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Physiological and Pathological Roles of the Mitochondrial Permeability Transition Pore in the Heart

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Prolonged mitochondrial permeability transition pore (MPTP) opening results in mitochondrial energetic dysfunction, organelle swelling, rupture, and typically a type of necrotic cell death. However, acute opening of the MPTP has a critical physiologic role in regulating mitochondrial Ca^{2+} handling and metabolism. Despite the physiological and pathological roles that the MPTP orchestrates, the proteins that comprise the pore itself remain an area of ongoing investigation. Here, we will discuss the molecular composition of the MPTP and its role in regulating cardiac physiology and disease. A better understanding of MPTP structure and function will likely suggest novel cardioprotective therapeutic approaches.

Introduction

Mitochondria are critical mediators of cellular life through energy production as well as death through induction of apoptosis and necrosis. These diametrically opposed functions are especially relevant in the heart, where mitochondria supply 90% of the cardiomyocyte's ATP (Harris and Das, 1991), and cell death due to mitochondrial intrinsic killing mechanisms underlies a host of cardiac diseases (Lesnefsky et al., 2001). While many death pathways converge on mitochondria, there has been an increasing body of evidence indicating that the mitochondrial permeability transition pore (MPTP) acts as a key nodal point in mediating cardiac dysfunction and cell death.

Mitochondrial permeability transition is the phenomenon whereby the inner membrane suddenly allows free passage of solutes up to 1.5 kDa in size (Haworth and Hunter, 1979; Hunter and Haworth, 1979a, 1979b). MPTP opening results in inner membrane potential collapse, respiratory chain uncoupling, halt of mitochondrial ATP synthesis, and eventually mitochondrial swelling, rupture, and cell death (Halestrap, 2009).

Given the intimate links between the MPTP, mitochondrial function, and cell death, permeability of the inner membrane is a critical decision point between cellular life and death. Thus, the MPTP is an attractive target for cell death prevention in a host of disease states. Indeed, MPTP inhibition via targeting cyclophilin D (CypD), the best-characterized regulator of the MPTP, produced mice with protection from cell death in an array of tissues in response to select disease stimuli (Baines et al., 2005; Martin et al., 2009; Millay et al., 2008; Ramachandran et al., 2011). However, despite our understanding of the pathological consequences of MPTP opening, the field currently lacks an understanding of the complete molecular constituents of the MPTP complex as well as its ultimate physiological role in cardiac function and metabolism.

The Molecular Identity of the MPTP

The MPTP was initially characterized by Hunter and Haworth (Haworth and Hunter, 1979; Hunter and Haworth, 1979a, 1979b) as a non-selective channel with a peak conductance of ~1.3 nS (Szabó and Zoratti, 1992). Pore opening is activated by Ca^{2+} together with phosphate and reactive oxygen species (ROS) and is inhibited by numerous factors including adenine nucleotides, low pH, divalent cations like Mg^{2+}, and CypD inhibitors such as cyclosporine A (CsA) and sanglifehrin A (Halestrap et al., 2004). MPTP activation may also be subject to additional layers of regulation through modulation by kinases as well as post-translational modification of CypD, as previously reviewed more comprehensively (Elrod and Molkentin, 2013; Vagnozzi et al., 2012).

While the biophysical properties of the MPTP are well established, identification of the molecular constituents of the MPTP has proven to be a conundrum that remains unresolved. Historically, biochemical studies originally suggested that the MPTP was comprised of the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, the adenine nucleotide translocator (ANT) in the inner mitochondrial membrane, and CypD as its regulator in the matrix of the mitochondria (Crompton et al., 1998) (Figure 1). In the past decade, however, genetic studies have systematically tested the requirement of each of these originally proposed components to MPTP structure and function, resulting in a dramatically different model (Figure 1). Below we will summarize work on the molecular identification of the pore to date and highlight evidence for new candidates that may serve as either direct pore-forming components of the MPTP or that simply function as critical regulators as part of a larger complex.

