



Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell?

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Mitochondrial dynamics is a recent topic of research in the field of cardiac physiology. The study of mechanisms involved in the morphological changes and in the mobility of mitochondria is legitimate since the adult cardiomyocytes possess numerous mitochondria which occupy at least 30% of cell volume. However, architectural constraints exist in the cardiomyocyte that limit mitochondrial movements and communication between adjacent mitochondria. Still, the proteins involved in mitochondrial fusion and fission are highly expressed in these cells and could be involved in different processes important for the cardiac function. For example, they are required for mitochondrial biogenesis to synthesize new mitochondria and for the quality-control of the organelles. They are also involved in inner membrane organization and may play a role in apoptosis. More generally, change in mitochondrial morphology can have consequences in the functioning of the respiratory chain, in the regulation of the mitochondrial permeability transition pore (MPTP), and in the interactions with other organelles. Furthermore, the proteins involved in fusion and fission of mitochondria are altered in cardiac pathologies such as ischemia/reperfusion or heart failure (HF), and appear to be valuable targets for pharmacological therapies. Thus, mitochondrial dynamics deserves particular attention in cardiac research. The present review draws up a report of our knowledge on these phenomena.

Keywords: mitochondrial dynamics, cardiomyocytes, adult, energetic metabolism, cytoarchitecture

INTRODUCTION

Mitochondria, which have long been regarded only as energy producers, are actually recognized at the crossroads of many cellular functions. Obviously, they play a crucial role in energy production in cells, but they are involved in other phenomena such as ion homeostasis, free radical production, and ultimately cell death. Many of their characteristics, as morphology, location in the cell, proximity to other organelles are important parameters that have to be considered to understand the mitochondrial functions. This is particularly true in the adult cardiac cells where mitochondria, which produce 90% of ATP, occupy 30% of cardiac cell volume and are embedded in a dense and complex organization. This interlinking reflects the challenging function of the heart which requires rhythmic contractions of the pump throughout the life, and consequently needs a fast and effective intracellular energy delivery to the ATP consumers of the cardiomyocyte (for review, see Ventura-Clapier et al., 2011). Moreover, the main phosphorylated metabolites do not vary with the increase in work (Balaban, 2012). This metabolic homeostasis and the tight coupling between mitochondria and ATP consumer sites are two of the peculiarities of the cardiac cell and require an optimized cellular organization to ensure efficient energy fluxes. Any modification of the cellular architecture, but also of the internal organization of mitochondria

could thus impair cell energetic and as a consequence cell function.

In the majority of cells, mitochondria are able to adjust their morphology and their location depending on energy needs and metabolic conditions (Hackenbrock, 1966; Bereiter-Hahn, 1990; Karbowski and Youle, 2003; Rossignol et al., 2004; Mannella, 2006; Benard et al., 2007; Soubannier and McBride, 2009). This “mitochondrial dynamics” seems to be particularly important during cell division and for mitochondrial quality control; it may also play a role under pathological conditions. At some point, mitochondrial network morphology actually is the result of several processes, including fusion and fragmentation of the organelles which are usually controlled by a complex protein machinery (for review, see Liesa et al., 2009). It is generally admitted that a connected mitochondrial network is observed in active metabolic cells (Skulachev, 2001) while the mitochondria are rather fragmented in quiescent cells (Collins et al., 2002). However, it should be kept in mind that mitochondria exhibit a high structural and functional tissue specificity in connection with cell functions. Thus, any finding on mitochondrial organization and functioning cannot be directly extrapolated before being considered in the framework of the considered cell or tissue. For example in adult cardiac cells, the relationship between mitochondrial morphology and function does not seem to be rigorous,

since cardiomyocytes are metabolically active but exhibit an apparently fragmented network (Kuznetsov et al., 2009).

Mitochondrial dynamics, however, depends on the cellular environment and architecture constraints. In adult cardiomyocytes, the large amount of myofilaments, the presence of a rigid cytoskeleton and the densely packed mitochondrial network clearly impedes mitochondrial movements (Vendelin et al., 2005). Moreover, the arrangement of the different organelles between them is so crucial for cardiac cell function that mitochondrial morphology has to be efficiently controlled (Wilding et al., 2006; Piquereau et al., 2010). Although abundance of proteins of the mitochondrial dynamics can appear paradoxical in cardiac cells, where the mitochondrial network appears to be frozen, it may be less rigid than believed. Proteins of the mitochondrial dynamics are involved in multiple processes and can thus be important for cardiac physiology out of their role in mitochondrial network organization. In this review, we present some evidences for the importance of these proteins in physiological and pathological situations.

MITOCHONDRIAL FUNCTIONS IN THE ADULT CARDIAC CELL: TANGLED IN A COMPLEX ARCHITECTURE

ROLE OF THE INTERNAL ORGANIZATION OF MITOCHONDRIA

Since the discovery of the chemiosmotic mechanism of ATP synthesis by PD Mitchell in the 1960s, the importance of mitochondrial internal organization and of a local regulation of energy production has been pointed out by many authors. Indeed, organization of internal membranes of mitochondria is critical for an optimal function of respiratory complexes and ATP formation (Davies et al., 2011). The existence of a large inner membrane folded in cristae, where oxidative-phosphorylation coupling occurs, is a prerequisite for local proton gradient generation and maximal ATP synthase functioning (Strauss et al., 2008). Phospholipids, and in particular cardiolipins, are involved in the formation of these cristae (Khalifat et al., 2008). However, the all set of elements inducing cristae generation is far from being elucidated. For example, the presence of oligomers of ATP synthase (Davies et al., 2012) and of dynamin proteins (Hinshaw, 2000) could also play a role in the cristae organization. Different dynamin proteins, such as the optic atrophy protein 1 (Opa1) or Mitofilin, have been recognized as important actors regulating cristae formation (Frezza et al., 2006; Hoppins et al., 2011a). Those cristae are dynamic structures which can rapidly and reversibly fuse and divide, depending on energetic state (Mannella, 2006), from orthodox to condensed conformations upon activation of ATP synthesis (Hackenbrock, 1966; Mannella, 2008). Thus, optimal mitochondrial function seems to require the morphological control of inner membrane organization. This is particularly evident for mitochondria of the adult cardiomyocyte that exhibit the highest density of cristae (Vafai and Mootha, 2012). Finally, it is also well-known that changes in mitochondrial morphology can play a role in different events, in particular in mitochondrial permeability transition pore (MPTP) function, and in apoptosis (Nogueira et al., 2005; Wasilewski and Scorrano, 2009; Campello and Scorrano, 2010). So it is now evident that all the phenomena that modify mitochondrial morphology can modify mitochondrial functions, and possibly cell functions.

