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VDAC: The channel at the interface between mitochondria and the cytosol

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Abstract

The mitochondrial outer membrane is not just a barrier but a site of regulation of mitochondrial function. The VDAC family of proteins are the major pathways for metabolite flux through the outer membrane. These can be regulated in a variety of ways and the integration of these regulatory inputs allows mitochondrial metabolism to be adjusted to changing cellular conditions. This includes total blockage of the flux of anionic metabolites leading to permeabilization of the outer membrane to small proteins followed by apoptotic cell death. (Mol Cell Biochem **256/257**: 107–115, 2004)

Key words: mitochondrion, outer membrane, apoptosis, isoforms, metabolism

Introduction

Mitochondria live, function, and reproduce in a very rich and friendly environment, the cytosol of the eukaryotic cell. Just as the plasma membrane is the interface between the interstitial space and the cytosol, so the mitochondrial outer membrane is the interface between the cytosol and the mitochondrial spaces. Both separate the cell or the organelle from its environment and both act as selective barriers to the entry and exit of matter. These membranes are also logical sites for control. While the plasma membrane has long been known to be the site of action of many chemicals and electrophysiological signals, a similar role for the outer membrane is just beginning to be recognized. One way in which the cell can control mitochondrial function is through controlling the flux of metabolites. This could obviously influence both mitochondrial growth and activity and overall cellular activity dependent on mitochondrial energy transduction and mitochondria-derived compounds. The common pathway for the translocation of metabolites through the outer membrane is the VDAC channel [1–3]. This article will examine ways in which VDAC can influence metabolite flux through gating and interaction with other proteins and the consequences of changing outer membrane permeability on cell survival. For a review that addresses VDAC's structure and gating mechanism (see ref. [4] and updated information in ref. [5] for this author's view, see also ref. [6] for an alternative view). Information on VDAC can also be found on the VDAC web page: www.life.umd.edu/vdac.

The VDAC channels: Conservation and specialization

VDAC channels (Fig. 1) from a wide variety of sources (plants [7–9], animals [1, 3, 10–12], fungi [4, 13], and protists [14, 15]) have been studied to different levels of detail.[1] The picture that is emerging is one of a highly functionally-conserved archetypal VDAC and isoforms that retain some of the basic properties but are different in ways that probably indicate specialized functions. The conservation is not as evident in the primary sequence as it is in the secondary structure deduced from that sequence [16]. The alternating polar-non-polar

[1]VDAC is often referred to as mitochondrial porin implying that it is part of a larger family of channels located at the outer membranes of gram negative bacteria. While the evolutionary origin of VDAC is unclear, experimental evidence strongly indicates that the structure and properties of VDAC are quite different from the rather varied structures and properties of the family of bacterial channels collectively referred to as porins. Any similarity is likely coincidental. Hence the term "mitochondrial porin" is misleading.

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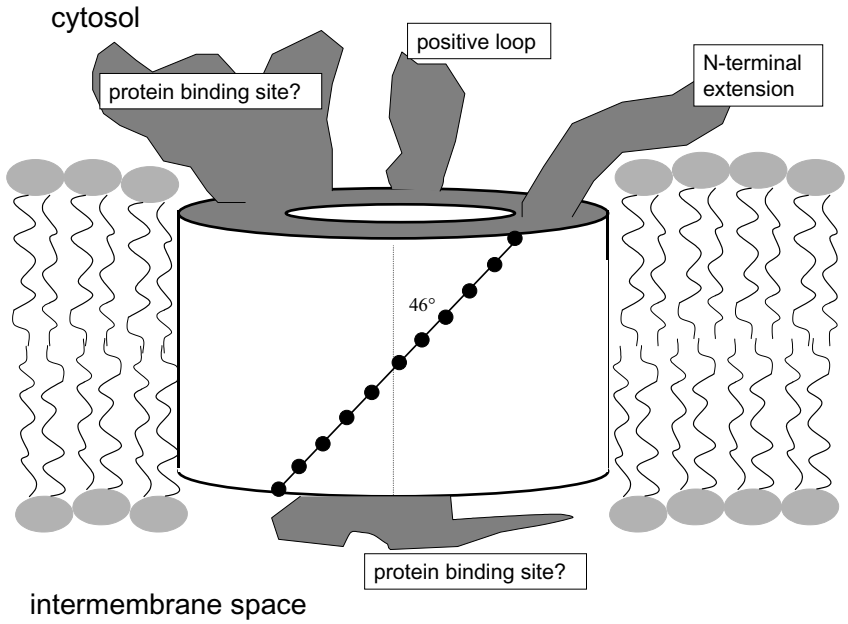

