# Chapter 11
# Mitochondria and Heart Disease

**Elinor J. Griffiths**

**Abstract** Mitochondria play a key role in the normal functioning of the heart, and in the pathogenesis and development of various types of heart disease. Physiologically, mitochondrial ATP supply needs to be matched to the often sudden changes in ATP demand of the heart, and this is mediated to a large extent by the mitochondrial $Ca^{2+}$ transport pathways allowing elevation of mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$). In turn this activates dehydrogenase enzymes to increase NADH and hence ATP supply. Pathologically, $[Ca^{2+}]_m$ is also important in generation of reactive oxygen species, and in opening of the mitochondrial permeability transition pore (MPTP); factors involved in both ischaemia-reperfusion injury and in heart failure. The MPTP has proved a promising target for protective strategies, with inhibitors widely used to show cardioprotection in experimental, and very recently human, studies. Similarly mitochondrially-targeted antioxidants have proved protective in various animal models of disease and await clinical trials. The mitochondrial $Ca^{2+}$ transport pathways, although in theory promising therapeutic targets, cannot yet be targeted in human studies due to non-specific effects of drugs used experimentally to inhibit them. Finally, specific mitochondrial cardiomyopathies due to mutations in mtDNA have been identified, usually in a gene for a tRNA, which, although rare, are almost always very severe once the mutation has exceeded its threshold.

**Keywords** Ischaemia • Ischemia • Reperfusion • Cardiomyopathy • Hypertrophy • Congenital heart disease • Heart failure • Calcium • Permeability transition pore • Mitochondrial DNA • Calcium uniporter • Cyclosporine A

## Abbreviations

| | |
|---|---|
| mCU | Mitochondrial calcium uniporter |
| mNCX | Mitochondrial sodium calcium exchanger |
| MPTP | Mitochondrial permeability transition pore |
| ROS | Reactive oxygen species |
| RuR | Ruthenium red |

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## 11.1 Introduction: Heart Diseases and Mitochondria

Mitochondria provide over 90% of the ATP required for the heart to function normally (Harris and Das 1991). The heart also has to have a way of increasing ATP supply rapidly upon increases in demand, such as occurs during increased workload or adrenergic stimulation. To give and idea of how quickly this has to happen, in a canine heart the entire ATP pool is turned over in 1 min under normal conditions, and in about 10 s under conditions of high workload (Khouri et al. 1965; Balaban 2009; Katz et al. 1989). Oxygen is not rate limiting for ATP synthesis until below about 20 μM (McCormack et al. 1990), a level much lower than that occurring physiologically. Pathologically, however, $O_2$ levels can decrease for a variety of reasons, leading to partial or total ischaemia, which will then impair ATP synthesis. This occurs when arteries become narrowed during atherosclerosis, either causing angina or a heart attack, in the case of unstable plaques that rupture and block the artery completely. Ischaemia also occurs during cardiac surgery when the aorta is cross-clamped, the heart stopped and the body placed on a heart-lung bypass machine. Reperfusion of the heart is obviously necessary but paradoxically can cause further damage. Clinically reperfusion damage can therefore occur during cardiac surgery, spontaneously following a myocardial infarction (MI), and in patients undergoing thrombolysis or angioplasty for treatment of MI.

Mitochondria, in particular mitochondrial $Ca^{2+}$ overload and oxygen free radical formation, are associated with the transition from reversible to irreversible damage following ischaemia: The discovery that the mitochondrial permeability transition pore (MPTP) plays a key role in the development of reperfusion injury has opened the way for protective strategies targeted at the pore – these are discussed below. Similarly the oxidative stress cause by reactive oxygen species (ROS) has been shown to contribute to the pathogenesis of both reperfusion injury and lately to the development of heart failure; free radical scavengers have been used experimentally to prevent such damage, and recently scavengers targeted specifically at the mitochondria have been designed that afford better protection; see Sect. 11.3.1.

Some specific cardiomyopathies arising from defects in mitochondrial DNA (mtDNA) have been identified, and these will be highlighted below. However, there are a myriad of other diseases arising from defects in mtDNA that affect the nervous system and muscular tissue. This chapter will not deal with these non heart-specific defects, which are covered in the chapter on inherited diseases (see Chap. 8 by Finsterer). Neither will I cover congenital heart disease arising from defects in cardiac structure or function during development since these are not specifically mitochondrial; for reviews see Bruneau (2008), Nemer (2008).

## 11.2 The Central Role of Mitochondria in Cardiac Ischaemia/Reperfusion Injury

### *11.2.1 Regulation of ATP Supply and Demand in the Heart*

Normal mitochondrial physiology including oxidative phosphorylation is covered in Chap. 1 (by Papa) of this volume and has been reviewed recently (Balaban 2009; Denton 2009; Griffiths 2009). However, I will discuss the parts relevant to understanding the role of mitochondria in heart disease, particularly the mitochondrial $Ca^{2+}$ transport pathways.

Oxygen acts as the final electron acceptor in the respiratory chain; as oxygen becomes limiting, electron carriers can no longer be re-oxidised, resulting in a build up of NAD(P)H and $FADH_2$ generated by dehydrogenases in the citric acid cycle. This has been measured using autofluorescence at the relevant wavelength in both whole hearts and isolated myocytes (Katz et al. 1987; Heineman and