Another important feature of mitochondria, especially in cardiac cells, is the existence of local control of energy production in the intermembrane space by different phosphotransfer enzyme systems. For example, we and others have shown that the mitochondrial creatine kinase isoform (miCK), which is located in the vicinity of the adenine nucleotide translocase (ANT), allows a local supply of ADP, a local control of proton concentration, and an efficient transfer of energy via phosphocreatine (PCr) diffusion (Joubert et al., 2000, 2001a, 2002a; Saks et al., 2000; Guzun et al., 2012). Such enzymes also play a role in the structural organization of the intermembrane space by bridging inner and outer membranes (Wallimann et al., 1992; Speer et al., 2005). Any modification of this energetic microdomain organization by alteration of mitochondrial morphology could affect local regulation of energy production.

IMPORTANCE OF CELL ARCHITECTURE FOR ENERGY TRANSFER AND COMMUNICATION BETWEEN ORGANELLES

Adult cardiomyocytes are characterized by a complex cytoarchitecture which allows an efficient and synchronized contraction of the entire cell. The maintenance of this cytoarchitecture is ensured by the cytoskeleton which holds sarcomeres in lateral register, mechanically couples myofibrils of adjacent myocytes, and transduces mechanical stress signals. Mitochondria are an integral part of this cytoarchitecture; different populations of those organelles are usually defined according to their location: intermyofibrillar mitochondria, subsarcolemmal mitochondria and perinuclear mitochondria. Those mitochondria are easily identifiable by electron microscopy (**Figure 1**). Intermyofibrillar mitochondria are strictly ordered between rows of contractile proteins, apparently isolated from each other by repeated arrays of T-tubules, and in close contact with myofibrils and sarcoplasmic reticulum (SR) (Vendelin et al., 2005; Kuznetsov et al., 2009; Guzun et al., 2012); they are mainly devoted to the energy supply of myosin and

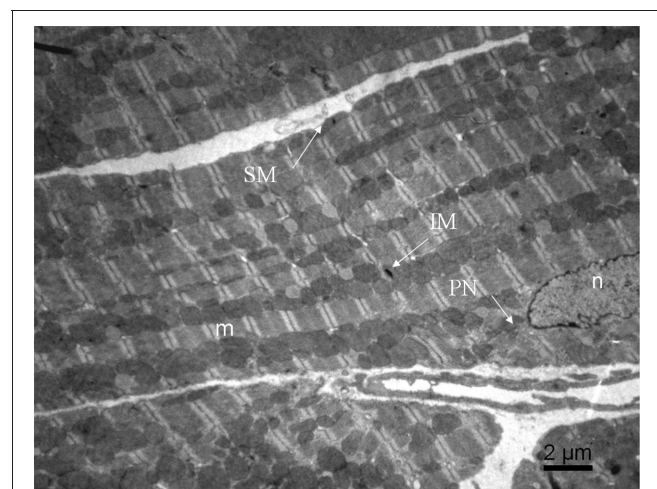


FIGURE 1 | Image of adult mouse cardiomyocyte obtained by electron microscopy. Three subpopulations of mitochondria are observed: intermyofibrillar mitochondria (IM) along the contractile proteins, the subsarcolemmal mitochondria (SM) just beneath the sarcolemmal and perinuclear mitochondria (PN) around the nucleus. m, myofibrils; n, nucleus.

SR-ATPases. The subsarcolemmal mitochondria present a lower degree of organization and are probably mainly involved in other roles such as ion homeostasis or signaling pathways. Finally, perinuclear mitochondria are organized in clusters and are most probably involved in transcription and translation processes. Concurrently to these functional differences, those mitochondria do not exhibit exactly the same morphology; for example, some authors have shown that intermyofibrillar mitochondria can be larger in certain situations (Ong et al., 2010).

Thus, the mitochondria in muscle cells seem separated from each other and exhibit a unique feature of a highly ordered crystal-like structure which appears optimized for maximal efficacy of energy supply and sustained contraction (Vendelin et al., 2005). Due to spatial constraints and isolation of mitochondria from each other, the energetic regulation of contraction would be confined in the small regions surrounding each sarcomere where the intracellular components interact with each other and compose the «Intracellular Energetic Units» (ICEU) (Saks et al., 2001; Guzun et al., 2012). The family of creatine kinases (CK), specifically located on the inner-membrane of the mitochondrion, but also at the M-line of myofibrils and on the outer surface of the SR, is an important component of these ICEUs and allows efficient local control over adenine nucleotides and fast energy transfer (Joubert et al., 2001b, 2002b; Tepp et al., 2011), thus emphasizing the importance of subcellular organization and compartmentalization of energy transfer (Joubert et al., 2002c; Piquereau et al., 2010).