Fig. 1. Schematic diagram of VDAC in the mitochondrial outer membrane. The barrel that spans the membrane is composed of 1α helix and 13β strands [5]. The staves tilt at 46° to the axis of the pore [85]. Loop regions between the transmembrane strands are likely the sites of interaction with proteins and other soluble factors. A conserved unusually long loop facing the cytosol is an attractive candidate for a protein binding site. There is also a highly positively charged loop in some forms of VDAC including the mammalian isoforms. While the overall length of VDAC is highly conserved, there are N-terminal extensions in some VDACs. These additions may serve a recognition function or as sites of attachment.

pattern of the amino-acid side chains in the primary sequence is characteristic of β strands separating the polar environment within the channel from the non-polar membrane phase. This pattern in 10-amino-acid stretches, sufficient to span the membrane, can be easily identified using a simple computer algorithm and a suitable hydrophobicity scale [17]. The resulting 'beta pattern' is remarkably conserved even when the percent homology is below the level of statistical significance (16). Nevertheless, not all predicted β strands turned out to be transmembrane when tested experimentally. Three lines of experimental evidence [5, 18, 19] points to the conclusion that the barrel of the channel is formed by 1 α helix and 13 β strands (Fig. 2.). It is also established that a single polypeptide chain forms one channel [20, 21].

The archetypal VDAC is often labeled as VDAC1. Its fundamental properties (single-channel conductance, selectivity, voltage dependence) are highly conserved in all eucaryotic kingdoms [12]. However, detailed studies have revealed important differences. For example, mammalian VDAC is sensitive to La^{3+} [22] while *N. crassa* VDAC is not [23]. Conversely, *N. crassa* VDAC is modulated by G-actin while mammalian VDAC is not [24]. These differences undoubtedly reflect differences in the regulatory functions within the different cell types.

Additional specialization takes the form of VDAC isoforms obtained both by the expression of distinct autosomal genes and by alternative splicing [25]. So far more isoforms seem to be present in more complex multicellular organisms. *N. crassa* seem to have only one form of VDAC, *S. cereviciae* (yeast) has 2 [26], and mice and wheat have 3 [8, 27]. Beyond just numbers, the knock out of just one of the 3 VDAC isoforms in mice causes serious and surprising effects but no single isoform is required for cell viability [28]. For example, the knock-out of VDAC3 in mice results in male sterility due to non-motile sperm [29] while the knock-out of either VDAC1 or VDAC2 yields mice with a 30% reduction in respiratory capacity [28]. The lack of VDAC1 results in embryonic death of some mice between 10.5 and 11.5 days after conception [25]. More frequent embryonic death occurs in mice lacking both VDAC1 and VDAC3 and these mice also show serious growth retardation. The growth of yeast cells lacking yeast VDAC1 and expressing one of the three wheat VDAC isoforms varied depending on the isoform with wheat VDAC3 expressing cells being the slowest growing [9]. The findings did not seems to be related to expression levels or amount of functional protein in the mitochondrion but to the molecular properties of the isoforms. Results such as these strongly indicate functional specialization rather than redundancy.