Cyclophilin D

The mitochondrial matrix petidyl-prolyl *cis-trans* isomerase CypD is a genetically verified and undisputed regulator of MPTP function. CypD's involvement in MPTP regulation was discovered through the investigation of the immunosuppressive drug CsA, which reduces mitochondrial swelling and was later shown to bind and inhibit CypD activity (Crompton et al., 1988; Fournier et al., 1987; Halestrap and Davidson, 1990). Definitive

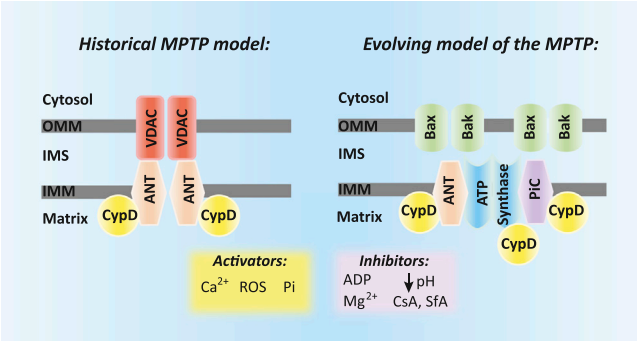

Figure 1. The Molecular Structure of the MPTP
The original paradigm of the MPTP featured VDAC, ANT, and CypD as the core constituents of the complex (left). Genetic evaluation of putative MPTP components has shown that ANT, PiC, and CypD serve as pore regulators, while the BH3-domain pro-apoptotic proteins Bax/Bak function in the outer membrane to permit mitochondrial swelling and rupture once the inner membrane complex opens. The F_1F_0 ATP synthase has been suggested to be a candidate for the inner membrane pore-forming unit of the MPTP (right). IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; IMS, intermembrane space.

proof of CypD's role in MPTP function first came from three independent studies by Baines and colleagues, Nakagawa and colleagues, and Basso and colleagues where the gene encoding this protein was ablated in mice. These studies showed that mitochondria lacking CypD had reduced sensitivity to Ca^{2+} and oxidative stress-induced MPTP opening. However, at high levels of Ca^{2+}, permeability transition could still be elicited, suggesting that CypD controls the sensitivity of MPTP opening (Baines et al., 2005; Basso et al., 2005; Nakagawa et al., 2005). Indeed, pharmacological inhibition of CypD or deletion of the gene encoding this protein reduced MPTP opening and subsequently reduced cell death in numerous tissues after ischemic injury and degenerative disease processes (Baines et al., 2005; Martin et al., 2009; Millay et al., 2008; Ramachandran et al., 2011; Schinzel et al., 2005; Wissing et al., 2010). However, to date the precise mechanism whereby CypD controls MPTP opening has not been determined. Indeed, while CypD can directly bind a number of mitochondrial inner membrane proteins implicated in affecting permeability transition (see below), the consequences of these interactions are still not known.

VDAC

The VDAC was first suggested to serve as the outer membrane component of the MPTP due to the fact that the electrophysiological properties of VDAC were similar to those of the MPTP and that VDAC functions as a nonselective pore to anions and cations (Szabó et al., 1993; Szabó and Zoratti, 1993). The involvement of VDAC in MPTP formation was further bolstered by the fact that VDAC, ANT, and CypD could be reconstituted into proteoliposomes to yield a CsA-sensitive pore (Crompton et al., 1998). However, genetic studies showed that mitochondria lacking all three VDAC isoforms retained an unaltered ability to undergo permeability transition (Baines et al., 2007), and further, instead of being protective, loss of VDAC2 expression in cultured cells potentiated cell death (Baines et al., 2007; Cheng et al., 2003), collectively suggesting that VDAC is not an essential component of the MPTP.

