 Experimental studies implicating changes in mitochondrial morphology in cardiovascular physiology and disease

Study	Condition	Cell type	Change in mitochondrial morphology	Other findings
Brady *et al.*[85]	Ischaemia–reperfusion injury	HL-1 cells	Mitochondrial fission	Pharmacological inhibition of p38MAPK activation at reperfusion allows mitochondrial re-fusion
Cribbs and Strack[20]	β-Adrenergic stimulation by isoproterenol or exercise	Adult murine heart	Not investigated	Phosphorylation and inhibition of Drp1 at Ser656
Chung *et al.*[83]	Cardiac differentiation	Embryonic stem cells	Mitochondrial fusion	Mitochondrial fusion is required to support oxidative phosphorylation
Yu *et al.*[98]	Hyperglycaemia	H9C2 rat myoblast	Mitochondrial fission	
Chen *et al.*[89]	Post-MI heart failure and dilated cardiomyopathy	Adult rat and human heart	Mitochondrial fragmentation	↓OPA1
Makino *et al.*[103]	Diabetes	Murine coronary endothelial cell	Mitochondrial fission	↓OPA1, ↑Drp1
Williamson *et al.*[104]	Diabetes	Adult murine diabetic heart	Mitochondrial fission	Increased mPTP opening and lower mitochondrial membrane potential
Ong *et al.*[84]	Ischaemia–reperfusion injury and cardioprotection	HL-1 cells, adult heart	Mitochondrial fission	Inhibiting mitochondrial fission cardioprotective
Hom *et al.*[87]	Cytosolic calcium overload	Neonatal cardiomyocytes and adult heart	Mitochondrial fission	

elongated structures once more, suggesting that p38MAPK activity during reperfusion may have contributed to the detrimental changes in mitochondrial morphology.[85] We have gone on to demonstrate in HL-1 cells that ischaemia-induced mitochondrial fragmentation is associated with the translocation of Drp1 from the cytosol to the mitochondria (a process which is required for its pro-fission activity) and that the fragmentation process can be largely prevented by transfection with $Drp1_{K38A}$ (the dominant-negative construct of Drp1).[84] Furthermore, in the adult murine heart, we have demonstrated the fragmentation of IF cardiac mitochondria (*Figure 5C* and *D*).

The mechanisms underlying mitochondrial fragmentation during ischaemia are currently unclear, although we speculate that cytosolic calcium overload and reactive oxygen species (ROS) may be contributory factors. One can speculate that by interfering with cellular respiration, mitochondrial fragmentation may result in the production of ROS. In this respect, using primary renotubular cells, Plotnikov *et al.*[86] demonstrated that pre-treatment with an anti-oxidant could prevent ischaemia-induced mitochondrial fragmentation. Interestingly, in that particular study, insulin was also reported to prevent ischaemia-induced mitochondrial fragmentation, although the mechanism was not further investigated. In the context of diabetes, mitochondrial fragmentation induced by hyperglycaemia has also been reported to result in the production of ROS (see Section 6.6); however, the actual interplay between mitochondrial fragmentation, ROS production, and mitochondrial respiration requires further investigation.

6.4.1 Calcium-induced changes in mitochondrial morphology

Studies have established that calcium can induce changes in mitochondrial morphology through two potential pathways. Cereghetti *et al.*[21] found in HeLa cells that inducing mitochondrial membrane depolarization using either arachidonic acid (an mPTP opener) or a protonophore resulted in cytosolic calcium overload which activated the phosphatase, calcineurin, which in turn dephosphorylated and activated Drp1, thereby allowing the latter to translocate to the mitochondria and induce mitochondrial fission. An alternative pathway in which calcium can induce Drp1 translocation to the mitochondria is through the activation of Ca^{2+}/CaMKIα in cultured hippocampal neurons.[22] Han *et al.*[22] found that calcium influx through the voltage-gated calcium channels (activated by extracellular potassium) activated CaMKIα, which phosphorylated and activated Drp1 at Ser600, allowing it to translocate to mitochondria to induce mitochondrial fission.