Three VDAC-like proteins have been identified in *D. melanogaster* [30] (also Brett Graham and William Craigen, unpublished). These have sufficient similarity to the canonical VDAC to be recognized but also contain major differences. These are in addition to the clearly recognized *D. melanogaster* VDAC protein whose properties match those

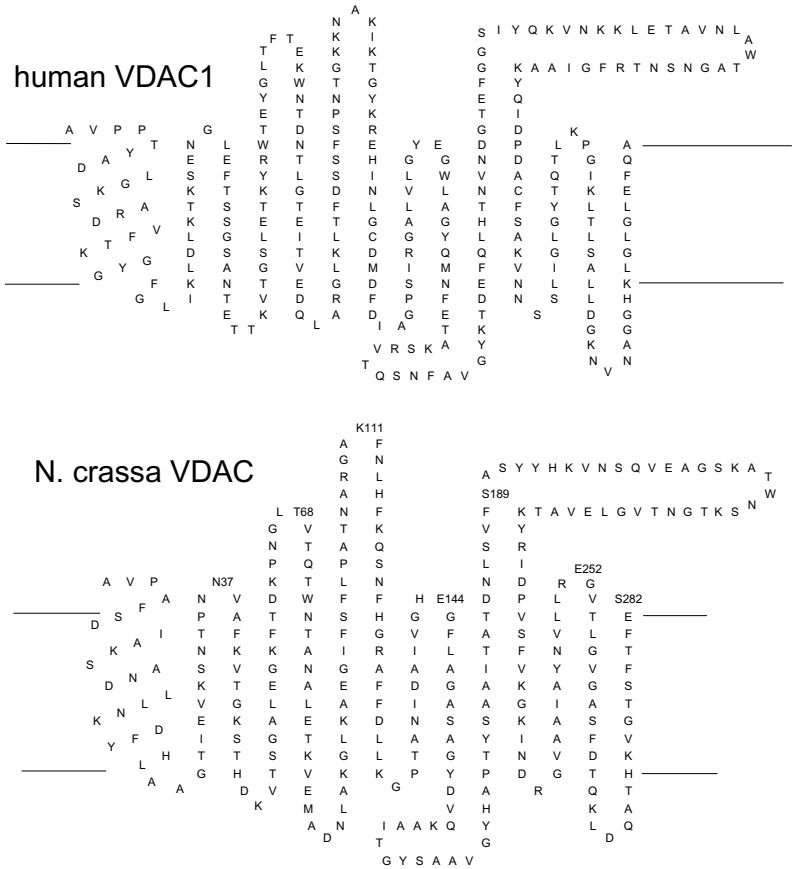

Fig. 2. Deduced transmembrane folding pattern of human VDAC1 isoform and VDAC from *N. crassa*. The *N. crassa* pattern is from ref. [5] and that for human VDAC1 was folded in a homologous manner.

of VDAC1 [26]. The role of these variants is unclear although 2 can generate channels in membranes (Alexander Komarov, unpublished results).

Detailed studies of the electrophysiological properties of VDAC isoforms reveal both minor and major changes in the fundamental properties of these molecules. In the yeast isoforms, only VDAC1 (the most abundantly expressed form) makes channels in phospholipid membranes [26]. Despite hints to some channel formation in isolated mitochondria [2], no channels were reconstituted into phospholipid membranes using VDAC2. Perhaps some factor present *in vivo* was missing. Examination of the mouse isoforms [3] showed that all formed channels with VDAC1 and VDAC2 having similar properties while VDAC3 showed far less channel formation and a broader range of properties. Interestingly, mouse VDAC2 expressed in yeast showed the presence of 2 populations with distinct properties. The structural basis for this difference is not known. The VDAC isoforms from wheat expressed in yeast were all capable of forming channels when reconstituted into planar membranes but their detailed electrophysiological properties were quite different [9]. Additional modification of the properties of these channel-formers came from tightly associated modulating proteins that copurified along with VDAC. These produced pronounced rectification and altered the gating properties.