Another evidence of the importance of cardiac cell architecture is the existence of microdomains of Ca^{2+} at the interface of the organelles. Different authors have shown that the close association between mitochondria and SR provides “hot spots” of very high calcium concentration in the vicinity of mitochondria (Dorn and Maack, 2013). At energetic level, the existence of direct adenine nucleotide canalization (DANC) (Kaasik et al., 2001) has also been shown to represent a pathway complementary to phosphotransfer enzymes to directly transfer and regulate ATP and ADP levels in microdomains at the interface of the organelles. Interestingly, this cytoarchitecture develops at the time of early cardiac ontogenic development (Piquereau et al., 2010), and when disturbed, the efficiency of this direct canalization is decreased (Wilding et al., 2006). Moreover, a change in osmotic pressure which can induce swelling or shrinkage of mitochondria can modulate the function of other compartments (Kaasik et al., 2004, 2010). Any change of cell architecture, and particularly of mitochondrial morphology, can therefore impact mitochondrial function and energy transfer (Joubert et al., 2008; Piquereau et al., 2012). This is why many authors address the question of how mitochondrial dynamics play a regulatory role in energy production and cell signaling in the heart.

MITOCHONDRIAL DYNAMICS PROTEINS IN THE HEART

Mitochondria are dynamic organelles able to change their morphology in response to different signals. Phenomena that govern mitochondrial morphology aroused real interest since the 1970s when the first fusion events have been described (Kimberg and Loeb, 1972; Wakabayashi et al., 1975; Wakabayashi and Green, 1977). For the last four decades, the mechanistic knowledge of

mitochondrial dynamics has been well-developed and, even if some phenomena involved in mitochondrial fusion and fission remain shrouded in mystery, the major proteins governing these processes have been identified. The complete protein machinery of mitochondrial fusion and fission exists in cardiac cells and specifically regulates mitochondrial morphology and size. The currently identified of such proteins and their possible roles in the adult heart are summarized next.

PROTEINS INVOLVED IN FUSION AND FISSION OF MITOCHONDRIA

All studies agree so far that fusion of the outer and inner mitochondrial membranes occurs separately and involves distinct molecules. The outer membrane fusion is governed by mitofusins (Mfn1 and Mfn2) (Santel and Fuller, 2001; Legros et al., 2002) while the inner membrane fusion involves Opa1 (Alexander et al., 2000). Interestingly, these two kinds of proteins (Mfns and Opa1) exhibit strong similarities in their structure and their mode of action. Indeed, these proteins contain a GTPase domain, a transmembrane domain allowing the anchorage of the proteins to the outer (Mfns) or inner (Opa1) membranes, and a coiled-coil domain. Whereas their GTPase domain implies a GTP-dependence of the phenomena involved in the mitochondrial fusion (Chen et al., 2003; Ishihara et al., 2004; Olichon et al., 2007), the coiled-coil domain also plays a major role since it allows homotypic (Mfn1-Mfn1, Mfn2-Mfn2 et Opa1-Opa1) or heterotypic (Mfn1-Mfn2) interaction of these proteins (Chen et al., 2003). The non-transmembrane part of Mfns, which is located in the cytosol, allows, through the interaction of the coiled-coil domains of two distinct Mfn proteins, the formation of a physical link between the outer membranes of two neighboring mitochondria (Koshiba et al., 2004; Chan, 2006). Thus, these two outer membranes become closer and the fusion of the involved mitochondria is initiated. In a similar way, the formation of Opa1-Opa1 homotypic complexes leads to the fusion of the inner membranes of the mitochondria engaged in the fusion process (for review see Liesa et al., 2009).

The specificity of action of these proteins is partly due to their anchorage to the membranes of which they control the fusion. However, the specific localization of each protein does not seem to be exclusive since Opa1 exists in a soluble form, which has been detected in an isolated outer mitochondrial membrane fraction, indicating that this Opa1 isoform is able to interact with the outer membrane (Satoh et al., 2003). Besides, this observation combined to the description of a direct interaction between Mfns and Opa1 (Guillery et al., 2008) could in part explain the synchronization of Mfns and Opa1 action. Indeed, the coordination of these proteins is still mysterious because no clear mechanism has been claimed and no equivalent to Ugo1, the yeast protein known to be involved in the coordination of Mfns and Opa1 yeast orthologs (Fzo et Mgm1, Hales and Fuller, 1997; Alexander et al., 2000), has been described in mammals.

Whereas the mechanisms involved in mitochondrial fusion seem to be relatively well-understood, the processes regulating mitochondrial fission still raise some questions. During the end of the 1990s and the beginning of the 2000s, many studies were interested in the dynamin-related protein 1 (Drp1), also called Dynamin-like protein 1 (Dlp1), and the mitochondrial fission

protein 1 (Fis1), which were considered as the major actors of mitochondrial fission. Drp1 is a cytosolic protein which comprises a GTPase domain and migrates to mitochondria using dynein and the microtubule (Varadi et al., 2004) or the actine network (De Vos et al., 2005), depending on the fission initiating factor. After this migration, Drp1 is specifically found at the level of the future fission site where it oligomerizes to form a ring, the GTP-dependence constriction of this ring leading to mitochondrial division (Yoon et al., 2001; Ingerman et al., 2005). Interestingly, while phosphorylated Drp1 stays inactive in the cytosol, Drp1 activity can be modulated by calcium through the activation of calcineurin which participates in Drp1 mitochondrial recruitment by dephosphorylating it. This mechanism could be of high significance in muscle cells where calcium is cyclically released from the SR (Cribbs and Strack, 2007; Cereghetti et al., 2008). Knowing that this protein does not exhibit any transmembrane domain necessary for its anchorage to the mitochondrial membranes, its mitochondrial localization requires a docking receptor on the outer mitochondrial membrane. Fis1 was the first protein described as the mitochondrial receptor (Yoon et al., 2003). Fis1 which is anchored in the outer mitochondrial membrane has an intracellular facing domain containing five α -helices which allow oligomerization (for the first α -helix) and direct or indirect interaction with Drp1 (for the all set of α -helices) (Jofuku et al., 2005).