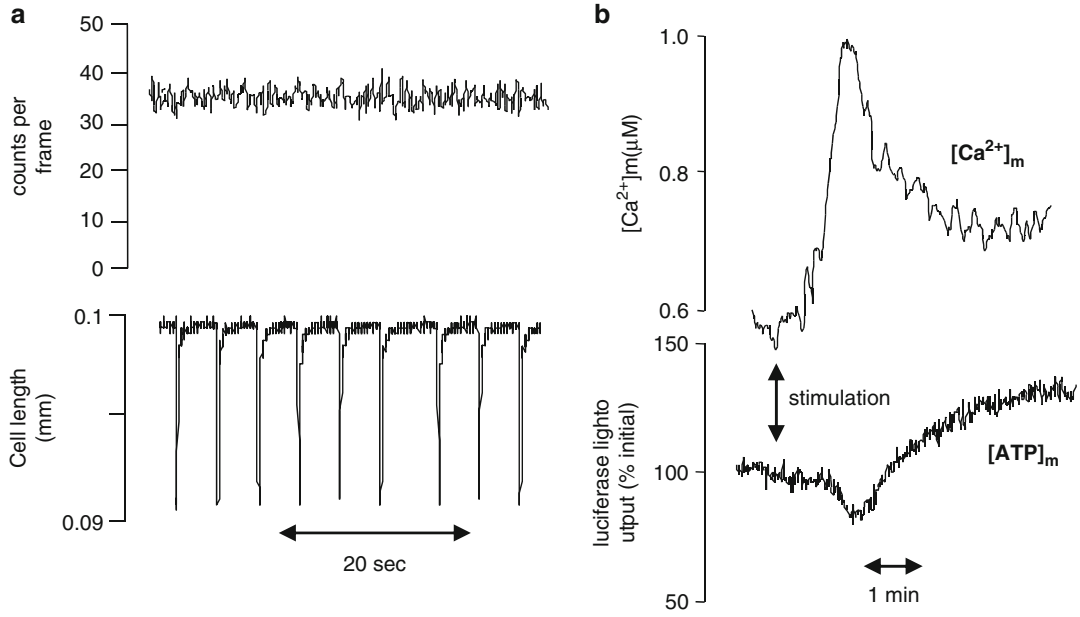


**Fig. 11.1** *ATP and mitochondrial [$Ca^{2+}$] levels in beating cardiomyocytes.* (**a**) $[ATP]_m$ was measured using targeted luciferase in synchronously beating adult cardiomyocytes stimulated to contract at 0.2 Hz. Parallel cell length measurements were taken from a single cell to highlight the lack of change in $[ATP]_m$ during a single contraction, (**b**) $[Ca^{2+}]_m$ and $[ATP]_m$ were measured using targeted aequorin and luciferase, respectively, in parallel experiments on small populations of cells stimulated to contract at 2 Hz from rest in presence of isoproterenol. In this figure the average $[Ca^{2+}]_m$ is shown – see text for discussion (Figure based on work of the author data first published in Bell et al. 2006)

Balaban 1993; White and Wittenberg 1993; Griffiths et al. 1997). The importance of ensuring adequate $O_2$ supply for maintaining supply-demand balance was illustrated when initial studies on whole hearts found an increase in the NAD(P)H/$NAD(P)^+$ ratio fluorescence upon increased workload – however, this was subsequently found to be due to inadequate perfusion of the hearts; in vivo and in well-oxygenated hearts, there was no such increase (Katz et al. 1987; Heineman and Balaban 1993).

Thus there has to be a mechanism of ensuring that ATP supply is matched exactly to demand, and early studies in isolated mitochondria found that the ADP/ATP ratio was the main regulator of ATP production (Chance and Williams 1956). However later studies in beating hearts found that the ratio did not change in well-oxygenated hearts even during large increases in workload (Katz et al. 1989; Neely et al. 1972). In support of this, we found recently, using targeted luciferase, that ATP levels in beating cardiomyocytes were remarkably constant in both cytosolic and mitochondrial compartments (Bell et al. 2006); and see Fig. 11.1. The discovery by Denton and McCormack that $Ca^{2+}$ could activate the mitochondrial dehydrogenases – pyruvate dehydrogenase (PDH), oxoglutarate dehydrogenase (OGDH) and isocitrate dehydrogenase (ICDH) – in the physiological range lead them to propose a parallel activation model where an increase in intramitochondrial free [$Ca^{2+}$] ($[Ca^{2+}]_m$) activated the dehydrogenases to increase NADH and hence ATP production (McCormack et al. 1990; Denton 2009); see Fig. 11.2. The observation that ATP levels do not change on a beat to beat basis, or under conditions of increases workload in the heart imply that $[Ca^{2+}]_m$ plays the key role under physiological conditions in the heart. However, Balaban has argued that, given the importance of ensuring a rapid response system of ATP synthesis in the myocardium, more than one mechanism is likely to operate to coordinate ATP supply and demand (Balaban 2009). This can be seen under conditions where the heart was stimulated to beat rapidly from rest when there is an initial drop in ATP before it recovers, the timecourse of which correlates with the time taken for mitochondria to take up $Ca^{2+}$ (Fig. 11.2).

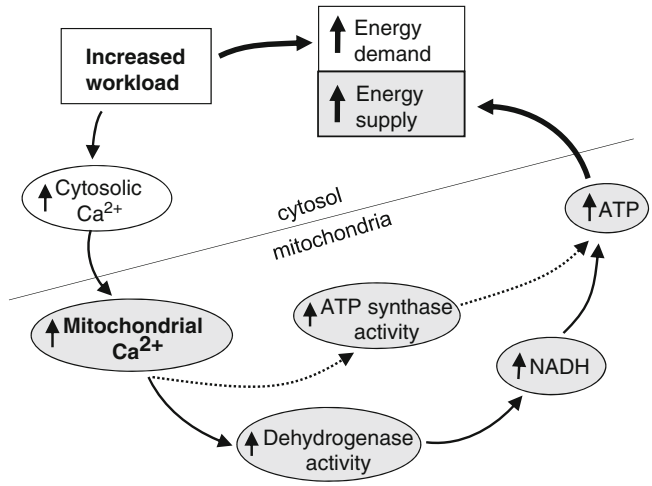


**Fig. 11.2** *Parallel activation model of dehydrogenase activation by mitochondrial [$Ca^{2+}$].* Increases in cytosolic $Ca^{2+}$ resulting form increases in heart workload or adrenergic stimulation are relayed to the mitochondrial matrix via the mitochondrial $Ca^{2+}$ uniporter (mCU). $[Ca^{2+}]_m$ activates dehydrogenase of the citric acid cycle and possibly the ATP synthase to increase ATP supply in line with the increased demand. See text for further details

Role of $Ca^{2+}$ in regulating mitochondrial function is discussed in more detail in Chap. 3 (by Brini), and in recent reviews (Griffiths 2009; Maack and O'Rourke 2007; Dedkova and Blatter 2008). Briefly, mitochondrial $Ca^{2+}$ uptake occurs by a uniporter (mCU), and efflux via a sodium calcium exchanger (mNCX); a summary of the mitochondrial pathways for $Ca^{2+}$ transport together with known inhibitors is shown in Fig. 11.3. Studies using isolated mitochondria found that the kinetics of the channels indicated they were too slow to play any role in intracellular $Ca^{2+}$ signalling during excitation-contraction (EC) coupling of the heart (reviewed in Nicholls and Crompton 1980; Gunter and Pfeiffer 1990) and it was predicted that net $Ca^{2+}$ influx would occur only when external $[Ca^{2+}]$ rose above about 500 nM (Nicholls and Crompton 1980; Gunter and Pfeiffer 1990), much higher than the resting cytosolic free $[Ca^{2+}]$ ($[Ca^{2+}]_c$) of 100–200 nM. However, evidence from non-cardiac and more recently cardiac cells has revealed that the mCU is located in close proximity to the sarcoplasmic reticular (SR) $Ca^{2+}$ release channel, at least in some subcellular populations of mitochondria, and therefore is exposed to a much higher but very localised $[Ca^{2+}]_c$ than previously thought (Griffiths 2009; Maack and O'Rourke 2007; Dedkova and Blatter 2008). It therefore may change on a rapid timescale and be able to modulate EC coupling.

The $Ca^{2+}$-induced mitochondrial permeability transition pore (MPTP), first described as an increase in inner membrane permeability in 1976 (Hunter et al. 1976), can act as a $Ca^{2+}$ efflux mechanism but is not specific for $Ca^{2+}$, allowing transport of small molecules with a molecular weight of less than about 1.5 kDa. The MPTP also requires additional factors such as adenine nucleotide depletion, oxidative stress and elevated phosphate; this makes a physiological role for the MPTP unlikely but these conditions are exactly those that occur during ischaemia/reperfusion injury; this is discussed further below.