VDAC's ability to control metabolite flux through gating

Detailed studies of the gating properties of VDAC have revealed that these channels don't simply act as doors that can be opened or closed. Under special conditions the channels can be closed completely or almost completely [31, 32]. However, generally the gating involves a rather mild reduction in overall conductance (generally 50–60% reduction) but a dramatic change in selectivity. The high conducting state, referred to as the open state, shows preference for anions and this is important for its role as a conduit of metabolites as these are generally anions. Channel closure and the associated motion of the positively-charged voltage sensor out of the chan-

nel [33, 34] results in a smaller pathway [35, 36] that favors cations [37–39]. For small cations, the change in selectivity more than compensates for the reduction in channel diameter resulting in an increase in cation flux [40]. In contrast, the selectivity inversion greatly reduces anion flux. Not only is the electrostatic charge on the walls of the channel unfavorable for the translocation of anions but the smaller pore diameter forces the permeating ions to be closer to the wall of the channel increasing the energy barrier to anion translocation. This is a stronger barrier to larger anions such as ATP and, in fact, ATP becomes virtually impermeant [41]. Other anionic metabolites follow suit (Fig. 3). Thus closure of VDAC channels refers to the ability of the closed states to inhibit the flux of metabolites.

The reverse is expected for cations. This is of particular interest with regard to the flux of Ca^{2+} as it shuttles rapidly between the cytosol and ER storage compartments and the mitochondrial matrix. VDAC closure should actually favor Ca^{2+} flux. The studies of Ca^{2+} flux into and out of mitochondria in mammalian cells is now complicated by the finding that blockers of Ca^{2+} flux traditionally believed to be acting only at the inner membrane have been shown to inhibit flux through mammalian VDAC [32]. The use of these pharmacological tools to tease apart the process has now become far more complicated.

Closure of any single VDAC channel can occur in many ways. First of all closure occurs in response to either a positive or a negative potential but the structural states achieved are quite different and channels closed by a positive potential need to reopen before they can close in response to a negative potential [42]. This highly conserved symmetrical response to voltage can be eliminated by a single point mutation [43] or by the use of a pure lipid environment (e.g. diphytanoyl PC) instead of the more natural mixed lipids. This indicates the presence of a strong selective pressure to conserve this property. Beyond the sign of the applied voltage, channel closure occurs in a large variety of possible structures as indicated by a variety of conductance states and selectivity of these states. This variety is seen not just among individual channels but in recordings from a single channel [38, 42]. This variability is greatly reduced in the presence of modulating proteins and polyanions [33, 44]. A similar effect was seen with Bcl–x_L addition (XiaoXian Li, unpublished). It is possible that the variability in structure is important for VDAC's interaction with different modulating agents.

It is difficult to gain direct evidence for the role of VDAC gating in intact cells. By equating changes in the permeability of the outer membrane with VDAC gating, one can find such evidence. The removal of growth factor from a cell line requiring such factor results in a dramatic drop in the permeability of the outer membrane of mitochondria to metabolites in a way that is consistent with VDAC closure [45]. Most impressive is the dramatic rise in mitochondrial phosphocreatine levels that requires the outer membrane to be permeable to creatine but not phosphocreatine. This is characteristic of the electrostatic barrier generated upon VDAC closure [4, 33, 45]. Jonas *et al.* [46] observed as much as a 60-fold increase in mitochondrial channel activity in the presynaptic terminal of the squid following a train of action potentials. The observed conductances were consistent with VDAC gating.

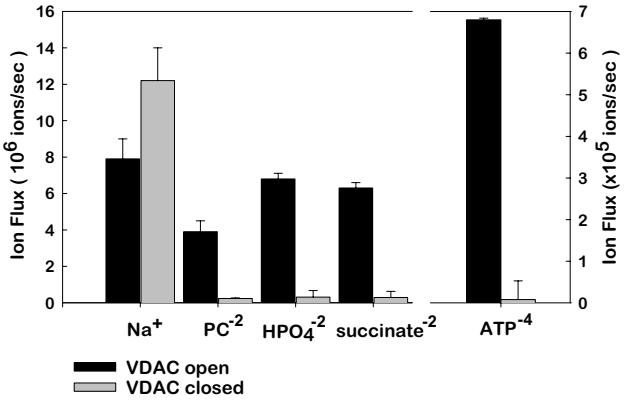

Fig. 3. VDAC closure results in a selectivity change from anion preference to cation preference. The bars show the calculated ion flux through a single channel determined in the presence of a gradient of the indicated salts. These fluxes represent the ion flow in the absence of an electrical potential and in the presence of a concentration difference of 100 mM. The error bars are S.E.M. For further information see refs [40, 41, 45].