ANT

The ANT is a 32 kDa inner membrane transporter responsible for the import of ADP into the mitochondrial matrix in exchange for ATP and as such is an integral component of the mitochondrial ATP synthesis machinery (Klingenberg, 2008). ANT was hypothesized to be the inner membrane pore-forming component of the MPTP given that adenine nucleotides and bongkrekic acid, the latter being an ANT inhibitor that stabilizes the "m" conformation of the protein, inhibit MPTP activity while carboxyatractylate, an ANT effector that promotes the "c" conformation of the protein, stimulates MPTP opening (Haworth and Hunter, 2000; Klingenberg, 1989). Further, ANT is a direct binding partner of CypD (Crompton et al., 1998; Woodfield et al., 1998), and reconstitution of ANT alone or in combination with CypD and VDAC into proteoliposomes yielded pores with MPTP characteristics (Brustovetsky and Klingenberg, 1996; Brustovetsky et al., 2002; Crompton et al., 1998; Rück et al., 1998). However, in vivo genetic inactivation of the most abundant ANT isoforms (*Ant1* and *Ant2* genes) in liver resulted in mitochondria that were still able to undergo Ca^{2+} induced permeability transition, although higher concentrations of Ca^{2+} were required (Kokoszka et al., 2004). An additional ANT gene, *Ant4*, was recently identified in both humans and mice (Brenner et al., 2011; Dolce et al., 2005), thus introducing the possibility that ANT ablation in the liver was incomplete in studies conducted by Kokoszka and colleagues. However, since murine *Ant4* is mainly expressed in pluripotent stem cells and germ cells, this additional isoform of ANT might not contribute significantly to the previous MPTP studies conducted in the liver (Kokoszka et al., 2004). Finally, there is evidence that ANT may represent a major site of oxidant stress and thiol modulation of MPTP function (Halestrap et al., 1997). Collectively, studies on ANT suggest that it is not a requisite component of the MPTP, but it clearly regulates MPTP activity.

Phosphate Carrier

The mitochondrial phosphate carrier (PiC) is an inner membrane solute carrier that is the primary transporter of inorganic phosphate (Pi) into the mitochondrial matrix (Kolbe et al., 1984; Palmieri, 2004). As such, PiC plays a key role in mitochondrial oxidative phosphorylation (OXPHOS) and energy production as it supplies mitochondria with the Pi required for ATP synthesis (Kolbe et al., 1984; Palmieri, 2004). PiC was introduced as a regulator of cell death and the MPTP because PiC overexpression in cultured cells induced death (Alcalá et al., 2008), and Pi has long been known to facilitate MPTP opening (Al-Nasser and Crompton, 1986; Beatrice et al., 1980; Crompton and Costi, 1988; Varanyuwatana and Halestrap, 2012). PiC was further proposed to be a candidate for the inner membrane component of the MPTP because it was also a CypD binding partner (Leung et al., 2008), and it can form pores in vitro when reconstituted into liposomes (Schroers et al., 1997). However, the essential nature of PiC's contribution to MPTP structure and function was called in to question given the observation that partial reduction of PiC protein with an siRNA approach resulted in no effect on MPTP opening (Gutiérrez-Aguilar et al., 2014; Varanyuwatana and Halestrap, 2012), suggesting that PiC was not required for permeability transition. However, cardiomyocyte-specific deletion of the gene encoding PiC protein, which resulted in an

even a greater loss of total PiC protein from mitochondria, blunted Ca^{2+} overload-induced MPTP opening (Kwong et al., 2014). This attenuation of MPTP opening conferred protection against cardiac ischemia-reperfusion injury (Kwong et al., 2014). However, since the MPTP retained full function under phosphate-free conditions, these findings collectively suggest that the PiC is not a direct pore component of the MPTP, but instead its ability to alter matrix Pi levels secondarily impacted pore opening.

F_1F_0 ATP Synthase

The mitochondrial F_1F_0 ATP synthase has more recently emerged as a strong candidate to be the core component of the MPTP in the inner membrane (Figure 1). The F_1F_0 ATP synthase is a multi-subunit enzymatic complex that couples proton translocation across the inner membrane to ATP synthesis. The F_0 proton-translocating domain of the ATP synthase is embedded in the inner membrane and is connected to the F_1 catalytic domain through the central and peripheral stalks (Jonckheere et al., 2012). CypD was initially found to modulate ATP synthase activity by binding to the OSCP subunit of the peripheral stalk (Giorgio et al., 2009, 2013), and purified dimers of the F_1F_0 ATP synthase reconstituted into lipid bilayers recapitulated channel activity similar to that of the MPTP (Giorgio et al., 2013). Reconstitution of just the purified c subunit ring of the F_0 domain into liposomes could also produce a high-conductance voltage-gated channel that was sensitive to adenine nucleotides but not CsA (Alavian et al., 2014). Similarly, reconstitution of complete F_1F_0 ATP synthase monomers including the OSCP subunit restored CypD/CsA-regulated channel activity. Finally, shRNA-mediated reduction of the c subunit prevented Ca^{2+}-mediated MPTP opening and conferred protection against Ca^{2+} overload-induced death in cultured cells and glutamate excitotoxic death in cultured neurons (Alavian et al., 2014; Bonora et al., 2013). Together, these studies provide compelling evidence that the ATP synthase is required for proper MPTP function. However, further work is required to reconcile two potential models: ATP synthase dimers forming the MPTP versus ATP synthase monomers alongside the c subunit ring forming the pore (Alavian et al., 2014; Giorgio et al., 2013). Moreover, it also remains possible that the ATP synthase alters the activity of an as yet unidentified component that is the actual pore itself. In vivo genetic loss-of-function analyses of the various ATP synthase components will be helpful in resolving this issue.