### *11.2.2 Mitochondrial Dysfunction and $Ca^{2+}$ Transport During Ischaemia and Reperfusion*

Whether the result of gradual or sudden ischaemia, mitochondrial ATP production will become progressively less as oxygen levels and substrate supply decrease. The ionic changes that result from reduced ATP supply are summarised in Fig. 11.3, and reviewed in Suleiman et al. (2001), Murphy and

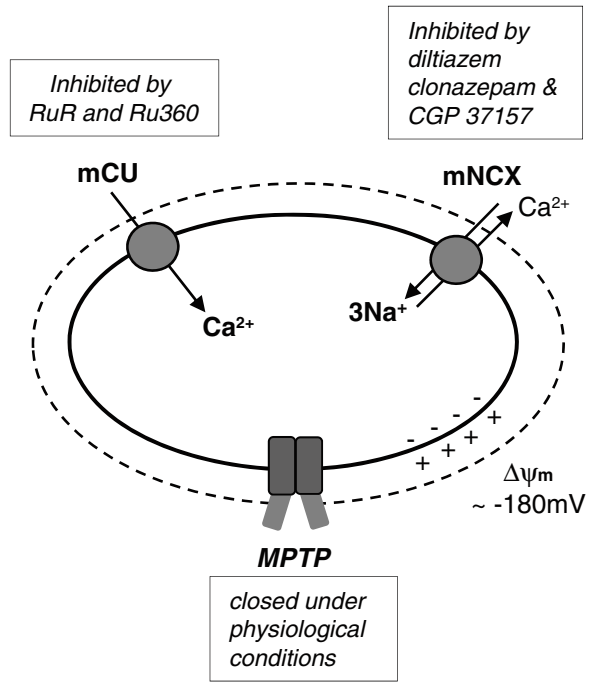


**Fig. 11.3** *$Ca^{2+}$ transport pathways of mitochondria. mCU* Calcium uniporter, *mNCX* mitochondrial sodium calcium exchanger, *MPTP* mitochondrial permeability transition pore, *RuR* ruthenium red. $\Delta\psi_m$ – mitochondrial membrane potential, approximately −180 mV in actively respiring mitochondria (inside negative). *Solid oval* depicts the inner membrane and *dashed oval* the permeable outer membrane. The MPTP is probably closed under physiological conditions but when open can act as an efflux pathway for $Ca^{2+}$; is also permeable to other small molecules of less than about 1,500 Da. The MPTP is shown here as consisting of both inner and outer membrane proteins; see text for a discussion of pore components

Steenbergen (2008a). The reduced ATP levels lead to failure of the $Na^+/K^+$-ATPase, $Na^+$ loading (Haigney et al. 1994), and subsequently $Ca^{2+}$ loading via reversal of the $Na^+/Ca^{2+}$ exchanger (Silverman and Stern 1994), and build up of lactic acid causes intracellular acidosis. The ionic changes occurring during ischaemia/reperfusion and the role of mitochondria are summarised in Fig. 11.4 and reviewed in Suleiman et al. (2001), Murphy and Steenbergen (2008b). In isolated myocytes, $[Ca^{2+}]_m$ only increased following ATP depletion-dependent rigor contracture. Reperfusion of hearts or myocytes before rigor-contracture causes reversible damage "stunning", but usually recovery with time. Whether or not cardiomyocytes recovered from hypoxia depended on the level of $[Ca^{2+}]_m$ achieved at the end of the hypoxic period: cells having $[Ca^{2+}]_m$ greater than about 250–300 nM invariably hypercontracted upon reperfusion (Miyata et al. 1992; Griffiths et al. 1998). Similar increases have been observed in mitochondria isolated from whole hearts following ischaemia/reperfusion – $[Ca^{2+}]_m$ rose from pre-hypoxic values of 160–360 and 570 nM after 50 and 80 min of hypoxia, respectively (Allen et al. 1993). But a much greater increase in $[Ca^{2+}]_m$ occurs upon reperfusion (Delcamp et al. 1998; Chacon et al. 1994), for example in whole hearts following 80 min hypoxia, reperfusion led to a 10-fold increase in $[Ca^{2+}]_m$ (Allen et al. 1993). Although normally mitochondria have the capacity to take up huge amounts of $Ca^{2+}$ (Nicholls and Crompton 1980; Gunter and Pfeiffer 1990) and thus could potentially remove toxic levels of $Ca^{2+}$ from the cytosol, such accumulation of $Ca^{2+}$ can eventually damage mitochondria both by competing for ATP production and more importantly by inducing the mitochondrial permeability transition pore (MPTP). Other factors upon reperfusion also lower the threshold of $[Ca^{2+}]_m$ needed for the MPTP to open; for example low adenine nucleotides, free radical generation, and return to normal pH. Hence the MPTP can open at values of $[Ca^{2+}]_m$ that may not be much higher than physiological.

But it should be remembered that limited (rather than excessive) $Ca^{2+}$ uptake by mitochondria on reperfusion has the capacity to be protective: for example, by activation of PDH. Increased glucose