Although many experimental studies have established an undeniable role of Fis1 in mitochondrial fission, the recruitment of Drp1 to mitochondria is not affected by Fis1 deficiency (Lee et al., 2004; Wasiak et al., 2007). Thus, Fis1 does not appear to be the only factor involved in Drp1 mitochondrial recruitment. Three other proteins have been described like the mitochondria fission factor (Mff) (Otera et al., 2010), the mitochondrial dynamics protein of 49 kDa (MiD49) or 51 kDa (MiD51) (Palmer et al., 2011). As Fis1, these three proteins are anchored to the outer mitochondrial membrane (Gandre-Babbe and van der Bliek, 2008; Palmer et al., 2011). The involvement of each protein in mitochondrial fission is, however, not yet clearly defined, and the interaction of these proteins with Drp1 could require other intermediary proteins. This is the case in the yeast in which the interaction of Drp1 and Fis1 orthologs needs several other proteins like Mdv1 and Caf4 (Tieu et al., 2002; Griffin et al., 2005); however, no such proteins have been described in mammals so far.

If a specific machinery of the inner mitochondrial membrane fission exists, it remains unknown. Although, it is suggested that the MDM33 protein is involved in the fission of the inner membrane of *Saccharomyces cerevisiae* mitochondria (Messerschmitt et al., 2003), no MDM33 ortholog has been found in mammals. Even if a study proposes that a protein called Mtp18 (Mitochondrial protein 18) could be an actor of this unknown inner mitochondrial membrane fission machinery (Tondera et al., 2005), this clearly requires further investigations.

Finally, mechanisms governing mitochondrial fragmentation seem to be less specific than those involved in mitochondrial fusion. Indeed, Drp1, Fis1, and Mff which are responsible for mitochondrial fission are also implicated in peroxisome fission (Koch et al., 2003, 2005; Gandre-Babbe and van der Bliek, 2008); however, the synchronization or the joint regulation of these two

phenomena has never been explored. The fact that the fission of these two organelles involves the same proteins should not be coincidental. It can be noticed that mutations in genes encoding these proteins have been involved in serious diseases, and in particular in neurological diseases such as Charcot-Marie-Tooth type 2A or autosomal dominant optic atrophy (ADOA) (Alexander et al., 2000; Delettre et al., 2001; Zuchner et al., 2004). In heart failure (HF), recent data also suggest their possible implication in the progression of the pathology (Chen et al., 2009), suggesting a role of these proteins in cardiac tissue.

WHAT ABOUT MITOCHONDRIAL DYNAMICS IN THE HEART?

Whereas it has long been suggested that adult cardiomyocytes would show a limited mitochondrial dynamics because of the complex cytoarchitecture of this cell, the high expression level of dynamin proteins in the heart (Alexander et al., 2000; Delettre et al., 2001; Santel et al., 2003; Gandre-Babbe and van der Bliek, 2008) implies that these actors could play roles which would not be anecdotal. Thus, many research groups have tried to explain mitochondrial dynamics in the heart for a few years. However, knowing that the initial experiments were done with immortalized cardiac cell lines (H9c2, HL-1) or with neonatal cardiomyocytes (Shen et al., 2007; Parra et al., 2008; Twig et al., 2008a) in which mitochondria face an environment very different from the adult one (Leu et al., 2001; Piquereau et al., 2010), the first data about mitochondrial fusion and fission obtained in the mature heart are relatively new and consequently these phenomena are not clearly understood yet.

Existence of mitochondrial dynamics in the adult heart

Mitochondrial dynamics comprises two main notions, one is the capacity of mitochondrial to move within the cell and the second relates to the capacity to undergo fusion and fission, these two notions not being mutually exclusive. As presented above, the adult cardiac muscle cell is an extremely organized cell in which the mitochondrial movements are greatly restricted (Beraud et al., 2009; Hom and Sheu, 2009). Moreover, the fusion/fission events appear to be greatly slowed compared to neonatal cardiomyocytes, and mitochondria are poorly connected. It has been recently suggested that the fusion/fission cycle would last 14–16 days in adult cardiomyocytes (Chen et al., 2011). Thus, mitochondrial dynamics could seem irrelevant, although mitochondria have a limited life span, being subjected to biogenesis and autophagy/mitophagy, which are strictly dependent on fusion and fission phenomena (Diaz and Moraes, 2008; Twig et al., 2008b). Consequently, even if fusion or fission events have never been observed in real time, this mitochondrial turnover imposes mitochondrial dynamics as an essential cog of cardiac physiology.

Phenotypic examination of genetically modified mice has recently substantiated our knowledge. Major changes in mitochondrial morphology have been described in mice with inducible cardiac Mfn2 ablation (Papanicolaou et al., 2011), or decrease in Opa1 protein content (Piquereau et al., 2012). However, the observation of larger mitochondria in Mfn2 and Opa1 deficient mice made by Walsh's group (Papanicolaou et al., 2011) and our team (Piquereau et al., 2012) is surprising because

it contradicts the previously published data. Indeed, while several studies on non-cardiac cells (Chen et al., 2003; Olichon et al., 2003; Yoon et al., 2003; Cipolat et al., 2004; Stojanovski et al., 2004; Griparic et al., 2007) evidenced that a decrease in expression of a fusion protein would lead, respectively, to mitochondrial network fragmentation, in these animal studies, deficiency in the fusion proteins Mfn2 or Opa1 led to paradoxically larger cardiac mitochondria. Thus, it appears that the specific architectural organization of this cell impact on the phenomena and presumably affects the mode of action of these proteins. On the other hand, the cardiac-specific Mfn1-null mice showed a fragmentation of the mitochondrial network (Papanicolaou et al., 2012); this observation reinforces the idea that Mfn1 and Mfn2 play different roles. Finally, the conditional combined Mfn1/Mfn2 ablation in adult hearts induces mitochondrial network fragmentation (Chen et al., 2011). Thus, mitochondrial dynamics exists in the heart tissue, but is a complex process that depends on the specific cell architecture.