Bax/Bak

The pro-death Bcl-2 family member proteins Bax and Bak are central players in apoptotic cell death. In response to apoptogenic stimuli, Bax/Bak oligomerize to permeabilize the mitochondrial outer membrane, thereby permitting cytochrome *c* release and the subsequent steps in mediating apoptosis (Tait and Green, 2010). In addition to this role in apoptotic cell death, Bax/Bak have been implicated in MPTP regulation through their ability to induce a level of outer mitochondrial membrane permeability that allows MPTP opening to progress to organelle swelling and eventual rupture (Karch et al., 2013). Indeed, mitochondria lacking Bax/Bak are resistant to MPTP opening, and reconstitution of Bax/Bak-deficient cells with apoptosis/oligomerization-impaired Bax mutants restored both MPTP opening (Karch et al., 2013; Whelan et al., 2012) and outer mitochondrial membrane permeability (Karch et al., 2013). Moreover, absence of Bax/Bak is protective against cardiac ischemia-reperfusion injury (Karch et al., 2013; Whelan et al., 2012) and, combined with CypD deficiency, did not confer additional protection (Whelan et al., 2012). Prior to this more recent work, Bax and Bak overexpression were originally shown to regulate MPTP opening in isolated mitochondria, in a CsA- and bongkrekic acid-dependent manner (Narita et al., 1998). Collectively, these findings support the hypothesis that Bax/Bak are essential for MPTP to progress to organelle swelling and rupture at the level of the outer mitochondrial membrane once the inner membrane components of the MPTP are engaged in response to Ca^{2+} or ROS stimulation.

TSPO and C1qbp

The translocator protein (TSPO), also known as the peripheral benzodiazepam receptor, is an outer mitochondrial membrane protein that was initially suggested to regulate MPTP function due to the effects of TSPO ligands on MPTP opening (Chelli et al., 2001; Li et al., 2007; Pastorino et al., 1994). TSPO was further found to associate with VDAC and ANT, thereby linking TSPO directly to regulatory components of the MPTP (McEnery et al., 1992). However, a recent mouse model for the conditional deletion of TSPO from the liver and heart definitively showed that this protein does not participate in MPTP regulation (Šileikytė et al., 2014).

C1qbp (complement 1q binding protein) was initially identified as a plasma membrane receptor for complement 1q that could also form homotrimeric pores (Jiang et al., 1999) with the ability to insert within the mitochondrial inner membrane and possibly be part of the MPTP (Starkov, 2010). Overexpression of C1qbp in rat fibroblasts induced mitochondrial swelling, dysfunction, and cell death (Chowdhury et al., 2008). However, analyses of C1qbp gain- and loss-of-function phenotypes in mouse embryonic fibroblasts actually suggested this protein was an inhibitor of the MPTP (McGee and Baines, 2011). Hence, it is unlikely that that this protein serves as a direct pore-forming component of the MPTP.

Physiological Function of the MPTP in Metabolism

Mitochondria are central to cellular metabolism and energy production. With genetic studies confirming ANT (Kokoszka et al., 2004) and PiC (Kwong et al., 2014) as MPTP regulators and the F_1F_0 ATP synthase as a candidate for the pore-forming component itself (Alavian et al., 2014; Giorgio et al., 2013), there appears to be a connection between the MPTP and mitochondrial energy metabolism (Figure 2). The F_1F_0 ATP synthase, ANT, and PiC are not only functionally coupled for mitochondrial ATP production, but physically coupled into an inner membrane supercomplex termed the ATP synthasome (Ko et al., 2003). Loss of any one of the ATP synthasome components results in impaired mitochondrial oxidative phosphorylation (Bakker et al., 1993; Graham et al., 1997) and energy production (Kwong et al., 2014; Tatuch and Robinson, 1993). Indeed, mice lacking either PiC or ANT1 protein in the heart develop a severe mitochondrial cardiomyopathy characterized by hypertrophy, ventricular dilation, cardiac dysfunction, and mitochondrial hyperproliferation (Graham et al., 1997; Kwong et al., 2014; Narula et al., 2011). Human patients with mutations in the skeletal muscle isoform

