Journal of Molecular and Cellular Cardiology 46 (2009) 850–857

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

The molecular composition of the mitochondrial permeability transition pore

Christopher P. Baines *

Dalton Cardiovascular Research Center, Department of Biomedical Sciences, University of Missouri-Columbia, Columbia, Missouri 65211, USA
Department of Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, Missouri 65211, USA

ARTICLE INFO

Article history:
Received 17 December 2008
Received in revised form 30 January 2009
Accepted 5 February 2009
Available online 20 February 2009

Keywords:
Mitochondrial permeability transition
Voltage-dependent anion channel
Adenine nucleotide translocase
Cyclophilin-D
Mitochondrial phosphate carrier

ABSTRACT

Uncontrolled cell death is a fundamental cause of organ disease in humans. However, despite the need for us to delineate the molecular machinery that underlies cardiomyocyte death, our knowledge of these lethal cellular processes is still limited. The discovery that mitochondrial dysfunction, and in particular the mitochondrial permeability transition (MPT) pore, is often a common cause of the cardiac cell mortality that underlies numerous cardiac diseases has been a first crucial step. The purpose of this review is to outline our current understanding of the molecular identity of the MPT pore and the many questions that still need to be answered.

Contents

1. Introduction

Uncontrolled cell death is a fundamental cause of organ disease in humans. Being primarily post-mitotic, the heart is particularly sensitive to the loss of its constituent cells and even a relatively small increase in cardiomyocyte death can result in pathology. Consequently, there is a desperate need for us to delineate the molecular machinery that underlies cardiomyocyte death, whether it is the acute, extensive damage seen during myocardial infarction or the more insidious, cumulative cell loss that leads to cardiomyopathy. Despite this, our knowledge of these lethal cellular processes is still limited. The discovery that mitochondrial dysfunction is often a common cause of the cardiac cell mortality that underlies the etiology of numerous cardiac diseases has been a first crucial step towards a better understanding of these mechanisms. In particular, research studying the mitochondrial permeability transition pore and its role in cardiac disease has proven fruitful. However, much work remains to be done especially with regard to the actual molecular composition of the mitochondrial pore, and the purpose of this review is to outline what we know and perhaps more importantly what we don't know about the protein components of this fascinating entity.

* Dalton Cardiovascular Research Center, Department of Biomedical Sciences, University of Missouri-Columbia, 134 Research Park Drive, Columbia, MO 65211, USA.
E-mail address: bainesc@missouri.edu.

0022-2828/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2009.02.007

2. The mitochondrial death pathway

Cardiac stresses, such as ischemia/reperfusion, oxidative stress, and cytotoxic drugs induce a cascade of events at the level of the mitochondrion that, if left unchecked, will kill the myocyte. Initially this involves excessive production of reactive oxygen species, and Ca^{2+} overload of the mitochondrial matrix [1–4]. This in turn causes permeabilization of the inner mitochondrial membrane. This phenomenon, termed the mitochondrial permeability transition (MPT), dissipates the proton electrochemical gradient ($\Delta\Psi_m$) that drives many mitochondrial functions, leading to ATP depletion, further reactive oxygen species production, and ultimately swelling and rupture of the organelle [1–4]. This in turn releases pro-apoptotic intermembrane space proteins, most notably cytochrome *c*, Smac/DIABLO, and endonuclease-G (endoG). Cytochrome *c* binds to the cytosolic protein apaf1 and the resultant "apoptosome" activates the caspase protease system [1,5]. Smac/DIABLO activates caspases by sequestering caspase-inhibitory proteins, whereas endoG mediates DNA fragmentation. Therefore, activation of the mitochondrial pathway may initially induce apoptosis. However, if the stress is severe and/or prolonged, ATP will be depleted and the cell will instead undergo necrosis. These sequelae, plus the ability to inhibit them, indicate the existence of a regulated circuitry within the mitochondrion that underlies the cell death process — one that could potentially be exploited therapeutically.

3. The mitochondrial permeability transition pore and cardiac disease

The MPT pore, a non-specific channel originally thought to span both mitochondrial membranes, mediates the increases in mitochondrial permeability associated with cell death [1–3]. The pore itself is permeable to solutes up to 1.5 kDa. This causes equilibration of H^+ across the inner membrane, which dissipates $\Delta\Psi_m$ and inhibits ATP production. A concomitant influx of water causes swelling of the mitochondria, which stretches the membranes to the point where the outer membrane fails. The mitochondrial pore is redox, Ca^{2+}, voltage, adenine nucleotide, and pH sensitive [1–3]. In particular, increases in matrix Ca^{2+} and reactive oxygen species induce pore opening, whereas adenine nucleotides inhibit the pore; indeed many cardiac diseases, especially ischemia/reperfusion injury, are associated with increases in MPT activators e.g., Ca^{2+} and oxidative stress, and reductions in MPT inhibitors, e.g., ATP/ADP [1–4]. Moreover, studies have shown that inhibition of the MPT pore blunts the loss of cardiac myocytes that underlies several cardiac pathologies: myocardial ischemia/reperfusion injury [6–9], Ca^{2+}-induced cardiomyopathy [10], diabetic cardiomyopathy [11], muscular dystrophy [12], and the cardiotoxic actions of anti-cancer agents [13]. Unfortunately, despite these substantial advances regarding our understanding of the MPT pore and its role in cardiovascular disease, the precise molecular architecture of the MPT pore remains unknown.

