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Mitochondrial Channels Revisited

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When the *Journal of Bioenergetics and Biomembranes* adopted its new format four years ago, the inaugural minireview series was on "mitochondrial channels" (Mannella and Tedeschi, 1992). The current issue of *JBB* returns to this topic, containing reviews and original research articles intended to update this still-young field. As the new papers show, the intervening years have seen a steady unfolding of knowledge about the permeability properties and regulation of ion channels that have been discovered in mitochondrial membranes by electrophysiology and other means (see Fig. 1). At the same time, readers familiar with the field may note that progress has been slower in other areas, in particular, linking mitochondrial channel activities to specific proteins or physiological functions, and understanding how these channel activities correlate with the physical organization of mitochondria. This introduction is an attempt to summarize briefly the situation with respect to these two important topics and to indicate possible future directions of research.

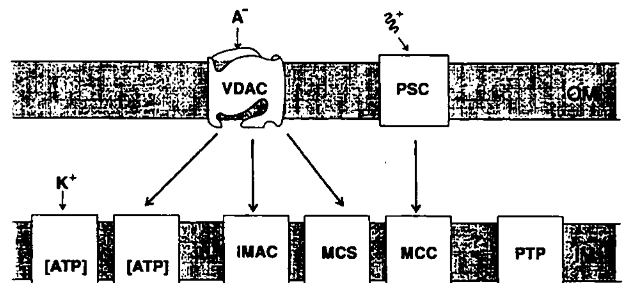

Fig. 1. Schematic diagram showing channels identified in the mitochondrial outer membrane (OM) and inner membrane (IM). Channel names: VDAC, voltage-dependent, anion-selective channel (also, mitochondrial porin); PSC, peptide-sensitive channel; IMAC, inner membrane anion channel; MCS, 107-pS channel; MCC, multiple conductance channel (also, mitochondrial megachannel); PTP, permeability transition pore. Structure of VDAC is from Guo *et al.* (1995); other channels are indicated as squares. Unnamed channels at far left represent other classes of inner-membrane cation- (e.g., K^+) and anion-selective channels, some of which are ATP-sensitive as indicated by [ATP]. Other symbols: A^-, anion; squiggle, positively charged targeting peptide.

CORRELATING MITOCHONDRIAL CHANNELS WITH KNOWN PROTEINS AND FUNCTIONS

VDAC, the large-conductance, voltage-gated channel of the mitochondrial outer membrane, is being characterized in detail at the protein and DNA levels. Molecular genetic and electrophysiological (Peng *et al.*, 1992), electron microscopic (Guo *et al.*, 1995), and computational (Mannella *et al.*, 1996; Song and Colombini, 1996) approaches are converging on a model of a β-barrel pore that can undergo structural rearrangements involving movements of segments (β-strands, α-helix) in and out of the lumen. It is usually assumed that this channel represents the main permeability pathway for ions and metabolites across the outer membrane. However, the simplistic notion that this channel is a nonselective molecular sieve has been made untenable in recent years. The discovery of isoforms of the channel [at least four in human cells (Blachly-Dyson *et al.*, 1994; also, Huizing *et al.*, 1996), two in yeast (M. Forte, personal communication), and two in plants (Heins *et al.*, 1994; also, Carbonara *et al.*, 1996)] suggests as yet undefined functional differentiation. In mammalian cells, part of the diversity of VDAC isoforms appears to involve hexokinase binding (Blachly-Dyson *et al.*, 1993). Whether other differences relate to functions like protein import (see PSC below) or targeting to other membranes (Thinnes,

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1992; Yu and Forte, 1996) remains to be determined. Also, endogenous modulators have been found in mitochondria (Liu and Colombini, 1992) and elsewhere (Heiden *et al.*, 1996) which cause VDAC to switch to lower conducting substates. Since these substates are impermeable to adenine nucleotides (Benz *et al.*, 1988), the functional status of VDAC may impact on rates of oxidative phosphorylation. The low frequency of detecting obvious VDAC transitions in outer membranes attached to mitochondria led Moran and Sorgato (1992) to question whether VDAC normally occupies the fully open state observed in synthetic systems. Recent experiments involving labelling of fungal mitochondria with antibodies specific for segments of VDAC expected to be inaccessible in the open state also suggest that at least some VDAC channels in the outer membrane are closed (Stanley *et al.*, 1995).