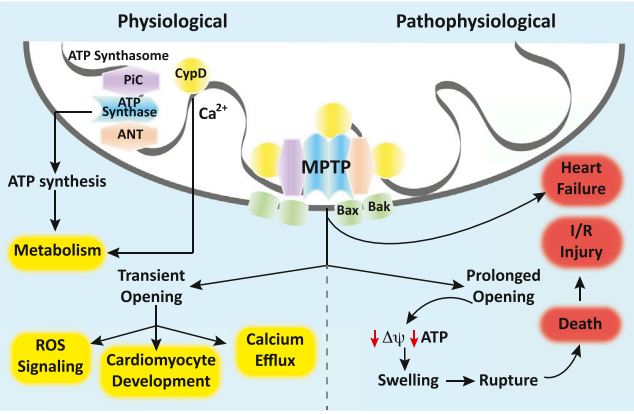

Figure 2. Physiological and Pathophysiological Roles of the MPTP in the Heart

Transient opening of the MPTP is implicated in ROS signaling, cardiomyocyte development, and mitochondrial Ca^{2+} efflux that affects metabolism. Key components of the MPTP (PiC, ANT, and the F_1F_0 ATP synthase) comprise the ATP synthasome, thereby providing a direct link to mitochondrial energy metabolism. Prolonged MPTP opening leads to loss of mitochondrial membrane potential, cessation of ATP synthesis, mitochondrial swelling, rupture, and death. Unregulated MPTP opening has been found to contribute to cardiac ischemia-reperfusion injury and the development of heart failure. $\Delta\psi$, inner membrane potential.

of PiC develop muscle weakness, lactic acidosis, hypertrophic cardiomyopathy, and shortened lifespan (Mayr et al., 2007, 2011). Similarly, patients with ANT1 protein deficiency can present with hypertrophic cardiomyopathy, muscle myopathy, lactic acidosis, and exercise intolerance (Bakker et al., 1993; Palmieri et al., 2005). Finally, mutations in the mitochondrial *MT-ATP8* gene are associated with hypertrophic cardiomyopathy (Jonckheere et al., 2010; Ware et al., 2009).

In further support of the MPTP being linked to metabolism, mice lacking CypD protein showed increased activity of two key matrix dehydrogenases of the tricarboxylic acid cycle (pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) (Elrod et al., 2010). Moreover, in-depth proteomic analyses of these CypD-deficient mice revealed alterations in enzymes involved in pyruvate and branched-chain amino acid metabolism, as well as the Kreb's cycle (Menazza et al., 2013), suggesting that MPTP desensitization produces global reprogramming of cellular metabolism. Taken together, these studies suggest that the MPTP may be a nodal point of cell life and death decisions by integrating energy metabolism with the cell death machinery.