In the cardiac tissue, Hom *et al.*[87] have recently demonstrated that cytosolic calcium overload induced by either thapsigargin (a pharmacological inhibitor of calcium into the sarcoplasmic reticulum) or KCl (which opens L-type calcium channels via membrane depolarization) resulted in mitochondrial fragmentation in neonatal rat ventricular cardiomyocytes, an observation which was antagonized by transfection with $Drp1_{K38A}$. Importantly, these authors were then able to demonstrate calcium-induced mitochondrial fragmentation in adult rat ventricular cardiomyocytes, using electron microscopy to assess mitochondrial morphology in the adult heart.[87]

It will be important to determine whether ischaemia-induced mitochondrial fragmentation is dependent upon the activation of calcineurin, the dephosphorylation of Drp1, and Drp1 translocation to mitochondria. In subsequent studies, we have gone on to investigate the potential consequences of ischaemia-induced fragmentation in terms of susceptibility to IRI.

6.4.2 Changes in mitochondrial morphology and cardioprotection

Given that ischaemia appeared to induce mitochondrial fragmentation, we were interested to investigate the relevance of this change in mitochondrial morphology to the susceptibility to IRI, and whether by preventing this process, one might be able to protect the heart from IRI. We found that transfecting HL-1 cells with

$Drp1_{K38A}$, Mfn1 or Mfn2, promoted mitochondrial elongation and reduced cell death following simulated IRI (SIRI), suggesting that preventing ischaemia-induced mitochondrial fragmentation is cardioprotective.[84] Conversely, transfection with the mitochondrial fission protein hFis1 promoted mitochondrial fragmentation and increased cell death following SIRI.[84] Crucially, pre-treatment of HL-1 cells with the mitochondrial division inhibitor, mdivi-1 (a recently described small molecule Drp1 inhibitor), was equally beneficial, confirming the cardioprotective effects of inhibiting mitochondrial fission.[84] The ability to pharmacologically inhibit mitochondrial fission using mdivi-1 has allowed us to demonstrate in adult rodent cardiomyocytes and the adult rodent heart reduced cell death post-SIRI and decreased *in vivo* myocardial infarct size.[84] In addition, *in vivo* pre-treatment with the Drp1 inhibitor appeared to increase the proportion of elongated IF mitochondria (defined by mitochondria >2 μm in length, the average length of a sarcomere), assessed by electron microscopy.[84] Interestingly, similar findings have been described in the kidney in a study by Brooks *et al.*[88] who demonstrated that Drp1-dependent mitochondrial fission was an underlying mediator of a variety of forms of acute renal injury including IRI, and renoprotection could be achieved by inhibiting mitochondrial fission.

Our recent data suggest that Akt activation using either a transgenic approach or the treatment with the cytokine, erythropoietin, can induce mitochondrial elongation, prevent mPTP opening, and promote cell survival in the HL-1 cardiac cell line (unpublished data). Whether Akt induces mitochondrial fusion by regulating Drp1 in a similar manner to PKA and PKG or acts independently of Drp1 and mediates its effect through the mitochondrial fusion proteins is unknown.

6.4.3 Changes in mitochondrial fusion and fission proteins with IRI

Whether IRI induces changes in expression or protein levels of the mitochondrial fusion and fission proteins is unclear, and whether these changes are able to account for the observed ischaemia-induced mitochondrial fragmentation is unknown. However, investigating the individual role of these mitochondrial-shaping proteins may not be so straightforward, given that the expression of these proteins, the protein levels, any post-translational modifications, and finally their subcellular localization may all influence their activity. Chen *et al.*[89] have shown in the cardiac myogenic H9c2 cell line that ischaemia-induced mitochondrial fragmentation was associated with a reduction in protein levels of the mitochondrial fusion protein, OPA1. Furthermore, blocking OPA1 with siRNA induced mitochondrial fragmentation and apoptotic cell death, although the over-expression of OPA-1 did not protect the cells against ischaemia-induced apoptosis.[89] Of note, the pre-treatment of cells with cyclosporin A (CsA), a known mPTP inhibitor,[90] prevented the reduction in OPA1 induced by simulated ischaemia,[89] suggesting that the opening of the mPTP may influence levels of OPA1. However, CsA is also known to inhibit calcineurin, an effect which has been reported to prevent Drp1-mediated mitochondrial fragmentation,[21] and one cannot exclude this mechanism from being responsible for the apparent effects of CsA on OPA1 levels.