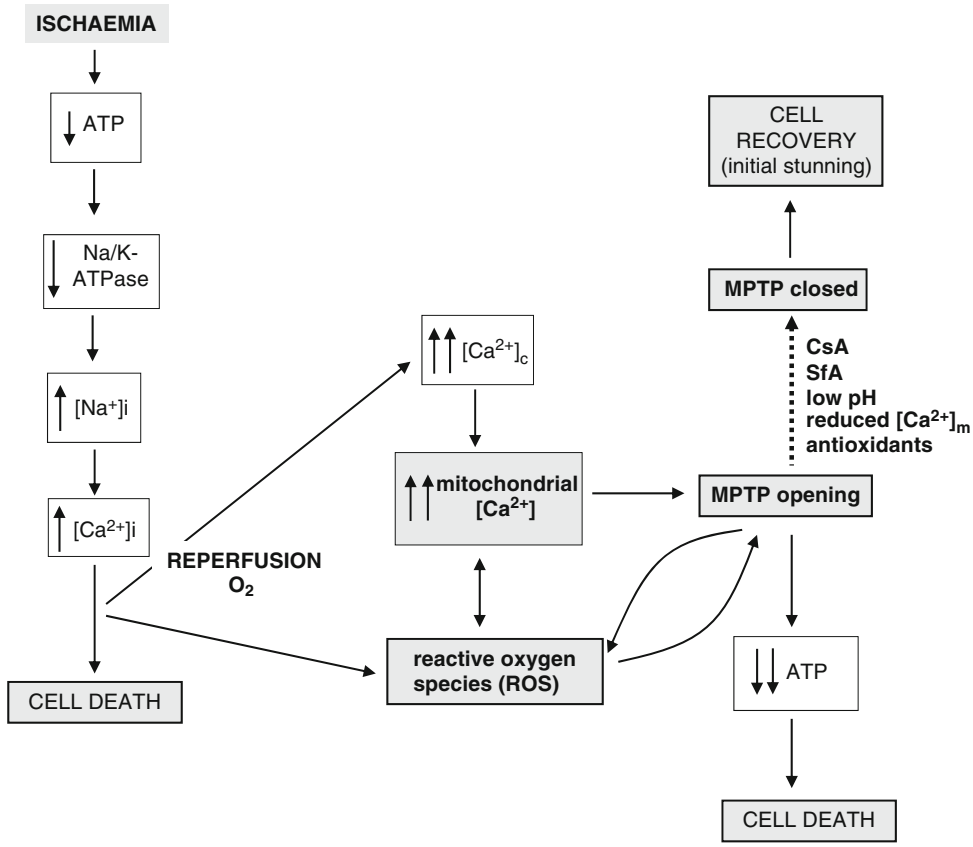


**Fig. 11.4** *Ionic changes during ischaemia and reperfusion injury showing central role of mitochondrial [$Ca^{2+}$] and the MPTP. Ischaemia* leads to an inhibition of oxidative phosphorylation, the resulting decrease in ATP causes ion channel dysfunction, leading to increases in $[Na^+]_i$, $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$. Of ischaemia is of short duration, the cell can recovery as the changes will not be severe enough to cause opening of the MPTP, although stunning (reversible injury) may occur. However, following prolonged ischaemia, the increase in $[Ca^{2+}]_m$, reactive oxygen species and low ATP levels cause opening of the MPTP. ATP levels can then not recover and ROS production is exacerbated, leading to cell death. Agents that either prevent the increase in $[Ca^{2+}]_m$, inhibit pore components (SfA and CsA), or inhibit the pore indirectly (low pH and free radical scavengers), will allow the pore to close, or prevent opening, shifting the balance towards cell recovery. See text for further discussion

oxidation on reperfusion improves recovery of the heart, and drugs like ranolazine, which increase PDH activity, can improve post-ischaemic recovery (Clarke et al. 1993). So in designing protective strategies, it is a matter of getting the balance right between beneficial versus harmful effects of mitochondrial $Ca^{2+}$ uptake. The situation is further complicated by the fact that many other factors on reperfusion affect this balance.

Although several studies had reported an increase in $[Ca^{2+}]_m$ during hypoxia (see above), the mechanism of entry of the $Ca^{2+}$ was either not discussed, or assumed to be the mCU. But mitochondrial membrane potential ($\Delta\Psi_m$) depolarises during hypoxia (Di Lisa et al. 1995), and this would be expected to inhibit $Ca^{2+}$ uptake through the mCU. However, we found that the increase in myocyte $[Ca^{2+}]_m$ during hypoxia could *not* be prevented by RuR (at concentrations of RuR, 20 μM, that could inhibit $[Ca^{2+}]_m$ increases in response to a cytosolic $Ca^{2+}$ load) but instead the $[Ca^{2+}]_m$ increase could be prevented by clonazepam, an inhibitor of mNCX. By contrast, upon reoxygenation, RuR once again inhibited $Ca^{2+}$ uptake, whilst clonazepam inhibited efflux (Griffiths et al. 1998). This allowed us to propose the following model: during hypoxia $Ca^{2+}$ entry into mitochondria occurs via mNCX, and the mCU is largely inactive. Upon reoxygenation, however, the transporters regain their normal directionality.

We have provided further evidence that entry of $Ca^{2+}$ during hypoxia occurs via mNCX by showing, using a model of simulated hypoxia, that the process is $Na^{+}$-dependent (Griffiths 1999). And a reversal of the mNCX under conditions of metabolic inhibition was also found in a study on cultured renal epithelial cells, supporting our hypothesis (Smets et al. 2004).

### *11.2.3 The Mitochondrial Permeability Transition Pore*

It has been known for many years that $Ca^{2+}$-induced mitochondrial dysfunction during ischaemia is associated with the transition from reversible to irreversible cell damage; see above and Bush et al. (1980), Sordahl and Stewart (1980), Fleckenstein et al. (1983): Mitochondria accumulate deposits of calcium phosphate, become swollen, and eventually rupture. This sequence of events, whereby mitochondrial $Ca^{2+}$ uptake leads to mitochondrial dysfunction and prevents oxidative phosphorylation was later found to be due to $Ca^{2+}$-induced opening of the MPTP. The MPTP plays a key role in cell death by both necrosis and apoptosis, and has been discussed in recent reviews (Hajnóczky et al. 2006; Rasola and Bernardi 2007; Roy and Hajnóczky 2008; Leung and Halestrap 2008; Pinton et al. 2008). In absence of specific inhibitors of mitochondrial $Ca^{2+}$ uptake that can be used on reperfusion, which, as mentioned above, may not be ideal since some $Ca^{2+}$ would be needed to activated mitochondrial dehydrogenases, the discovery of relatively specific inhibitors of the MPTP have provided firstly a useful tool for investigation of the role of the MPTP, and secondly as therapies for ischaemia/reperfusion damage.

However, the exact components of the MPTP are still not known; for a number of years the MPTP was generally thought to consist of the outer membrane voltage-dependent anion channel (VDAC), the inner membrane adenine nucleotide translocase (ANT) and the matrix protein cyclophilin D (CyP-D). Studies on transgenic mice confirmed the role of CyP-D as having a critical regulatory role in pore-opening (Baines et al. 2005), but have shown that neither the ANT (Kokoszka et al. 2004) nor VDAC (Baines et al. 2007) are essential components of the pore, though they may also play a regulatory role; the composition and regulation of the pore are discussed fully in recent reviews (Di Lisa and Bernardi 2006; Halestrap 2009; Javadov et al. 2009). The latest and more compelling evidence indicates that the inner membrane phosphate carrier (PiC) rather than the ANT is likely to be a pore component (Leung et al. 2008), and a working model has been proposed by Halestrap (2009). There is a suggestion that there is no single protein responsible for pore formation, but rather it is due to aggregation of misfolded proteins, or proteins damaged by oxidative stress (He and Lemasters 2002). However, the specific properties of the pore; $Ca^{2+}$ activation, inhibition by CsA, and modulation by activators and inhibitors of the ANT or PiC make this unlikely (Halestrap 2009).