Beyond the fact that these genetically-manipulated mice show obvious changes in cardiac mitochondrial morphology, significant deleterious consequences on their cardiac function were observed under stress (Papanicolaou et al., 2011; Piquereau et al., 2012), suggesting a direct involvement of the mitochondrial morphology in the heart function. At present, it is complicated to determine how and to which extent the morphology of mitochondria affects cardiac efficiency. For example, our group has already evidenced that changes in the mitochondrial volume may directly impact the force developed by myofibrils (Kaasik et al., 2004) as well as the direct energetic transfers between mitochondria and myofilaments (Piquereau et al., 2012). Even if these studies are insufficient to conclude that these mechanisms are responsible for the cardiac alterations observed in the previously described models, they suggest a direct link between mitochondrial morphology and cardiac contractile function. However, it is not easy to get an overall understanding of the mechanisms governing mitochondrial fusion and fission in the heart, even if it obviously seems that mitochondrial dynamics exists in this organ on a very slow time-course in the normal heart.

Impact of mitochondrial dynamics alterations on respiratory capacities

It is generally accepted that fusion-fission processes impact on the mitochondrial energetics in cultured cells (Chen et al., 2003). Disruption of mitochondrial dynamics by overexpression or suppression of fusion (Mfn2, Opa1) (Olichon et al., 2003; Chen et al., 2005) or mitochondrial fission (Benard et al., 2007), can cause alterations in mitochondrial metabolism (Chen and Chan, 2005), according to the degree of differentiation of the considered cell type. These changes may be accompanied by the modulation of the mitochondrial membrane potential, and of the expression of complex I, IV, and V subunits (Pich et al., 2005; Chan, 2006; Liesa et al., 2009). However, in patients and in mouse models, direct effect of dynamin mutations on mitochondrial function gave conflicting results, probably because of the diversity of the cell types and the mutations studied (Olichon et al., 2006). In general, it is considered that fragmentation induced by an increase in Drp1 or a decrease in Mfn2 or Opa1 is harmful and leads

to metabolic disorders (Parra et al., 2011). In contrast, fusion is generally considered rather beneficial. But a careful look at the literature shows that there is no clear link between mitochondria morphology and respiratory capacities, especially in mature cardiac cell. Indeed, in three different studies, no obvious alterations of respiratory chain function were observed when Opa1, Mfn1 or Mfn2 were separately genetically downregulated (Papanicolaou et al., 2011, 2012; Piquereau et al., 2012), except for free fatty-acid utilization in Opa1^{+/-} mice (Piquereau et al., 2012). In another study where a different Opa1 mutation was studied, mitochondrial function alterations were only observed in old mice (Chen et al., 2012). Finally, when complete/double KO (Mfn1/Mfn2) was used to suppress fusion proteins, decreased oxygen consumption or increased oxidative stress was observed (Chen et al., 2011; Dorn et al., 2011). Thus, compensatory mechanisms with other dynamin proteins may exist and attenuate the consequences of one dynamin protein deficiency, but complete loss or aging will be detrimental for mitochondrial function. In addition to an effect on respiratory chain function, other mitochondrial functions can also be affected by mitochondrial dynamics protein alterations.

Implication in mitochondrial biogenesis

As mentioned above, the mechanisms involved in mitochondrial dynamics in the cardiomyocyte seem not to be exactly similar to those observed in proliferative cells. However, this complexity is easily understood when the cardiomyocyte is regarded as a cell presenting a strictly organized intracellular architecture which is not in favor of mitochondrial plasticity as in dividing cells. It is thus important to keep in mind this notion when extrapolating results obtained during the early development to adulthood. Obviously, the role of mitochondrial dynamics is different in prenatal and neonatal cardiomyocytes which proliferate and consequently need the synthesis of new mitochondria to assure a suitable repartition of these organelles between daughter cells obtained after mitosis, and in non-dividing adult cardiomyocytes. Not surprisingly, we already described a remarkably high level of expression of mitochondrial biogenesis and dynamics genes during the early stages of postnatal development in the heart (Piquereau et al., 2013). Thus, this important stimulation of mitochondrial biogenesis and therefore of mitochondrial dynamics during development could explain why the cardiac-specific ablation of dynamins during prenatal or early postnatal development quickly leads to HF and death while several months are needed when the ablation occurs at the beginning of adulthood (Chen et al., 2011; Papanicolaou et al., 2012; Dorn, 2013). More generally, this could explain the lethality of the total dynamin knock-out models which die *in utero* (Alavi et al., 2007; Chen et al., 2011), a period during which the mitotic activity is undeniably substantial.

Globally, it would seem that mitochondrial dynamics is particularly important when the heart is under conditions which require the synthesis of new mitochondria. This is the case during development, but also in stress conditions like ischemia (Ong et al., 2010) or pressure overload (Piquereau et al., 2012) or ageing (Chen et al., 2012) which induce increased energetic demand and mitochondrial damages and consequently requires an adequate mitochondrial turnover. That is certainly the reason

why the mice genetically manipulated to abolish Mfn2 expression or to decrease Opa1 expression exhibit a high sensitivity to stress. This highlights the fact that the reduced mitochondrial dynamics can be partly compensated under baseline conditions while under stress the mitochondrial turnover cannot be ensured and precipitates cardiac dysfunction. Finally, inducible cardiac-specific double ablation of Mfn1 and Mfn2 genes quickly led to dilated cardiomyopathy before inducing death within 9 weeks after gene ablation. Interestingly, the heterozygous mutation of the Drp1 gene also induced a dilated cardiomyopathy in mice (Ashrafian et al., 2010). The cardiac dysfunction observed in these genetically-manipulated mice clearly shows that these proteins play a fully-fledged role in cardiac muscular cell physiology.

Other roles of mitochondrial dynamics proteins

All dynamins described above are of course widely known for the role they play in mitochondrial dynamics; however, these proteins could have a broader field of action than it seems (Figure 2). Several studies assigned them an effect on the property of the MPTP, a non-selective pore which induces, in particular situations, a high permeability of the mitochondrial membranes and can lead to cell death (Di Lisa and Bernardi, 2009). Indeed, we and others showed that the adult cardiomyocytes partially deficient in Opa1 (Piquereau et al., 2012) or totally deficient in Mfn1 or Mfn2 (Papanicolaou et al., 2011, 2012) exhibit a delayed Ca^{2+} -induced MPTP opening. Knowing that these observations have been obtained in isolated cardiomyocytes with enlarged or fragmented mitochondria, they suggest that the individual volume of the mitochondria are not directly involved and that Opa1 and mitofusins could facilitate MPTP opening under normal conditions.

