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Perspectives on the Mitochondrial Multiple Conductance Channel

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A multiple conductance channel (MCC) with a peak conductance of over 1 nS is recorded from mitoplasts (mitochondria with the inner membrane exposed) using patch-clamp techniques. MCC shares many general characteristics with other intracellular megachannels, many of which are weakly selective, voltage-dependent, and calcium sensitive. A role in protein import is suggested by the transient blockade of MCC by peptides responsible for targeting mitochondrial precursor proteins. MCC is compared with the peptide-sensitive channel of the outer membrane because of similarities in targeting peptide blockade. The pharmacology and regulation of MCC by physiological effectors are reviewed and compared with the properties of the pore hypothesized to be responsible for the mitochondrial inner membrane permeability transition.

KEY WORDS: Mitochondria; multiple conductance channel; mitochondrial megachannel; permeability transition; protein import; peptide-sensitive channel; intracellular channel; patch clamp.

INTRODUCTION

Mitochondria are double-membrane organelles that fulfill most of the cellular requirements for energy by oxidizing substrates and producing ATP. An inner membrane that is highly impermeable is required for this energy transduction to occur. Several channels have been identified in both the inner and outer mitochondrial membranes (see recent reviews by Benz, 1994; Beavis, 1994; Campo *et al.*, 1996; Mannella and Kinnally, 1996; Sorgato and Moran, 1993; Tedeschi and Kinnally, 1994; Zoratti and Szabó, 1994). In keeping with the notion of a high-resistance permeability barrier, the inner membrane channels are quiescent unless activated (Kinnally *et al.*, 1992). Many mitochondrial channels are voltage gated and/or responsive to nanomolar concentrations of substances, some of biological significance, suggesting ligand gating as well. Although the functions of these channels are still poorly understood, it is clear that they are highly regulated, suggesting that they play an important role in the physiology of the cell.

The multiple conductance channel (MCC, also called MMC for mitochondrial megachannel) is a voltage-dependent, high-conductance channel recorded under voltage clamp conditions from mitoplasts (mitochondria with the inner membrane exposed by French press treatment or by brief exposure to hypotonic media). A high-resistance gigaseal is formed on the mitoplast membrane with a glass micropipette (0.3–0.5 μm tip diameter) either spontaneously upon contact or after application of a slight negative pressure. Measurements are usually made on membrane patches excised by pulling the micropipette away from the mitoplast attached to the glass slide. Current traces are recorded under voltage clamp conditions with voltages

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reported relative to the mitochondrial matrix: $V = V_{bath} - V_{pipette}$ in excised inside-out patches (for complete details on patch-clamp techniques, see Lohret and Kinnally, 1995b).

Recently, we found MCC is transiently blocked specifically by synthetic peptides whose sequences correspond to targeting regions of mitochondrial precursor proteins (Lohret and Kinnally, 1995a). The effect of these targeting peptides suggests MCC may play a role in the import of mitochondrial precursor proteins synthesized in the cytoplasm. Szabó and Zoratti (1992) have proposed that mammalian MCC may be responsible for the mitochondrial permeability transition first described by Haworth and Hunter (e.g., 1980). A further examination of this proposal as well as a comparison of the behavior of MCC with that of a selected group of intracellular channels will be the focus of this review.

GENERAL CHARACTERISTICS OF MCC

MCC is an ion channel activity with multiple conductance levels that has been recorded by patch-clamp techniques in mitoplasts from a variety of organs from several mammals as well as yeast (Kinnally *et al.*, 1989; Petronilli *et al.*, 1989; Lohret and Kinnally, 1995a,b). An array of transition sizes with a peak conductance of 1–1.5 nS is a hallmark characteristic of this activity with nanoSiemen transitions observed in about 20% of the patches with MCC activity (e.g., see Kinnally *et al.*, 1989; Petronilli *et al.*, 1989; Zorov *et al.*, 1992b). MCC transitions in the range of 300–600 pS predominate in mammalian and yeast mitoplasts, although yeast MCC has fewer subconductance levels (see Fig. 1) (Lohret and Kinnally, 1995b). MCC can be detected in the same patch as the inner membrane anion channel referred to as mCS (mitochondrial centum picoSiemen channel), suggesting that the two classes of channels coexist in the mammalian mitochondrial inner membrane (Kinnally *et al.*, 1992).

While the various conductance levels observed might correspond to more than one channel, they have been attributed to a single class. They are activated by similar conditions, e.g., calcium and voltage, and respond similarly to the pharmacological agents noted below (Kinnally *et al.*, 1989, 1991, 1992, 1993; Antonenko *et al.*, 1991; Campo *et al.*, 1992; Petronilli *et al.*, 1989; Szabó and Zoratti, 1991, 1992; Zorov *et al.*, 1992a, 1992b). In addition, MCC activity can be reconstituted into liposomes after detergent solubilization of inner membranes while retaining its variety of subconductance levels (Fig. 1 and Lohret and Kinnally, 1995b). Furthermore, Petronilli *et al.* (1989) showed that conductance levels between 300 and 1300 pS are probably substates of a single channel since several small openings sometimes close in a single large step.