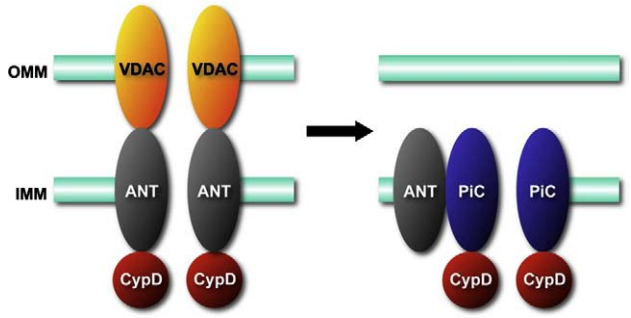

Fig. 1. Molecular models for the mitochondrial permeability transition (MPT) pore. Left, The original model for the MPT pore, consisting of the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane (OMM), the adenine nucleotide translocase (ANT) in the inner mitochondrial membrane (IMM), and cyclophilin-D (CypD) in the matrix. Right, Revised models in light of recent findings in gene-targeted mice. VDAC is no longer part of the model and it appears that an outer membrane component may not even be necessary for this process. ANT now appears to be more of a regulatory protein, and only CypD remains as an established component. In contrast, the mitochondrial phosphate carrier (PiC) has been added to model as a potential candidate for the pore-forming unit of the MPT pore.

4. Molecular composition of the MPT pore: the original paradigm

Based upon biochemical and pharmacological studies, the pore was proposed to consist of the voltage-dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocase (ANT) in the inner membrane, plus CypD in the matrix [1–4] (Fig. 1). VDAC, ANT, and CypD interact at membrane contact sites and reconstitution of this complex in vesicles yields a Ca^{2+}-sensitive channel reminiscent of the MPT pore [14]. Moreover, pharmacological inhibitors of ANT and CypD, e.g., bongkrekic acid and cyclosporine-A, respectively, can inhibit MPT [6–9,15,16]. However, recent genetic studies have questioned the validity of this paradigm.

4.1. VDAC is not a component of the MPT pore

The most abundant protein in the outer mitochondrial membrane, VDAC (also known as porin) facilitates the efficient transport of ATP/ADP across the outer leaflet [17,18]. The VDAC family consists of 3 gene products (VDAC1, 2, and 3) that exhibit structural and functional homology [17–19]. In their open state the VDAC channel is huge, being permeable to solutes up to 5 kDa in size. Interestingly even when in the closed state, the channel is still 1.5 kDa permeable and becomes more selective for cations [18,20].

As discussed above, VDAC has always been considered a key component of the MPT pore. This was based on an original hypothesis by Zoratti et al. [21,22], who suggested that the electrophysiological properties of the MPT pore were reminiscent of those of the VDAC channel. However, there is only limited data substantiating this hypothesis. Shimizu et al. first reported that anti-VDAC antibodies, which were able to block VDAC channel activity, were effective in preventing Ca^{2+}-induced MPT and cytochrome *c* release in isolated liver mitochondria [23]. Such "inhibitory" antibodies were also reported to block arsenic trioxide-induced MPT [24]. By means of a drug library screen Bernardi's laboratory discovered a novel VDAC1-binding compound, Ro 68-3400, which blocked Ca^{2+}-induced MPT in isolated mitochondria [25]. Biochemically, Crompton's group was able to pull-down VDAC along with ANT when mitochondrial lysates were incubated with a GST-CypD fusion protein [14]. Moreover, reconstitution of this VDAC-ANT-CypD complex yielded a Ca^{2+}-dependent, cyclosporine-sensitive channel similar to the MPT pore [14]. Together, these studies indicated that VDAC was indeed a critical part of the MPT pore's molecular architecture.

However, there is now considerable evidence to indicate that the conclusions of these original studies were in fact incorrect. An oft forgotten point is that even its "closed" form, the channel of VDAC is still permeable to solutes 1.5 kDa in size, i.e., the same size as when the MPT pore is *open* [18]. From this alone it is hard to reconcile how closing VDAC would close the pore. Moreover, Colombini has shown that closure of VDAC has been shown to *increase* Ca^{2+} influx into mitochondria [20], which would have the net effect of inducing MPT rather than inhibiting it. In addition, the specificity of the VDAC "inhibitors" described in the previous studies has been seriously questioned. VDAC antibodies are known to recognize other non-VDAC proteins and therefore cannot be considered totally specific [18]. Moreover, despite the original postulation that Ro 68-3400 was able to bind VDAC1, a subsequent study by the same group found that radioactive Ro 68-3400 was still able to label a ~32 kDa protein in VDAC1$^{-/-}$ mitochondria and was still retained on a hydroxyapatite