The mechanisms leading to this protection are far from being elucidated. Moreover, the data obtained from adult heart deficient in the two mitofusins, which did not show any change in MPTP sensitivity in comparison with wild type (Chen et al., 2011), complicate the understanding even if the experimental conditions differ (isolated mitochondria vs. cardiomyocytes).

Whereas their involvement in the prevention of MPTP opening could indirectly confer to Opa1 and mitofusins an indirect role in cardiomyocyte death, it seems, however, that the role of the mitochondrial dynamics proteins in cell death is not really clear. It is well-known that mitochondria are central elements in cell death, and a link between mitochondrial network fragmentation and apoptosis has been already described in cell lines (Frank et al., 2001; Breckenridge et al., 2003) and in neonatal cardiomyocytes, suggesting an important role of fission protein, especially Drp1 (Parra et al., 2008; Wakabayashi et al., 2009), in this form of cell death. The fusion proteins could also be directly involved in cell death by acting in an original way. Even if the data obtained from studies realized with cell lines assert that fusion is prosurvival while fission is proapoptotic, Mfn2 could participate in apoptosis. The participation of Mfn2 to this particular death has been demonstrated in cardiomyocytes and smooth muscle cells (Guo et al., 2007; Shen et al., 2007). The details of this mechanism are still unclear, although an interaction between Mfn2 and the proapoptotic protein Bax has been described (Hoppins et al., 2011b). On the other hand, Opa1 would have an opposite role and could be an antiapoptotic factor. Indeed, this protein would regulate cell death by forming oligomers of two Opa1 proteins creating a “bottle neck”-like structure which allows cytochrome c sequestration in the cristae (Frezza et al., 2006). By this oligomerization, Opa1

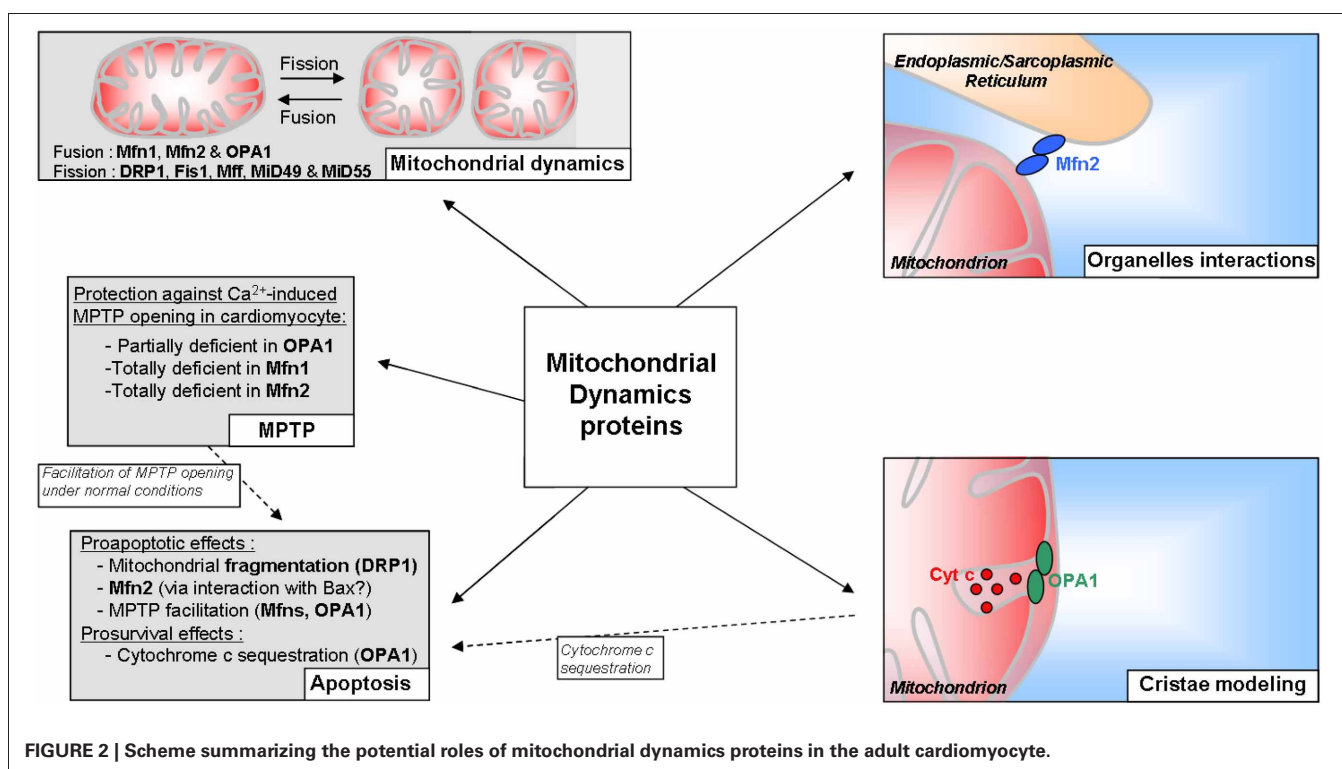


FIGURE 2 | Scheme summarizing the potential roles of mitochondrial dynamics proteins in the adult cardiomyocyte.

is in fact a crucial protein in the mitochondrial internal organization which is, as stated above, essential for the functions of these organelles.