MPTP in Ca^{2+} Efflux

In addition to energy production, mitochondria are major sites of intracellular Ca^{2+} signaling and accumulation (Figure 2). Increased Ca^{2+} content in the mitochondrial matrix directly enhances metabolic output by increasing the activity of tricarboxylic acid cycle dehydrogenases and the ATP synthase (Balaban, 2009; Hansford and Zorov, 1998). However, sustained mitochondrial matrix Ca^{2+} overload triggers prolonged high-conductance MPTP opening, leading to mitochondrial dysfunction and cell death (see below). Thus, maintenance of mitochondrial Ca^{2+} homeostasis is of critical importance. Mitochondrial Ca^{2+} influx is controlled by the mitochondrial calcium uniporter (MCU) (Baughman et al., 2011; De Stefani et al., 2011), while efflux is controlled by the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) (Palty et al., 2010) and the mitochondrial H^+/Ca^{2+} exchanger (Jiang et al., 2009; Tsai et al., 2014). Transient or low-conductance opening of the MPTP has also been proposed to serve as an additional mode of Ca^{2+} efflux that mitigates sustained matrix Ca^{2+} overload (Bernardi and von Stockum, 2012; Ichas and Mazat, 1998). In support of this hypothesis, both isolated mitochondria from CypD-deficient mice and intact cardiomyocytes treated with CsA display elevated matrix Ca^{2+} levels (Elrod et al., 2010). Additionally, transient asynchronous MPTP opening can allow for temporary membrane potential depolarization in limited populations of mitochondria while the vast majority of the mitochondria remain polarized and functional (Korge et al., 2011). In contrast to these studies, mitochondrial Ca^{2+} efflux rates measured in intact HeLa cells were completely unaffected by MPTP inhibition either by CsA or by siRNA-mediated reduction of the ATP synthase c subunit (De Marchi et al., 2014), suggesting that the MPTP may not play a role in Ca^{2+} efflux under all ex vivo assay conditions. Thus, the MPTP's participation in mitochondrial Ca^{2+} efflux under specific conditions or in certain cellular contexts needs further investigation.

MPTP in ROS Signaling

In addition to Ca^{2+} efflux, transient opening of the MPTP has been linked to mitochondrial ROS signaling (Zorov et al., 2000). Mitochondria are a major source of cellular ROS production, and although excessive ROS can contribute to accelerated aging and cell death, physiological levels of ROS can underlie processes ranging from ion channel homeostasis to gene expression (Figure 2). Through the use of a novel genetically encoded mitochondrial sensor, mt-cp-YFP (a matrix-targeted, circularly permuted yellow fluorescent protein), it has been suggested that mitochondria from resting adult cardiomyocytes (Li et al., 2012; Wang et al., 2008) or Langendoff perfused hearts (Wang et al., 2008) can release discrete bursts or "flashes" of superoxide. The MPTP was implicated in cardiac mitochondrial superoxide flash production as flashes were coincident with transient decreases in mitochondrial membrane potential (Li et al., 2012; Wang et al., 2008), and MPTP inhibition with bongkrekic acid, CsA, or by CypD knockdown greatly decreased flash frequency (Wang et al., 2008). It should be noted, however, that the MPTP's role in superoxide flashes may be tissue specific as neither CsA inhibition (Pouvreau, 2010) nor CypD protein loss (Wei et al., 2011) had an effect on flash frequency in skeletal muscle fibers. It also must be noted that instead of superoxide production, mt-cp-YFP flashes were suggested to reflect transient alkalinization of the matrix (Schwarzländer et al., 2012). While the precise nature of flashes is still not fully understood, they are linked to metabolic activity (Fang et al., 2011) and mitochondrial function (Wang et al., 2008), and there is increased incidence of flashes during the re-oxygenation phase of cardiac ischemia-reperfusion injury (Wang et al., 2008).

MPTP in Cardiac Development

The MPTP has also been suggested to play a novel role in cardiomyocyte development and mitochondrial maturation (Figure 2). Work by Horn and colleagues showed that during murine embryonic development, cardiac mitochondria underwent a profound structural and functional maturation that tracked with

cardiomyocyte differentiation (Hom et al., 2011). Mitochondria from immature E9.5 myocytes were fragmented with disorganized cristae, depressed mitochondrial membrane potential, and elevated ROS production, suggesting that the MPTP adopted an open conformation. However, by E13.5, the mitochondria were organized into tubular networks with defined cristae structure, higher membrane potential, and decreased ROS, suggesting that the MPTP was closed. In support of this hypothesis, MPTP inhibition by CsA or by CypD protein loss, as well as administration of ROS scavengers resulted in E9.5 myocytes displaying more mature myofibrillar organization and more developed mitochondrial networks (Hom et al., 2011).