### *11.2.4 Mitochondria as a Target for Cardioprotection During Ischaemia and Reperfusion*

#### 11.2.4.1 Studies Using Inhibitors of Mitochondrial $Ca^{2+}$ Transport

Ruthenium red (RuR) has been shown to protect hearts or myocytes from reperfusion/reoxygenation damage at concentrations ranging from 0.1 to 6 μM (Peng et al. 1980; Leperre et al. 1995; Miyamae et al. 1996; Grover et al. 1990; Benzi and Lerch 1992; Figueredo et al. 1991; Park et al. 1990; Carry et al. 1989; Stone et al. 1989). However, at these concentrations RuR can also inhibit $Ca^{2+}$ channels of the SR and myocyte contraction (Griffiths 2000), whereas much higher levels are required to inhibit mitochondria $Ca^{2+}$ uptake in myocytes (Griffiths et al. 1998). It therefore seems more likely that the protective effects were due to an energy sparing effect as a result of reducing $[Ca^{2+}]_c$ (Benzi and Lerch 1992). Unfortunately at higher concentrations RuR has non-specific damaging effects on hearts, making it unsuitable to accurately assess the contribution of mitochondrial $Ca^{2+}$ uptake to reperfusion-induced damage.

Ru360 is a more specific inhibitor of the mCU, although there are problems with permeability in isolated myocytes (Bell et al. 2006; Robert et al. 2001). It has, however, been reported to protect whole hearts against ischaemia/reperfusion injury: pre-treatment of isolated rat hearts with 10 μM Ru360 provided protection against reperfusion injury, as determined from infarct size and enzyme release (Zhang et al. 2006). But in another study lower concentrations of Ru360 were required: recovery of rat hearts from ischaemia was optimal between 0.25–1 μM Ru360 and declined at higher concentrations (de Jesus et al. 2005). $[Ca^{2+}]_m$ (as measured following isolation of mitochondria at the end of the perfusion protocol) was also decreased in Ru360 treated hearts (de Jesus et al. 2005).

With regard to inhibitors of the mNCX, diltiazem can protect hearts from ischaemia/reperfusion damage, but this has mainly been attributed to its effects on sarcolemmal L-type $Ca^{2+}$-channels (Winniford et al. 1985) or $Na^+$ channels (Takeo et al. 2004); both of which would indirectly preserve mitochondrial integrity. Clonazepam cannot be used in whole hearts as it appears to inhibit contractility by a non-myocyte effect (Griffiths, unpublished observation), even though it was protective and prevented the ischaemia-induced increase in $[Ca^{2+}]_m$ in isolated myocytes (Griffiths et al. 1998; Sharikabad et al. 2004), as discussed above. There have been no reports of the effects of CGP37157 in whole hearts; it has been used to inhibit $Ca^{2+}$ entry in isolated myocytes (Maack et al. 2006), although there can be problems with solubilising the compound (Griffiths et al. 1997); it has not been used in any models of ischaemia/reperfusion injury.

Intracellular acidification, such as occurs during ischaemia, can also decrease the rate of $Ca^{2+}$ uptake, probably as a consequence of reduced $\Delta\Psi_m$ (Gursahani and Schaefer 2004). Maintaining an acid pH upon reperfusion is known to delay intracellular $Ca^{2+}$ accumulation and protect against reperfusion injury (Panagiotopoulos et al. 1990), and additionally inhibits opening of the MPTP (Halestrap 1991).

#### 11.2.4.2 Inhibitors of the Permeability Transition Pore

A non-specific pore in the mitochondrial inner membrane that allowed permeability of molecules up to 1.5 kDa was first identified in the 1970s by Haworth and Hunter (Hunter and Haworth 1979a, b; Haworth and Hunter 1979). Crompton then determined that the MPTP was being regulated by $Ca^{2+}$ and oxidative stress (Crompton et al. 1987), and realised its implications for myocardial reperfusion injury (Crompton and Costi 1990). The discovery that opening of the MPTP could be inhibited by cyclosporine A (Crompton et al. 1988) suggested a possible protective strategy, and Crompton subsequently showed that cyclosporine A (CsA), a potent inhibitor of the pore in isolated mitochondria, was protective in a myocyte model of hypoxia/reoxygenation (Nazareth et al. 1991). We found CsA to be cardioprotective in a perfused rat heart model of IR injury (Griffiths and Halestrap 1993), and further that the MPTP opened only upon reperfusion, not during ischaemia (Griffiths and Halestrap 1995); see Fig. 11.4. Opening occurred during the first 5 min of reperfusion (Kerr et al. 1999), and giving CsA on reperfusion was also protective (Hausenloy et al. 2003). Cyclosporine A is an immunosuppressant commonly used in transplant operations. However its effect on the MPTP is by an entirely separate mechanism – it inhibits the peptidyl-prolyl cis-trans isomerase activity of CyP-D, an effect that greatly decreases the sensitivity of the pore to $Ca^{2+}$, although the inhibition by CsA can be overcome at high enough $Ca^{2+}$. We found that analogues of CsA that inhibit the MPTP but are not immunosuppressive are still cardioprotective (Griffiths and Halestrap 1993), indicating it is the mitochondrial effect that is important. Other inhibitors of the pore have subsequently been found to be cardioprotective, such as sanglifehrin A (SfA), which also acts on CyP-D, Debio-025(Gomez et al. 2007), pyruvate (a free radical scavenger and which maintains low pH), propofol (an anaesthetic and free radical scavenger), and low pH – more information on these compounds is given in recent reviews (Di Lisa and Bernardi 2006; Halestrap and Pasdois 2009).

It has nevertheless taken some time for these findings to be translated to a clinical setting: in a pilot trial patients presenting with acute ST-elevation following myocardial infarction (STEMI) were

given CsA or control saline before undergoing PCI, and administering CsA at the time of reperfusion was associated with a smaller infarct (Piot et al. 2008). Another study investigated the effect of a single dose of CsA administered at the time of reperfusion following MI on LV remodelling and function 5 days and 6 months following the MI; there was a reduction in infarct size at 6 months follow up in the CsA-group (Mewton et al. 2010). Both studies are promising but require a further large scale trial.

#### 11.2.4.3 Ischaemic Pre- and Post-conditioning

Ischaemic preconditioning is a protective strategy that has been widely used experimentally, where short periods of ischaemia protect against a prolonged period. Various end-effectors and mediators of the pathway have been proposed, including different signalling pathways and kinases, plus the mitochondrial ATP-sensitive $K^+$ ($mK_{ATP}$) channels (reviewed in Hausenloy and Yellon 2007; Ardehali and O'Rourke 2005; Lawrence et al. 2001). Ischaemic postconditioning (IPost) is another protective strategy that may be more clinically relevant (Hausenloy and Yellon 2007), since the short ischaemia episodes are applied following reperfusion. A full discussion of these pathways is beyond the scope of this review, but IPC is associated with a reduction in MPTP opening (Javadov et al. 2003), and so is relevant here. Several studies have now found IPC to be associated with a reduced $[Ca^{2+}]_m$ (Wang et al. 2001; Crestanello et al. 2000; Murata et al. 2001; Hausenloy et al. 2004a; Smart et al. 2006), which may then lead to a reduction in MPTP opening (reviewed in Halestrap et al. 2007). IPC and IPost both cause suppression of MPTP opening upon reperfusion (Javadov et al. 2003; Hausenloy et al. 2004b; Argaud et al. 2005).