Thus, mitochondrial dynamics proteins are clearly essential for the adaptation of those organelles to cell status. Moreover, some of them could be at the heart of the interaction between mitochondria and other components of the cell. Indeed, Mfn2, but not Mfn1, is also found at the endoplasmic reticulum (ER) membrane and thus can create physical links between ER and mitochondria (de Brito and Scorrano, 2008). This link has been recently shown between mitochondria and SR in muscle cells (Dorn and Maack, 2013); considering the calcium and energetic microdomains at the interface of mitochondria and SR described in the cardiomyocytes, Mfn2 could be a major actor of the contractile function of the heart.

Moreover, the physical links between ER and mitochondria were demonstrated to increase following ER stress (Csordas et al., 2006), a specific response triggered when ER homeostasis is disrupted and ER function is compromised. In the heart, ER stress has recently been recognized as an important contributor to the development of cardiac dysfunction (Groenendyk et al., 2013), and the link between ER stress and Mfns has started to be investigated. By using knock-out mice, Ngoh and colleagues demonstrated that cardiomyocyte-specific deletion of Mfn2, but not Mfn1, induces ER stress *in vivo*, leading to the conclusion that Mfn2 is a negative regulator of ER stress required for the homeostasis of the ER (Ngoh et al., 2012).

Even if the mechanism is not fully understood, Mfn2 is also involved in cardiac autophagic processes (Zhao et al., 2012). These authors showed that Mfn2 could participate in autophagosome-lysosome fusion. Due to the presence of Mfn2 in the ER and thus autophagosome membrane, it might act as an adaptor protein mediating autophagosome maturation. This link between Mfn2 and autophagy is reminiscent of the fact that mitochondrial dynamics proteins are also major actors of selective mitochondrial autophagy, i.e., mitophagy. Indeed, it is largely admitted that selective clearance of mitochondria are preceded by fission phenomena (Twig et al., 2008b). In fact, in the early event of mitophagy, Drp1 is recruited to mitochondria (Lee et al., 2011) and Mfns, present at the membrane of damaged mitochondria, are rapidly ubiquitinated (Ziviani et al., 2010). This Mfns ubiquitination could thus address these proteins to the proteasome or could interfere with mitochondrial tethering and prevent altered mitochondria to join the mitochondria network (Ziviani and Whitworth, 2010). Besides, in addition to the low mitochondrial potential allowing identification of mitochondria destined to mitophagy, these mitochondria would exhibit a low Opa1 amount at the inner membrane (Twig et al., 2008a). This creates non-fusing mitochondria which thus have only one destiny: degradation.

Finally, changes in the components of mitochondrial dynamics can also alter mtDNA. Observations in *S. cerevisiae* and MEF cells indicate that the normal activity of Opa1 (Jones and Fangman, 1992; Guan et al., 1993; Chen et al., 2007) or Mfns (Hermann et al., 1998; Rappaport et al., 1998; Chen et al., 2003, 2007) are crucial for maintaining the integrity of mtDNA nucleoids (Liesa et al., 2009). Part of Opa1 could be involved in the attachment of

mtDNA to the inner mitochondrial membrane and to promote mtDNA replication and distribution (Elachouri et al., 2011). In the mature heart, one study reported that heterozygous Opa1^{+/-} mice exhibit reduced mtDNA copy number (Chen et al., 2012), which could be involved in the development of cardiac mitochondrial dysfunction.

Finally, even if the number of studies devoted to the role of mitochondrial dynamics proteins in the heart is limited (Table 1), it can be asserted that these proteins are clearly integral part of cardiomyocyte life by ensuring fusion/fission processes and by participating in the several mechanisms described above. Interestingly, it seems that these proteins would be particularly important under stress conditions which are known to mobilize or affect mitochondrial functions. These kinds of situations exacerbate mitochondrial biogenesis and turnover which are relatively low under basal conditions, showing the major significance of the processes governed by these proteins in cardiac adaptations.

ALTERATIONS OF MITOCHONDRIAL DYNAMICS IN CARDIAC PATHOLOGIES AND POSSIBLE THERAPEUTIC APPROACHES

Chronic HF is associated with morphologic abnormalities of cardiac mitochondria including increased number, reduced organelles size, and compromised structural integrity (Schaper et al., 1991; Sabbah et al., 1992; Beutner et al., 2001), suggesting fragmentation of the mitochondrial network (Joubert et al., 2008). Mitochondrial damages as the depletion of the mitochondrial matrix and disruption of membranes positively correlate with the HF severity index (Sabbah et al., 1992) and it is recognized that mitochondria can determine the cellular fate (Di Lisa and Bernardi, 1998). In other pathologies, mega-mitochondria can appear (for review, see Wakabayashi, 2002 and Hoppel et al., 2009). Most of the time, heterogeneity of the size and distribution of cardiac mitochondria increases in HF, evidencing unbalanced fusion/fission cycles. Thus, an emerging hypothesis is that the mechanisms that control the shape of mitochondria may play a role in cardiac pathologies. In particular, a recent study suggested that Opa1 could be downregulated in HF (Chen et al., 2009; Chen and Knowlton, 2010). Another study observed a decrease in Mfn2, an increase in Fis1, and no change in Opa1 expression in rat hearts 12–18 weeks after myocardial infarction (Javadov et al., 2011). However, alteration of dynamin proteins could merely be the consequence of the alteration of mitochondrial biogenesis as it has been suggested (Garnier et al., 2005; Ventura-Clapier et al., 2011). Indeed, a strict relation exists between the PPAR gamma coactivator-1 (PGC-1 α), a master regulator of mitochondrial biogenesis, and the expression of these proteins (Garnier et al., 2005). Moreover, in most cardiac pathologies where defect in dynamin proteins are observed, a decrease in mitochondrial mass is also present (Ventura-Clapier et al., 2011; Parra et al., 2011). So it is not clear so far which protein alteration is responsible for a possible unbalance in fusion/fission, and the post-translational modifications of, for example, Drp1 (see above) could also be involved. Similarly, nothing is known about a possible implication of GTP/GDP supply in mitochondrial dynamics regulation.

Cell death is also an important pathophysiological process in both HF and in cardiac ischemia. However, the underlying mechanisms by which the heart loses myocytes in HF are not

Table 1 | Mitochondrial dynamic studies on the mature heart.