Pathophysiological Roles of the MPTP in Cell Death

A critical step in the activation of cell death programs, either apoptotic or necrotic, is mitochondrial membrane permeabilization (Kroemer et al., 2007). However, the mode whereby mitochondrial membranes are disrupted may be the deciding factor as to which death program the cell engages. In the classical mitochondrial apoptotic pathway (as previously reviewed more comprehensively by Spierings et al., 2005 and Tait and Green, 2010), outer membrane permeabilization by the pro-death Bcl-2 family member proteins Bax and Bak allows for apoptogenic factors like cytochrome *c*, Smac/DIABLO, and AIF to be released from the intermembrane space into the cytosol, leading to cell death by apoptosis. In contrast, stimuli such as Ca^{2+} overload or ROS cause MPTP opening leading to mitochondrial inner membrane permeabilization, membrane potential dissipation, impaired respiratory chain function, halt of mitochondrial ATP synthesis, organelle swelling, and outer membrane rupture. This ultimately results in necrotic cell death, characterized by loss of plasma membrane integrity and cell rupture (Golstein and Kroemer, 2007).

Certainly, there is considerable crosstalk between mitochondrial apoptotic and necrotic pathways as Bax/Bak participate in both apoptosis and necrosis at the level of the outer mitochondrial membrane (Karch et al., 2013; Wei et al., 2001; Whelan et al., 2012). However, while both apoptotic and necrotic pathways can contribute to cardiomyocyte loss in disease, the MPTP-necrosis pathway is distinct from apoptosis, as cells lacking CypD are protected against stimuli that induce MPTP opening (like Ca^{2+} and ROS overload) but retain sensitivity to apoptosis-inducing agents (Baines et al., 2005; Nakagawa et al., 2005). Bax/Bak-deficient cells are resistant to both forms of death, and re-introduction of Bax mutants that are unable to oligomerize in the outer membrane to generate large apoptotic pore can still increase the "micropermeability" of the outer membrane to facilitate regulated necrosis and organelle swelling once the inner membrane of the MPTP is opened (Karch et al., 2013).

Ischemia-Reperfusion Injury

During myocardial ischemia, oxygen deprivation arrests mitochondrial respiratory chain function and ATP synthesis, causing a marked drop in cardiac ATP content (Jennings and Reimer, 1991). This impaired mitochondrial metabolic flux forces pyruvate to be metabolized into lactic acid, leading to a drop in cellular pH and metabolic acidosis (Figure 2). To re-establish cytosolic pH and ionic balance, the plasma membrane Na^+/H^+ exchanger is activated to extrude H^+ and the Na^+/K^+ ATPase is engaged to remove excess cytosolic Na^+. Because cellular ATP is constrained, Na^+/K^+ ATPase activity is blunted, leading to a progressive rise in Na^+, which secondarily triggers the Na^+/Ca^{2+} exchanger to extrude Na^+ in exchange for Ca^{2+} influx. Moreover, because the major cellular Ca^{2+} pumping mechanisms require ATP, and the MPTP remains open as ATP levels dissipate, Ca^{2+} levels in the ischemic heart are further elevated. In addition to this Ca^{2+} elevation, ROS production is also increased in the ischemic heart (Murphy and Steenbergen, 2008). Thus, the ischemic heart is primed for prolonged MPTP opening that leads to cardiomyocyte death (Murphy and Steenbergen, 2008).

In light of the fact that the MPTP is open during reperfusion and that the MPTP has been proposed to contribute significantly to reperfusion-mediated cardiac damage (Griffiths and Halestrap, 1995), the MPTP is a prime target for therapies aimed at mitigating cell loss. Indeed, in genetic in vivo proof-of-concept studies, inhibition of MPTP opening by deletion of MPTP components, notably the genes encoding CypD (Baines et al., 2005; Nakayama et al., 2007), PiC (Kwong et al., 2014), and Bax/Bak (Karch et al., 2013; Whelan et al., 2012), reduced cardiac damage sustained in response to ischemia-reperfusion injury. Administration of the CypD inhibitors sanglifehrin A (Clarke et al., 2002; Lim et al., 2007) or CsA (Griffiths and Halestrap, 1993, 1995; Lim et al., 2007) reduced ischemic death and improved functional recovery in both ex vivo and in vivo models of cardiac ischemia-reperfusion injury. Similarly, non-immunosuppressive analogs of CypD such as [MeAla6]-cyclosporine (Griffiths and Halestrap, 1995), Debio-025 (Gomez et al., 2007), and NIM811 (Argaud et al., 2005) were strongly cardioprotective, further confirming the MPTP as a target for therapy. Importantly, a pilot clinical trial in patients presenting with myocardial infarction showed CsA infusion at the time blood flow was restored reduced infarct size by 40% compared to placebo (Piot et al., 2008). In a subsequent follow-up study with the same group of patients, the reduction in infarct size from the single dose of CsA was maintained 6 months post-treatment, and such patients displayed attenuated left ventricular remodeling with improved ventricular function (Mewton et al., 2010).