Control of the state of VDAC

Voltage gating

When VDAC was discovered in 1975, it stood out from other conductances observed because it had voltage-gating properties [14]. Over the years, the physiological role of the voltage-gating of VDAC has been quite controversial. How could a membrane that is as permeable as the outer mitochondrial membrane, maintain a membrane potential? Some have assumed that such a potential was not possible (no citation to avoid embarrassment). Others have proposed a variety of ways to generate a potential. One of these, the existence of a Donnan potential [1], is a natural consequence of the presence of charged macromolecules in the intermembrane space and cytosol. Since VDAC is not permeable to these, the free motion of their counterions will result in a potential across the outer membrane that depends on the ionic strength of the medium and the concentration of net charge carried by the macromolecules in the two compartments.

Another proposal takes into account the motion of charged substrates associated with mitochondrial metabolism [47]. Differential permeability of VDAC to metabolites would

result in a transmembrane potential. Theoretically, sizable potentials could be generated (tens of milliVolts) and this would depend on the level of mitochondrial metabolism. This would be a negative feedback process as an increase in metabolic rate would increase the potential resulting in channel closure and decrease access to metabolites.

An early proposal suggested that the potential across the inner membrane would somehow spill over to the outer membrane due to the proximity of both membranes [39]. While this specific proposal is rather improbable, metabolic cycling associated with ATP/ADP/Pi translocation could couple potential generation in the inner membrane to the formation of a potential in the outer membrane. This has been described in detail [48].

The fundamental question is: Is there experimental evidence for such a potential? Cortese *et al*. [49] measured the pH of the intermembrane space in isolated mitochondria and found that it was more acidic than the medium. The difference was 0.4–0.5 pH units in the condensed form (large intermembrane space) and 0–0.2 pH units in the orthodox state (small intermembrane space). These values did not vary much with medium pH. Since protons are highly mobile a pH gradient could only be maintained by a potential across the outer membrane. A pH difference of 0.4–0.5 corresponds to a 20–30 mV potential negative in the intermembrane space. Rosario Rizzuto *et al*. (personal communication) have used pH-sensitive GFP (green fluorescent protein) labeled proteins to measure the pH of the intermembrane space and cytosol of intact cultured mammalian cells. They find a pH difference: the cytosol was pH 7.4 and the intermembrane space 7.1. A pH difference of 0.3 corresponds to a 15–20 mV potential negative in the intermembrane space. These estimates are within the switching region of the channels when reconstituted in phospholipid membranes ($V_0 \approx 25$ mV). In this region the channel would be most sensitive to being controlled by other factors. An important question is whether this potential varies *in vivo* with changes in metabolic conditions.

Measurement of the state of the VDAC channel in living cells may have been achieved by Jonas *et al*. [46]. In the nerve terminal, they found very little conductance in patch recordings likely made on the outer membrane of mitochondria. This indicates that the channels are mainly closed and is consistent with measurements made of the permeability of the outer membrane of isolated mitochondria [50]. The effects they report for the action of NADH and Bcl-x_L on these patches shows that the conductances are sensitive to these agents as are VDAC channels [51, 52].