As mentioned above, mice deficient in CyP-D are resistant to pore opening, and have smaller infarcts in response to IR injury (Baines et al. 2005; Nakagawa et al. 2005; Lim et al. 2007). However, the CyP-D deficient mice could not be further protected by IPC, IPost, or pharmacological agents such as bradykinin (that mimics IPC), CsA or SfA (Lim et al. 2007), confirming the MPTP as essential in the cardioprotection afforded by either type of conditioning, and proposing it as an end-effector of the two pathways (Lim et al. 2007). The mechanism of this protection is not known, though various signalling pathways have been proposed, including involvement of Akt, GSK-3 and PKC -see review (Hausenloy and Yellon 2007). However, it appears unlikely that these act directly to phosphorylate mitochondrial proteins, but rather by reducing oxidative stress (Clarke et al. 2008). There is also evidence that transient opening of the MPTP may act as the trigger for IPC, since including CsA or SfA during the preconditioning ischaemia reduced the infarct-limiting effects of IPC (Hausenloy et al. 2004a).

Despite all the experimental studies showing protective effects of pre and post-conditioning, their translation to the clinical setting has been disappointing (Ludman et al. 2010). IPC (Murry et al. 1986) is clearly limited by the fact that it has to be applied before ischaemia, but IPost (Zhao et al. 2003) seems more promising. Clinically, IPost has been used by inflating/deflating an angioplasty balloon following insertion of a stent in the affected artery; reported to reduce infarct size at 6 months and improve function after 1 year (Thibault et al. 2008).

## 11.3 Mitochondria and Heart Failure

### *11.3.1 Role of Reactive Oxygen Species (ROS)*

Heart failure is a major cause of morbidity and mortality in developed countries (Cleland et al. 2001), and an increasing problem in aging populations (Tsutsui et al. 2008). It can result from a variety of causes, such hypertension or coronary artery disease, progressing from compensated hypertrophy

through to failure, or result from inherited or acquired valvular disease or cardiomyopathy. A link between mitochondrial dysfunction and heart failure was observed as early as 1962 using a guinea-pig model of heart failure induced by aortic banding, where a reduced oxidative phosphorylation capacity of mitochondria was observed. Although the authors could not prove a causal relationship, they suggested that mitochondria played a critical role in the development of the disease (Schwartz and Lee 1962).

Heart failure progresses by cardiac remodelling, where the myocytes enlarge, often preceded by compensated hypertrophy, followed by deterioration in pump function. Many experimental and clinical studies have now shown that the remodelling proceeds by myocyte loss via apoptosis – reviewed in van Empel et al. (2005). The role of mitochondria in apoptosis is well known, and covered by Chap. 7 in this volume (Mignotte). However, the role of oxidative stress induced by the generation of reactive oxygen species (ROS) in the pathogenesis of heart failure appears is also becoming increasingly well accepted (Tsutsui et al. 2008); these damaging species can causes deleterious effects on DNA (mitochondrial and nuclear), protein and cell structure, as well as acting as signalling molecules in their own right that contribute towards the development of the remodelling process, in hypertrophy and heart failure (Tsutsui et al. 2008; Seddon et al. 2007).

Mitochondria are a major source of ROS in the heart since the respiratory chain generates the superoxide anion, $O_2^-$, as part of normal respiration, and this can then trigger formation of other ROS (Murphy 2009). Mitochondria are more susceptible to ROS damage than nuclear DNA since they have poor DNA repair mechanisms and no protective histones; additionally $O_2^-$ generated by the respiratory chain is not easily membrane permeable so may become trapped within the mitochondriua (Tsutsui et al. 2009). The mutation rate of mtDNA is more than ten times that of nuclear DNA (Chen et al. 2006). Increased $O_2^-$ production by mitochondria was found in a canine model of heart failure (Ide et al. 2000), and markers of ROS generation in blood of patients with heart failure (Mallat et al. 1998). However ROS from other sources may also play a role, such as xanthine oxidase, NADPH oxidase, or uncoupled nitric oxide synthase in either myocytes or endothelial cells in the heart (Seddon et al. 2007). mtDNA encodes essential subunits of respiratory chain proteins (see below), and so damage to a mitochondrial gene can rapidly lead to deleterious effects on the whole cell. Recently, mitochondrially-targeted antioxidants have been produced, to try and effectively combat ROS-induced diseases (James et al. 2005; Murphy and Smith 2007). Mito$Q_{10}$ is a ubiquinol antioxidant with a triphenylphosphonium lipophilic tail that accumulates several 100-fold in mitochondria because of their highly negative membrane potential (James et al. 2005). In experimental studies, mostly on rat models of heart disease so far, Mito$Q_{10}$ has been shown to be beneficial in protecting against ischaemia/reperfusion damage (Adlam et al. 2005), hypertension and hypertrophy (Graham et al. 2009), and sepsis-induced cardiac dysfunction (Supinski et al. 2009). However, Mito$Q_{10}$ did not completely prevent hypertension, indicating that mitochondrial ROS production is not the only contributing factor (Graham et al. 2009).

The heart has various antioxidant defence mechanisms; these do not appear to be downregulated in heart failure, rather it is the increase in ROS production that overwhelms the anti-oxidant capacity (Tsutsui et al. 2008). Mitochondria contain several enzymes that detoxify ROS: manganese superoxide dismutase (Mn-SOD) converts $O_2^-$ to $H_2O_2$, and glutathione peroxidase and peroxiredoxins convert $H_2O_2$ to water (Murphy 2009). So as well as adding exogenous anti-oxidants, strategies that upregulate the endogenous defence pathways are candidates for the prevention or treatment of heart failure. One such enzyme is glutathione peroxidase, present in both cytosol and mitochondria, which scavenges $H_2O_2$ and prevents formation of hydroxyl radicals: overexpression of this enzyme in mice prevented the development of heart failure following myocardial infarction (Shiomi et al. 2004). Using a similar model in rats, dietary supplementation with vitamin E also protected against cardiac dysfunction leading to heart failure; this was associated with increased activities of catalase and glutathione peroxidase (Hill et al. 2005). Knocking out Mn-SOD in mitochondria similarly leads to dilated cardiomyopathy in mice, which die within 10 days of birth (Li et al. 1995). The role of oxidative stress in the heart is covered in more detail by recent reviews (Tsutsui et al. 2008; Seddon et al. 2007) and in Chap. 5 of this volume (by Lenaz).

Pacing induced heart failure is a common model of human dilated cardiomyopathy (Moe and Armstrong 1999), and is associated with defects in mitochondrial function such as reduced respiratory chain activity and beta-oxidation, and depletion of high energy phosphates (Marin-Garcia et al. 2001). A time course study in this model revealed parallel increases in markers of oxidative stress, apoptosis, and respiratory chain dysfunction (Marin-Garcia et al. 2009). Defects in complexes I, III and V were found in the left ventricle whereas in the left atrium only complex V was deficient, and markers of apoptosis were also found in both left ventricle and atrium (Marin-Garcia et al. 2009). However, there were no differences in the level of citrate synthase (a common marker of mitochondrial content), complex II or complex IV, indicating that the changes were specific for certain enzymes only, and not due simply to an overall decrease in mitochondrial content – rather there were selective changes in both nuclear-encoded and mitochondrial-encoded components of the respiratory chain. Thus it is clear that mitochondrial dysfunction occurs in heart failure and that mitochondrially generated ROS contribute to the development of the disease.