Establishing the function of ionic conductances associated with inner-membrane or mitoplast fractions is considerably more difficult than for VDAC since, at present, there is almost no information about the molecular identity of these channels. The earlier and current *JBB* miniseries issues contain suggestions that inner-membrane channels may participate in such activities as protein import (see below) and energy-state-related volume regulation (Szewczyk *et al.*, 1996; Ballarin and Sorgato, 1996). It has also been suggested that ion conductances detected by electrophysiology might correspond to channels or pores inferred from other studies, e.g., IMAC, the inner-membrane anion channel implicated in volume homeostasis (Beavis and Davatol-Hag, 1996); and PTP, the pore responsible for the so-called mitochondrial permeability transition, a marked increase in inner-membrane permeability induced by various combinations of agents (Bernardi and Petronilli, 1996; Novgorodov and Gudz, 1996; Sokolove and Haley, 1996). Strong correlations have been drawn between PTP and a multiple-conductance channel activity (MCC) detected by patch-clamping of mitoplasts (Szabo and Zoratti, 1992; Kinnally *et al.*, 1996). The latter channel also has been implicated in mitochondrial protein import, by virtue of specific transient blockade of its conductance by targeting peptides (Kinnally *et al.*, 1996). This kind of peptide sensitivity was first observed for another mitochondrial channel called PSC, which has electrical characteristics similar to MCC but which has been assigned to the outer mitochondrial membrane (Henry *et al.*, 1996; Bathori *et al.*, 1996). [MCC is considered an inner-membrane channel since it is detected in the same membrane patches as a voltage-gated 107-pS channel (Sorgato *et al.*, 1987) that has no close outer-membrane counterpart.] While these observations seem disjointed, they are consistent with a speculative scenario: PSC and MCC/PTP might represent hemichannels of gap-junction-like structures spanning the outer and inner membranes and involved in translocation of precursor proteins into mitochondria. At the same time, others point out that PTP (MCC?) has characteristics expected of a calcium-release channel (Bernardi and Petronilli, 1996) and so might function in calcium homeostasis. Might the same channel/pore serve multiple functions, perhaps in different states (e.g., Sokolove and Haley, 1996; Novgorodov and Gudz, 1996)? Or might there be more than one kind of PTP? One key to unraveling these puzzles lies in molecular genetic approaches just now being undertaken. For example, as the genes for known (and suspected) mitochondrial membrane transport proteins are identified and cloned, the channel phenotypes of the corresponding mutants (including knock-outs) are being tested. With this approach, it has recently been shown that MCC is not directly related to either the predominant isoform of yeast VDAC (Lohret and Kinnally, 1995) nor to yeast adenine nucleotide carrier (Lohret *et al.*, 1995), but may be related to the inner membrane protein mas6.1, involved in protein translocation (Lohret *et al.*, 1996).

CORRELATING CHANNELS WITH MITOCHONDRIAL STRUCTURE

In the previous *JBB* issue, there were several suggestions that mitochondrial ion channels might be located at sites of contact between outer and inner membranes. Aside from the possible involvement (noted above) of one or more of the channels in protein import [which occurs at contact sites (Van der Klei *et al.*, 1994)], other circumstantial evidence includes effects on inner-membrane channel activities of Ca^{2+} (Kinnally *et al.*, 1992), which increases the frequency of occurrence of intramitochondrial contacts (Bakker *et al.*, 1994), and of drugs which have receptors on the mitochondrial outer membrane (Kinnally *et al.*, 1993). Suggestions that ion channels occur at intramitochondrial contact sites persist in the current *JBB* issue but there is no new evidence for or against this localization. There is, however, a recent immunolabelling study suggesting that VDAC may occur in regions of contact between outer membranes of different mitochondria (Konstantinova *et al.*, 1995).

The question of the intramitochondrial location of ion channels also ties into broader issues about mitochondrial function and interior design. For example, recent results from electron tomography have provided new information about the organization of the inner membrane of rat-liver mitochondria (Mannella *et al.*, 1994). The cristae are not lamelliform sheets (as depicted in textbooks) but irregularly shaped internal compartments connected by long, narrow (ca. 20 nm diameter) tubes to each other and to the periphery of the inner membrane. [The tubular nature of the cristae of mammalian mitochondria had been suggested by earlier electron microscopic observations (Daems and Wisse, 1966; Brdiczka and Reith, 1987; Lea *et al.*, 1994).] The length of these tubes (hundreds of nanometers) and the common occurrence of constrictions in them make it possible that lateral ion gradients occur within cristae and between the intracristal and intermembrane spaces. Also, transient conductance increases in the peripheral region of the inner membrane (perhaps associated with opening of a protein import pore) might not fully depolarize transmembrane gradients on internal (intracristal) regions of the membrane. [That the chemisosmotic ion gradients are not totally delocalized as originally postulated by Mitchell (1979) has been suggested by others, e.g., Williams (1988).] Clearly, in order to understand the nature of the ion gradients inherent to chemisosmosis, it will be necessary (but not sufficient) to know the pathways for diffusion between the mitochondrial compartments and the location of all transport proteins (including channels) on the membranes defining the compartments.

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