In vivo *factors influencing the gating of VDAC*

The voltage gating of VDAC channels reconstituted into planar membranes would be greatly modified by conditions *in vivo*. The mere presence of impermeant macromolecules and their counterions in the environment (colloidal osmotic pressure) alters the gating properties [35]. These dilute the water in the medium resulting in equilibration of the water's chemical potential by the generation of negative pressures in the channel lumen. This negative pressure favors conductance states with reduced lumen volumes. The reduction in lumen volume upon channel closure means that the presence of impermeant macromolecules favors channel closure and this was observed. Addition of non-electrolyte polymers, dextran (20 and 500 kDa, [53]) and PEG (20 kDa, [54]), greatly inhibited mitochondrial outer membrane permeability to ADP as assessed by an increase in the K_M of adenylate kinase [53] or direct measurement of the outer membrane permeability [54]. These results are in harmony with effects observed on VDAC reconstituted into planar phospholipid membranes [35]. In intact mitochondria, however, these polymers could act by osmotically reducing the volume of the intermembrane space and thus increasing the concentration of its constituents including proteins that induce VDAC closure and likely contribute to the potential across the outer membrane. At low polymer concentrations (10% or less) there was no effect on state 3 or state 4 respiration in either study but higher concentrations resulted in inhibition perhaps due to reductions in the volume of the intermembrane space.

Some small molecules influence the gating of VDAC. Millimolar amounts of glutamate favor the closure of mammalian VDAC [22]. Of the variety of nucleotides and cyclic nucleotides tested, only NADH [51] and Mg-NADPH [54] influenced the gating properties of VDAC from various sources. The reduced form of NADH was without effect. Although the changes in the gating properties were relatively small, NADH induced a large decrease in the permeability properties of the outer membrane of isolated mitochondria to ADP [50]. This indicates that the VDAC channels in the isolated mitochondria were poised to produce a strong response upon addition of NADH, perhaps resulting from the presence of a transmembrane potential.

The presence of an ATP binding site in mammalian VDAC [41, 55] results in large effects on single-channel current noise [56] but no specific effects of VDAC gating [51]. This very low affinity site ($K_D \approx 70$ mM) is more likely associated with facilitating ATP translocation rather than being a regulatory site. Indeed, specific interactions exist that favor the flux of ATP but exclude molecules of similar size and charge [57].

Phosphorylation by protein kinase A of mammalian VDAC [58] resulted in an increase in the probability of closure at negative but not positive potentials. Thus phosphorylation only affects one of the two gating processes. The physiological role of phosphorylation is less clear. Phosphorylation of VDAC seems unrelated to metabolic state [59] in plant mitochondria.

A variety of proteins influence the gating of VDAC. Protein extracts from the intermembrane space of mitochondria

from diverse species contain highly protease-sensitive factors that profoundly favor VDAC closure [44, 60, 61]. These have been referred to as the VDAC modulator. The active constituents are highly conserved in that extracts from sources as diverse as mammals, fungi and higher plants act on VDAC channels isolated from all of these sources. The most potent ingredient has yet to be identified. There is also evidence for the closure of mammalian VDAC channels by cytosolic proteins [62, 63]. Some pure proteins have been demonstrated to modulate the properties of VDAC. These include: G-actin [24], Bcl-x_L [52], dynein light chain [64], heat shock protein mtHSP70 [64]. Some of these have only been tested on or are specific for VDAC from particular sources. Some favor channel closure while others favor the open conformation. C-Raf seems to bind to VDAC [65] without affecting its properties as reported for antibodies [66] but detailed studies were not described in the publication leaving room for weak effects as seen with Bcl-x_L.

The conductance of mammalian VDAC is dramatically reduced by the addition of either ruthenium red or La^{3+} ions [32]. The effect of the former is reversed by the presence of millimolar Ca^{2+}. The functional significance of this specialization is unclear but has been proposed to be related to the traffic of Ca^{2+} between the endoplasmic reticulum and mitochondria [32].

When multiple factors are present, the resulting gating properties of VDAC will depend on competition or synergy between the various factors present. This was also observed in isolated mitochondria in that addition of Bcl-x_L alone did not significantly increase the outer membrane permeability of rat liver mitochondria but its addition on top of NADH resulted in reversal of NADH's ability to induce a reduction in outer membrane permeability [52]. *In vivo*, multiple factors will be influencing VDAC and thus the resulting open probability will be some weighted sum of all these effects.