### *11.3.2 Mitochondrial [$Ca^{2+}$] and Heart Failure*

In a rabbit model of heart failure, mitochondrial $Ca^{2+}$ uptake was unchanged during the early stages of hypertrophy, but this was followed by a decrease in $Ca^{2+}$ uptake as the disease progressed (Sordahl et al. 1973). There was a parallel initial increase in respiratory chain activity in hypertrophy but which decreased on progression to failure (Sordahl et al. 1973); this decline in respiration fits with a lower $[Ca^{2+}]_m$, although that was not directly shown in this study. In myocytes isolated from hearts of cardiomyopathic hamsters that develop heart failure, there was a reduction in PDH activity, and also a reduced $[Ca^{2+}]_m$ in response to rapid electrical stimulation compared with control hearts (Di Lisa et al. 1993). This seemed likely due to a reduction in the systolic $Ca^{2+}$ transient, leading to reduced $[Ca^{2+}]_m$ and failure to activate PDH. The hearts also exhibited reduced developed pressure and adenine nucleotide content (Wikman-Coffelt et al. 1986). Mitochondria isolated from the hearts also showed a reduced $Ca^{2+}$ uptake (Lin et al. 2007) and this was associated with a lower $\Delta\psi_m$ and reduced activities of complexes I and IV. It is possible but untested that inhibiting the mNCX in these hearts would be beneficial in restoring $[Ca^{2+}]_m$ and activating PDH.

Recent work from O'Rourke's group has shown that dysregulation of $Na^+$ homeostasis in heart failure may be a primary cause of mitochondrial dysfunction (Maack et al. 2006; Liu and O'Rourke 2008): in a guinea-pig model of heart failure (induced by aortic constriction), intracellular $[Na^+]$ was 16 mM compared with 5 mM in control cells (Liu and O'Rourke 2008). Rapid pacing of the cells induced a decrease in NAD(P)H fluorescence, an indirect indicator of respiratory chain activity, whereas this was maintained in controls. An inhibitor of mNCX, CGP 37157, was able to prevent the decrease in NADH in the failing myocytes. It is thus likely to restore ATP levels in the failing hearts: earlier work showed that the mNCX is capable of regulating $[Ca^{2+}]_m$ and dehydrogenase activity since adding $Na^+$ to isolated mitochondria shifts the activation curves for PDH and OGDH by $Ca^{2+}$ to the right (Denton et al. 1980).

$[Ca^{2+}]_m$ may also play a role in regulating levels of oxidative stress: In the model of guinea-pig heart failure used above, increased workload resulted in a transient oxidation of NAD(P)H, but which was re-reduced as $[Ca^{2+}]_m$ increased (Kohlhaas et al. 2010). Concomitant with this was a rise in $H_2O_2$, measured using the fluorescent indicator $CMH_2$-DCF-DA. The ROS production was enhanced in the presence of Ru360 to block mitochondrial $Ca^{2+}$ uptake, or when $Ca^{2+}$-eflux was accelerated using increased $[Na^+]_i$. Myocytes from failing hearts showed elevated basal ROS production by the mitochondria, and this was prevented by inhibiting mNCX (Kohlhaas et al. 2010). The transient oxidation of NAD(P)H was closely associated with an increase in mitochondrial $H_2O_2$ formation. The authors argue that since NAD(P)H levels correlate positively with the glutathione redox state but inversely

with ROS formation (Aon et al. 2007), prevention of recovery of NAD(P)H by the reduced $[Ca^{2+}]_m$ is the underlying cause of the observed increase in ROS in these myocytes. They suggest therefore that mitochondrial $Ca^{2+}$ uptake is not just important for balancing energy supply with demand, but also for the ability of the mitochondria to scavenge free radicals by maintaining the redox state of the matrix (Kohlhaas et al. 2010).

However, the benzodiazepine inhibitors of the mNCX like clonazepam and diltiazem cannot be used in the whole heart as specific antagonists of mitochondrial $Ca^{2+}$ efflux, because of their effects on coronary vessels (diltiazem, for example, is used to reduce high blood pressure). Design of more specific inhibitors of the mNCX may be of benefit in states where ATP synthesis is impaired, such as in heart failure, since maintaining $[Ca^{2+}]_m$ at higher levels could in turn increase ATP production, and also reduce ROS formation by mitochondria (Kohlhaas et al. 2010). There is some precedent for the idea that inhibiting the mNCX can enhance [ATP]: in pancreatic islets CGP37157 increased oxidative phosphorylation, and potentiated glucose-stimulated insulin release (Lee et al. 2003), prompting the authors to suggest it as a novel insulin secretagogue.

## 11.4 Mitochondria and Inherited Cardiomyopathies

### *11.4.1 Mitochondrial DNA and Disease*

Mitochondrial DNA (mtDNA) is circular, double stranded, and encodes 13 subunits of oxidative phosphorylation, (in complexes I, III, IV and V), 2 rRNA subunits and 22 tRNA's; this is covered in detail in Chap. 2 of this volume (by Bai) and reviewed in (Tuppen et al. 2010). Mutations in mtDNA lead to diseases that predominantly affect the nervous system, skeletal and cardiac muscle. Defects in mitochondrial proteins, whether nuclear or mitochondrially encoded, can cause cardiomyopathy but also myopathy and neuropathy – this is not surprising and has been known for many years – for more information see reviews (Li et al. 1995; Naviaux 2000; Fosslien 2003). Although cardiac defects often form a part of the "mitochondrial disease", I will restrict this section to specific cardiomyopathies arising from mitochondrial defects – other diseases of mitochondria are the subject of another chapter in this issue (Chap. 8 by Finsterer).

### *11.4.2 Mitochondrial Cardiomyopathies*

Cardiomyopathies are diseases that cause cardiac dysfunction such as heart failure, arrhythmia, and sudden death; they represent a major cause of morbidity and mortality in both children and adults (Hughes and McKenna 2005). Types of cardiomopathy include dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) (Hughes and McKenna 2005). DCM is the most common cause of heart failure, affecting 40 people out of every 100,000 (Towbin and Bowles 2002). About 50% of individuals die within 5 years of diagnosis, either from pump failure or sudden death, although the situation us improving with development of new drugs. Although the aetiology is not always known, about 30–40% of cases of DCM have the familial form, with autosomal dominant inheritance the main form of inheritance. DCM and HCM can be cause by defects in contractile and structural proteins, for example in HCM most mutations are small (single point or small deletions/insertions) in genes for β-myosin heavy chain, cardiac troponin T or I, and myosin binding protien C (Marian et al. 2001). HCM is also a main cause of sudden death in young and apparently healthy individuals and athletes (Towbin and Bowles 2002).