Finally, there is evidence that mitochondrial association of the glycolytic enzyme hexokinase II can confer protection against cell death and reduce the extent of ischemic injury in the heart, possibly by limiting outer mitochondrial membrane permeabilization, cytochrome *c* release, and oxidative stress, thus secondarily constraining MPTP activation (Halestrap et al., 2014). Clearly, MPTP inhibition holds therapeutic promise for myocardial infarction-based injury, and as the molecular makeup of the MPTP becomes clearer, it will be important to explore pharmacological targeting of new pore components.

Heart Failure

Heart failure is associated with cumulative attrition of cardiomyocytes in cardiac disorders ranging from hypertension to MI. While a multitude of factors can contribute to pump dysfunction, key characteristics of failing cardiomyocytes include: dysregulation of Ca^{2+} homeostasis (Luo and Anderson, 2013), ATP insufficiency (Ingwall, 2009), and increased oxidative stress (Keith et al., 1998). Since these are conditions that favor MPTP opening, it has been hypothesized that the MPTP could be a point

of intervention in heart failure to prevent ongoing myocyte loss due to necrosis. In support of this concept, mice with cardiomyopathy due to Ca^{2+} overload mediated by overexpression of the β2a subunit of the L-type Ca^{2+} channel were protected by loss of the gene encoding CypD (Nakayama et al., 2007). In addition, CypD-deficient mice subjected to prolonged MI displayed reduced mortality and decreased infarct size (Lim et al., 2011), suggesting that MPTP inhibition could be beneficial.

In contrast, CypD-deficient mice displayed accelerated cardiac disease in response to a surgical model of chronic pressure overload as well as increased hypertrophy and mortality in response to forced exercise (Elrod et al., 2010), which are likely due to metabolic deficiencies in the mitochondria lacking CypD, as discussed earlier. Further, loss of CypD exacerbated the heart failure phenotype observed in the Ca^{2+}/calmodulin-dependent protein kinase IIδ-c model of cardiomyopathy, indicating maladaptive consequences of prolonged MPTP desensitization (Elrod et al., 2010). In line with these findings, as mentioned above, long-term cardiac loss of the MPTP regulators ANT1 and PiC in both mice and humans causes mitochondrial cardiomyopathy characterized by mitochondrial structural abnormalities, cardiac hypertrophy, and ventricular dysfunction, which again are likely reflective of a metabolic effect (Bakker et al., 1993; Graham et al., 1997; Kwong et al., 2014; Mayr et al., 2007; Narula et al., 2011; Palmieri et al., 2005).

Future Perspectives

In the past decade, the historical paradigm of MPTP structure has now given way to a new model that features CypD, ANT, PiC, Bax/Bak, and the ATP synthase in new roles. The MPTP remains a promising target for cell death prevention and cardioprotection. In particular, CypD inhibitors show promise in making the transition from bench to bedside as an approach to prevent cardiomyocyte loss following acute cardiac ischemic damage. Moving forward, it will be highly desirable to target additional constituents of the MPTP to provide greater inhibitory ability than is typically observed with cyclophilin inhibitors like CsA, which only desensitize the pore. For example, the therapeutic potential of indirect MPTP regulators such as hexokinase II could be further developed. Finally, there is an emerging theme that while central to cell death regulation, the MPTP is equally important in regulating cellular metabolism, as many MPTP constituents, namely the ATP synthase, ANT, and PiC, have primary roles in mitochondrial energy production. Further insight into how the MPTP contributes to cardiac physiology is needed as we consider other cardiac diseases where metabolism is more centrally involved, especially if we want to inhibit the MPTP long-term, such as in heart failure. Thus, understanding the interface between the physiological and pathophysiological roles of the MPTP will be important when employing current MPTP inhibiting agents or in developing new means of desensitizing or inhibiting MPTP opening in treating both acute post-ischemic tissue damage and long-term cardiomyocyte dropout in heart failure.

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