6.4.4 Changes in mitochondrial morphology and mPTP opening

The mPTP is a non-selective channel of the inner mitochondrial membrane which has been shown to open at the onset of myocardial reperfusion in response to mitochondrial calcium overload, ROS, and ATP depletion and results in cell death by uncoupling oxidative phosphorylation and inducing mitochondrial swelling.[91] Therefore, inhibiting mPTP opening using either CsA or sanglifehrin A (known mPTP inhibitors) can dramatically reduce myocardial infarct size in pre-clinical animal and human heart tissue models[92–95] and in patients undergoing primary percutaneous coronary intervention for an ST-elevation myocardial infarction.[96]

In terms of changes in mitochondrial morphology impacting on mPTP opening susceptibility, our preliminary data suggest that either inhibiting mitochondrial fission or inducing mitochondrial fusion can protect the heart from IRI by inhibiting mPTP opening.[84] Other studies in non-cardiac cells have also linked changes in mitochondrial morphology with mPTP opening susceptibility. Over-expressing the mitochondrial fission proteins Drp1 or hFis1 has been reported to increase the susceptibility to calcium-induced mPTP opening in COS epithelial cells,[97] whereas inhibiting Drp1 to prevent mitochondrial fission lowered mPTP opening susceptibility in response to hyperglycaemia and oxidative stress.[98] Neuspiel *et al.*[99] found that the over-expression of Mfn2 in COS-7 cells also inhibited the mPTP opening induced by free radicals. The mechanism through which inducing mitochondrial fusion actually prevents mPTP opening is not clear, although we speculate that: (i) elongated mitochondria may be able to accommodate a greater mitochondrial calcium load or withstand an increased burden of oxidative stress before undergoing mPTP opening when compared with fragmented mitochondria and (ii) mitochondrial fusion may generate mitochondria with greater respiratory capacity which are better equipped to withstand the metabolic and biochemical stresses associated with IRI. In this regard, it has been previously demonstrated that inhibiting the expression of mitochondrial fusion proteins Mfn1, Mfn2, or OPA1 generates fragmented mitochondria with impaired oxygen consumption, lower mitochondrial membrane potential, and decreased respiration.[64] Conversely, the over-expression of Mfn2 has been reported to induce elongated mitochondria with enhanced mitochondrial respiration capacity and hyperpolarized mitochondrial membrane potential in L6E9 myotubes.[63] In contrast, studies suggest that mPTP opening may induce changes in mitochondrial morphology, such that the induction of mPTP opening resulted in Drp1-mediated mitochondrial fission.[21] Clearly, further studies are required to investigate the link between changes in mitochondrial morphology and susceptibility to mPTP opening.

6.5 Changes in mitochondrial morphology and heart failure

Heart failure is associated with reduced mitochondrial oxidative phosphorylation and the production of oxidative stress from mitochondria. Whether changes in mitochondrial morphology contribute to the pathogenesis of mitochondrial dysfunction during heart failure is unknown. Disorganized small mitochondria have been observed in a variety of cardiac conditions such as dilated cardiomyopathy[75] and myocardial hibernation.[76] In a recent study, Chen *et al.*[89] also demonstrated disorganized small fragmented mitochondria in an adult rat model of post-MI heart failure. These authors found that the absolute number of mitochondria per area was significantly increased and the individual mitochondrial cross-sectional areas were significantly decreased in the adult rat heart failure hearts. In this heart failure model, protein levels of OPA-1 were reduced but there was no

change in the protein levels of Mfn1, Mfn2, Drp1, or hFis1.[89] Furthermore, mRNA expression of OPA1 was not changed suggesting that the reduction in OPA-1 proteins levels was probably due to post-transcriptional modification.[89] Finally, in this study, elevated protein levels of Mfn1, Mfn2, and Drp1, reduced levels of OPA-1, and no change in hFis1 were observed in human hearts with ischaemic cardiomyopathy, whereas in hearts with dilated cardiomyopathy, they demonstrated elevated protein levels of Mfn1, Mfn2, and Drp1, but no change in either OPA-1 or hFis1 levels.[89] The actual significance of these changes in protein levels of mitochondrial-shaping proteins with heart failure in terms of pathogenesis and if they are secondary to metabolic changes is unclear and requires further investigation. Whether inhibiting mitochondrial fission in the setting of heart failure would be beneficial in terms of improved mitochondrial and cardiac function is unknown.