Mitochondrial inheritance of cardiomyopathy like all mitochondrial diseases is complicated (Goldstein et al. 1999): inheritance is by the maternal line, since mitochondria in the embryo

derive almost entirely from oocyte mitochondria. However, manifestation of a disease varies due to heteroplasmy – the presence of different populations of mitochondrial DNA within the same cell. The offspring may inherit all, none, or intermediate amounts of the damaged mitochondrial genome from the mother. The disease may therefore not become apparent until the mutated mtDNA reaches a certain amount – the threshold effect (Chen et al. 2006).

mtDNA diseases that only affect the heart are rare and often fatal, but specific cardiomyopathies have been reported (Goldstein et al. 1999). More than 50 point mutations have now been identified that lead to diseases with cardiomyopathy; these are usually within genes for tRNA, and affect multiple systems since more than one mitochondrial protein is affected. Large deletions of mtDNA are present in patients with Kearns-Sayre syndrome, who also have a cardiac conduction block (rather than cardiomyopathy) (Goldstein et al. 1999).

An example of a point mutation causing disease occurred in two families with hypertrophic cardiomyopathy where clinical abnormalities were confined to the heart: an A to G transition in the $tRNA^{Ile}$ gene caused severely depressed respiratory chain enzyme activity (in complexes I and IV) (Terasaki et al. 2001). All family members had hypertrophy, some had or went on to develop LV dilation and failure; there were two childhood deaths, one heart transplant recipient and another family member awaiting one. Another study on an infant with cardiomyopathy found a mutation (C to T) in the mitochondrial $tRNA^{Leu}$ gene that lead to partial deficiencies of complexes I and IV (Goldstein et al. 1999) Although the same mutation could be detected in mother and siblings, they were asymptomatic, despite having up to 74% of the mutant genomes, whereas mitochondria from the infant with the cardiomyopathy contained 100% of the abnormal gene. The infant died aged 6 months following cardiac failure, and microscopic examination on autopsy revealed major abnormalities confined to the heart – hypertrophic cardiomyopathy; enlarged mitochondria but abnormal cristae – "whorls" (Goldstein et al. 1999).

Another patient with congestive heart failure showed a novel point mutation in the gene for mitochondrial $tRNA^{Lys}$ (Terasaki et al. 2001). In a biopsy taken from the left ventricular wall during an operation, electron microscopy showed that the failing tissue contained giant mitochondria surrounded by numerous smaller mitochondria, which had concentric circular cristae (Kanzaki et al. 2010). The giant mitochondria had possibly occurred via fusion of several mitochondria, possible in an attempt to compensate for the reduced function. By contrast normal tissue contained continuous rows of uniform mitochondria with the classical cristae appearance.

As well as mutations in mtDNA genes themselves, defective transcription can also have severe consequences: mitochondrial transcription factor (TFAM) is a nuclear-encoded transcription factor that binds to mitochondrial DNA. Cardiac-specific knockout of TFAM in mice caused dilated cardiomyopathy in addition to reduced mitochondrial copy number (Wang et al. 1999). Conversely, overexpressing TFAM in mice prevented the decline in mitochondrial copy number and attenuated heart failure following myocardial infarction (Ikeuchi et al. 2005). The restored respiratory chain activity could then reduce ROS production and the increases in mitochondrial copy number also maintained ATP synthesis (Tsutsui et al. 2009).

Mutations in nuclear DNA can also cause mitochondrial cardiomyopathies resulting from severe enzyme defects, since most proteins in mitochondria are encoded by the nuclear genome. Deficiencies in cytochrome c oxidase caused infantile hypertrophic cardiomyopathy (Servidei et al. 1994), and cardiomyopathy is the most common cause of death in infants with complex I deficiency associated with severe lactic acidosis (Goldstein et al. 1999). A cardiomyopathy was also seen in two patients with ATPase deficiency (Holme et al. 1992). Diseases associated with defects in nuclear-encoded mitochondrial proteins, including those involved in fatty acid synthesis or citric acid cycle enzymes are discussed more fully in Chap. 8 (by Finsterer).

There is an X-linked cardiomyopathy, Barth syndrome, which affects mitochondrial function: Barth syndrome is caused by a defect in the cardiolipin transacylase enzyme, tafazzin. Cardiolipin is a phospholipid characteristic of the mitochondrial inner membrane and synthesised in mitochondria but then remodeled to produce cardiolipin rich in unsaturated fatty acids, particularly linoleic acid (Hauff and

Hatch 2006; Claypool et al. 2008). Mitochondria have membrane protein contents much higher than those of other membranes (Claypool et al. 2008), and optimal cardiolipin content is essential for correct function and organisation of many mitochondrial enzymes, including those of the respiratory chain, the ATP synthase and adenine nucleotide translocase. Various mutations in the tafazzin gene have been described (Hauff and Hatch 2006), and lead to a reduced cardiolipin content of mitochondria, more saturated fatty acids in the cardiolipin, and an accumulation of monolysocardiolpin. Patients all show alterations in mitochondrial structure, and depressed oxidative phospgorylation (Claypool et al. 2008). Infants have LV dysfunction and dilation, and can succumb to sudden death although most survive infancy (Towbin and Bowles 2002).

There are also inherited diseases that cause electrophysiological disturbances in the heart, causing arrhythmias, long QT syndrome, and Brugada syndrome amongst others (Marcus 2000). However, although mitochondria, in particular mitochondrial $Ca^{2+}$ signalling, is becoming increasingly recognised as being capable of modulation EC coupling in the heart, few of the mitochondria ion transporters have been characterised, and there are as yet no known mutations in mitochondrial proteins that lead directly to arrhythmias.

## 11.5 Conclusions

It is clear that mitochondria play a major role in both normal and pathological heart function, either as a primary cause or in the development of heart disease. Very recently clinical trials utilising probes acting on mitochondria have begun: CsA, which acts on the MPTP, and the mitochondrially-targeted antioxidants. Particularly promising are strategies like CsA and IPost that can be used at the point of reperfusion.

Thus these carefully targeted strategies should give better clinical outcomes, than for example, generally antioxidants that may not reach the main site of ROS production, the mitochondria, effectively. It is essential to continue our basic research in this area: the MPTP was a curious phenomenon observed initially in isolated mitochondria, but it is now generally agreed to be a critical mediator of reperfusion injury, and targeted as the end-effector of various protective strategies. However, basic science findings need to be shaped by the constraints and practicalities of clinical applications, hence despite the numerous studies on IPC, it has proved limited as a clinical tool.

Finally, design of specifically targeted drugs against the MPTP or mitochondrial $Ca^{2+}$ transport pathways have I believe, tremendous potential, but first these elusive proteins need to be fully identified and characterised. There may also be as yet unidentified cardiomyopathies due to inheritance of abnormal genes for these proteins.

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