VDAC, the outer membrane, and apoptosis

A number of reports have associated VDAC with the initiation of the mitochondrial phase of apoptosis [45, 52, 67–70]. In *S. cerevisiae*, there is evidence that VDAC is not involved [71]. Its location in the outer membrane makes VDAC an attractive candidate for influencing the release of pro-apoptotic proteins from the mitochondrial intermembrane space. Some researchers have linked VDAC to the permeability transition pore (PTP) that results in the release of Ca^{2+} ions and metabolites from the matrix space [67–69, 72]. One view is that combining VDAC with a pore through the inner membrane results in a pathway for the transfer of solutes through both membranes. Another [73] proposes that the PTP (or MPT) results in osmotic swelling of the matrix compartment and consequently sufficient physical stress is applied to the outer membrane culminating in rupture of that membrane. Outer membrane components, like VDAC may modulate but are not required. Others have reported evidence that VDAC closure precedes the release of pro-apoptotic proteins from the intermembrane space [45, 74]. There is a failure to exchange adenine nucleotides between the intermembrane space and the cytosol and, most importantly, the remarkable accumulation of phosphocreatine in the intermembrane space. (The high negative charge of phosphocreatine makes it unable to permeate through the closed state of VDAC due to the presence of an electrostatic barrier.) This accumulation demonstrates that the outer membrane, not the inner, is the barrier to flux. It is not clear how this closure results in the release of proteins. However, antiapoptotic proteins can relieve this permeability barrier and rescue the cell. One of these, Bcl-x_L, has been shown to directly favor the open state of VDAC [52] reconstituted into planar membranes. In addition, when injected into the squid giant synaptic terminal, Bcl-x_L stimulated synaptic transmission in the same way as the injection of ATP suggesting that Bcl-x_L facilitates the flux of ATP out of mitochondria [75].

It is important to distinguish between permeability increases that allow faster metabolite exchange and those that allow protein flux. Bcl-x_L injection into the squid giant synapse [76] generates small conductance increases in what is presumed to be the mitochondrial outer membrane. Larger conductance increases were observed when a proapoptotic, truncated version of Bcl-x_L was injected. These differences are consistent with the notion of 2 types of permeability pathways, one for metabolites and one for proteins. Surprisingly, both are inhibited by NADH, an agent known to modulate VDAC and induce closure under certain conditions [50].

Other than outright tearing of the outer membrane, there are identified pathways that could facilitate the release of proteins from the intermembrane space: the proapoptotic proteins, Bid, Bax and Bak [77–81], a channel-forming activity (MAC) identified in the mitochondria of cells undergoing apoptosis [82], and channels formed by ceramide [83, 84]. The measured size of the pore formed by BAX and MAC are large enough to allow the efflux of cytochrome c but not the larger proteins known to be released from mitochondria [77, 82]. The reported ability of BAX to interact with VDAC to form a larger pore cannot be reproduced (Tatiana Rostovtseva, unpublished observations). However, the Bid/Bax combination has been reported to release 2 MDa dextran from liposomes [81]. Ceramide channels formed in the mitochondrial outer membrane show a cut-off based on SDS-denatured proteins of 60 kDa [84]. These large pathways are necessary to account for the protein efflux from the mitochondrial intermembrane space.

Concluding remarks

If, as Claude Bernard pointed out, the cellular systems strive to maintain an internal environment, a *milieu intérieur*, different from its external environment and appropriate to its function and survival, then the same can be said of mitochondria. However, the mitochondrion may strive not only to maintains its *milieu intérieur*, it may also strongly influence its external evnironment, the cellular cytosol. Clearly selection pressure must apply both to mitochondrial survival and cellular survival and these two formerly independent entities are now inextricably linked. This common survival requires elaborate communication and control systems. It is natural that VDAC, located at the interface between the cytosolic environment and the mitochondrial environment, would be a central player in both signal transduction and regulation.

Acknowledgements

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