Authors	Tissue	Model	Mitochondrial morphology	Alterations	Observations
Shahrestani et al., 2009	Drosophila	OPA1 ^{+/-}		Decreased heart rate and increased heart arrhythmia	Poor tolerance to stress induced by electrical pacing
Dorn et al., 2011	Drosophila	OPA1 RNAi	Decrease of mean mitochondrial size	Contractile abnormality and remodeling	Stimulation of mitochondrial biogenesis
Piquereau et al., 2012	Mouse (3–6 months)	OPA1 ^{+/-}	Enlarged mitochondria, cristae disorganization	No alteration of cardiac function nor change in QO_2 , but delay of MPTP opening and energy transfer alteration	More sensitive to transaortic constriction (TAC)
Chen et al., 2012	Mouse (12 months)	OPA1 ^{+/-}	Disorganization of mitochondrial network	Reduced mtDNA level, mitochondria and cell dysfunction	Increased of oxidative stress and late-onset cardiomyopathy
Dorn et al., 2011	Drosophila	Marf RNAi	Decrease of mean mitochondrial size	Contractile abnormality and remodeling	Stimulation of mitochondrial biogenesis
Papanicolaou et al., 2011	Mouse	Mfn2 KO	Enlarged mitochondria	No major cardiac and mitochondrial dysfunction, delay of MPTP opening	Protection against cell death induced injury and better recovery after I/R
Papanicolaou et al., 2012	Mouse	Mfn1 KO	Fragmented mitochondria	Normal cardiac and mitochondrial function, decreased of ROS induced MPTP opening	protection against ROS induced mitochondrial dysfunction
Chen et al., 2012	Mouse	Mfn1/Mfn2 DKO	Fragmented mitochondria	Cardiomyocyte and mitochondrial respiratory dysfunction	Progressive and lethal dilated cardiomyopathy
Ngoh et al., 2012	mice	Inducible Mfn2 KO	Fragmentation	Increased markers of the ER stress	Mfn2 is necessary for ER homeostasis
Ashrafian et al., 2010	Mouse	Drp1 mutation ^{+/-}		Reduced mitochondrial complexes levels and cardiac ATP depletion	Energy deficiency may contribute to cardiomyopathy
Chen et al., 2009	Rat	Heart failure	Decrease of mean individual mitochondrial size	decrease of OPA1 protein level	
Ong et al., 2010	Rat	Ischemia/reperfusion and drp1 inhibition by mdivi-1	Presence of elongated mitochondria in control heart	Mitochondrial fragmentation prevented by mdivi-1 after I/R	Decrease of infarct size in I/R after treatment with an inhibitor of Drp1

completely understood. Mitochondria have a critical role in regulating cardiac cell death. If fission is interrupted, large networks of fused mitochondria occur. If fusion fails, mitochondria become smaller and fragmented. Abnormalities in fission and fusion can lead to apoptosis (Lee et al., 2004; Cassidy-Stone et al., 2008) which is an important mechanism of cardiac myocyte loss in HF (Olivetti et al., 1997; Narula et al., 1999). So both HF and

ischemia could be associated with abnormalities of fission and fusion that would contribute to cardiac cell death and change of MPTP sensitivity. In particular, after ischemia-reperfusion, mitochondria of cardiomyocytes show heterogeneous damages in their morphology, in redox status and in calcium homeostasis, which could be related to an overproduction of local ROS (Ong et al., 2012).

Modulation of mitochondrial dynamics appears as a novel pharmacological strategy for cardioprotection, in particular to protect the heart after a heart attack, and in ischemia-reperfusion [see reviews by Ong et al. (2012) and Dorn (2013)]. Several studies have shown that changes in mitochondrial morphology, including targeting proteins of the mitochondrial dynamics could allow the heart to better recover from an ischemic insult (Ong et al., 2010; Papanicolaou et al., 2011). In the first study, the authors used a specific inhibitor of Drp1, Mdiv-1, to prevent mitochondrial fission, and observed a significant reduction in myocardial infarct size in the *in vivo* murine heart. In the second one, using a genetic model of Mfn2 KO mice, they observed an improvement of cardiac performance following *ex vivo* ischemia/reperfusion. In both cases, even if the strategy was different (decrease the fission in one case, decrease the fusion in the second case), they linked the protection to the inhibition of MPTP opening, which is a classical target of cardioprotection. However, it should be kept in mind that although inhibition of MPTP can be beneficial in short term, it could be detrimental on the long term (Elrod et al., 2010; Piquereau et al., 2012).

CONCLUSION

Generally, studies described above prove that mitochondrial dynamics proteins are necessary for normal mitochondrial functions in the cardiomyocyte. In this cell where the internal organization is an obstacle to organelles mobility, these proteins govern slow fusion/fission processes which ensure mitochondrial turnover required to maintain mitochondrial function and consequently organ function. It also seems that mitochondrial dynamics would be a key element of cardiomyocyte adaptation under stress which induce alteration of mitochondria and thus lead to generation of new mitochondria and degradation

of damaged mitochondria. Under such conditions, mitochondrial dynamics processes would be exacerbated and would assume greater significance, explaining why the genetically-manipulated mice for mitochondrial dynamics genes are particularly sensitive to stress. Therefore, the high expression level of dynamins under basal conditions, despite the slow fusion/fission cycle, could be a major element of cardiac adaptation. In addition, those proteins could be activated by post-translational modifications involved in signaling pathways activated in response to stress. It can be anticipated that those potential post-translational modifications would be the more efficient way to ensure an optimal reactivity of mitochondrial dynamics machinery under stress.

Finally, mitochondrial dynamics proteins are involved in several phenomena irrespectively of their role in mitochondrial dynamics. This aspect of these dynamins has to be kept in mind because they are essential for the adequate function of mitochondria and cell life. By their extended field of action, these proteins are clearly established as major components of cardiac physiology.

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