6.6 Changes in mitochondrial morphology in the diabetic heart

Patients with diabetes mellitus have an increased incidence of cardiovascular disease and experience worse clinical outcomes following cardiovascular disease. A major contributory factor linked to this increased risk of cardiovascular disease is hyperglycaemia-induced mitochondrial oxidative stress,[100,101] which can induce cellular injury and dysfunction. Interestingly, recent *in vitro* data suggest that hyperglycaemia induces mitochondrial fragmentation.[102] Yu *et al.*[98] demonstrated in the H9c2 rat heart myoblast cell line that sustained hyperglycaemia induced mitochondrial fragmentation and mitochondrial ROS production resulting in cell death by mPTP opening and apoptosis. Importantly, this detrimental process could be prevented by transfecting cells with $Drp1_{K38A}$, suggesting that the hyperglycaemia-induced mitochondrial fragmentation was a Drp1-dependent process.[98]

Makino *et al.*[103] have subsequently demonstrated that coronary endothelial cells isolated from the diabetic murine heart (Type I model of diabetes) displayed more mitochondrial fragmentation when compared with non-diabetic mice and this change in mitochondrial morphology was abolished by 4 weeks pre-treatment with an anti-oxidant. These findings were associated with reduced levels of OPA1 and increased levels of Drp1 (levels of Mfn1, Mfn2, and hFis1 were unchanged), although treatment with anti-oxidant therapy did not change the levels of these mitochondrial-shaping proteins.[103] These data suggested a role for oxidative stress as a mediator of mitochondrial fragmentation, which was confirmed by the fact that oxidative stress induced further mitochondrial fragmentation in coronary endothelial cells.[103] A recent study has examined SSC and IF mitochondria from a streptozocin-induced diabetic adult mouse heart and found that the IF mitochondria were smaller, had lower mitochondrial membrane potential, and were more predisposed to mPTP opening and apoptosis.[104]

Whether mitochondrial fission contributes to the cardiac dysfunction associated with diabetic cardiomyopathy is unclear. Furthermore, whether inhibiting mitochondrial fission can improve cardiac function in this setting is unclear.

7. Conclusions

Recent studies suggest that changes in mitochondrial morphology may impact on a variety of different aspects of cardiovascular biology including cardiomyocyte differentiation, the response to IRI and cardioprotection, heart failure, apoptotic cell death, and autophagy (*Figure 6*). In particular, it appears that mitochondrial fission may be detrimental in IRI, diabetes, hyperglycaemia, and heart failure. In the setting of IRI, inhibiting mitochondrial fission has been reported to be cardioprotective. However, it must be appreciated that mitochondrial fission can be a physiological requirement for cardiac differentiation and autophagy to replenish damage mitochondria. Furthermore, it must be remembered that some of the mitochondrial-shaping protein have effects which are independent of their ability to change mitochondrial morphology and it is important to distinguish these pleiotropic effects.

Many of the studies have been performed in cardiac cell lines, neonatal cardiomyocytes, and vascular cells where the arrangement of mitochondria readily allows the assessment of mitochondrial dynamics and changes in mitochondrial morphology. However, emerging studies suggest that changes in mitochondrial morphology may also be relevant to the adult heart, despite the unique arrangement of adult cardiac mitochondria. Electron microscopy has allowed the visualization of both elongated and fragmented mitochondria, but more sophisticated confocal imaging techniques such as super-resolution STED (stimulated emission depletion) confocal microscopy will be required to visualize directly the process of mitochondrial fission and fusion occurring *in vivo*. Crucially, recent studies suggest that changes in mitochondrial morphology can be induced by activating particular protein kinases such as PKA, PKG, and Akt or by using pharmacological agents such as mdivi-1, a small molecule inhibitor of Drp1, which has been used to inhibit mitochondrial fission, with cardioprotective effects. Therefore, changes in mitochondrial morphology may provide novel therapeutic targets for the future treatment of cardiovascular disease.

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