MCC has a slight selectivity for cations over anions. The relative permeability ratio for K^+/Cl^- Is ~6 for yeast MCC (Lohret and Kinnally, 1995b) and 3–10 for some of the subconductance levels of mammalian MCC (Kinnally *et al.*, 1989). It should be noted that Szabó and Zoratti (1992) found mammalian MCC

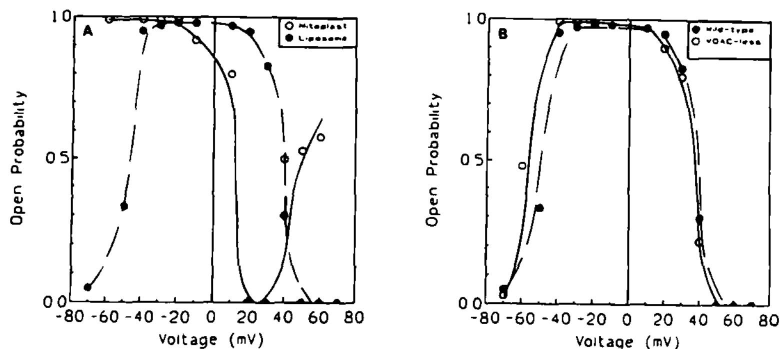

Fig. 1. Voltage dependence of MCC is sensitive to reconstitution and VDAC deletion. (A) Plots of open probability vs. voltage for MCC of mitoplasts from wild-type *Saccharomyces cerevisiae* (○) and from proteoliposomes (●) reconstituted using purified mitochondrial inner membranes from the same strain. (B) Plots of open probability vs. voltage for reconstituted MCC from wild-type (●) and from a VDAC-less strain of *S. cerevisiae* (○). Data from the study by Lohret and Kinnally (1995b).

to be unselective, possibly because of their use of a smaller KCl gradient.

VOLTAGE DEPENDENCE OF MCC

MCC is voltage dependent after its activation from a quiescent closed state (for example by calcium, see below). The peak conductance state is usually occupied at negative potentials relative to the mitochondrial matrix. Lower conductance levels are occupied at low positive potentials. Occupation of the peak level is often greater with high (usually >50 mV) compared to low positive potentials. This voltage profile (Fig. 1) is typically seen for mitoplast MCC from mammals and wild-type yeast. Zoratti and Szabó (1994) report a somewhat different voltage dependence. They report that MCC slowly closes after extended periods at negative potentials and closure is facilitated by higher than physiological salt (Szabó *et al.*, 1993). Closure of MCC with negative potentials is found after reconstitution of wild-type yeast MCC. It is also seen in mitoplasts from a yeast strain in which the voltage-dependent anion-selective channel (VDAC) is deficient (see Fig. 1 and Lohret and Kinnally, 1995b). Differences in voltage dependence may be related to the regulation of MCC by a variety of physiological effectors some of which might be lost during isolation and/or reconstitution (see below). Furthermore, MCC may reside in different membrane domains whose integrity may be sensitive to experimental conditions, e.g., contact sites (Kinnally *et al.*, 1992).

Voltage dependence can be expressed quantitatively in terms of gating charge and V_0. The gating charge (measure of the effective charge that moves across the membrane to fully open the channel) for MCC is approximately -5 at low positive potentials (Lohret and Kinnally, 1995b). V_0 is the voltage at which the open state is occupied half the time and it varies with the source of mitochondria. The V_0 for mammalian MCC is 20–30 mV while that of yeast is about 10 mV (Lohret and Kinnally, 1995b). Multiple open and closed states have been described for yeast MCC based on dwell time distributions at different voltages. These kinetic states have been summarized in a minimal model for yeast MCC with three open and three closed states (see Fig. 2) (Lohret and Kinnally, 1995b).

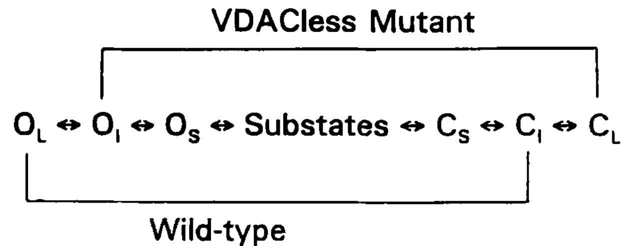

Fig. 2. Linear kinetic model for MCC from wild-type and VDAC-less yeast mitoplasts. MCC exhibits at least five kinetically distinct open (O) and closed (C) states, four of which are shared (O_i, O_s, C_i, C_s) and one which is strain specific (O_L or C_L). These open and closed states are defined by the duration of the dwell time constant τ where S is short (<3 msec), I is intermediate (3–100 msec), and L is long duration (>100 msec). Usually two to four of the possible five states are identified at voltages between -40 and 30 mV. A long-lived closed state (C_L) is identified in the mutant at higher positive voltages while a long-lived open state (O_L) is seen for the wild-type at higher negative potentials. Reproduced with permission from Lohret and Kinnally (1995b).

PHYSIOLOGICAL EFFECTORS

A variety of physiological effectors inhibit MCC activity (Szabó and Zoratti, 1992) including ADP, Mg^{2+}, and protons as shown in Table I. Based on *in vitro* results, normal cellular levels of calcium, ADP, and/or Mg^{2+} would probably render MCC quiescent under physiological conditions.

MCC can be activated from quiescence by other physiological effectors, e.g., calcium and voltage. Szabó *et al.* (1992) showed activation of MCC by 10^{-4} M calcium that could be reversed by EGTA addition

Table I. Comparison of MCC and PTP

	MCC	PTP
Pore diameter	>2.7 nm[a]	>1.4 nm[b]
Voltage dependent	Yes[c]	Yes[d]
Selectivity	Slight cation[a]	MW > 1500[f]
Calcium	Activate[g]	Activate[h]
Other divalent cations: Mg^{2+}, Sr^{2+}, Ba^{2+}	Inhibit[g]	Inhibit[i]
Low pH	Inhibit[g]	Inhibit[i]
ADP	Inhibit[g]	Inhibit[i]
Sulfhydryl reagents		
Oxidizing	Activate[j]	Activate[d]
Reducing	Inhibit[j]	Inhibit[d]

[a] Szabo and Zoratti, 1992. [b] Massari and Azzone, 1972. [c] Zorov *et al.*, 1992b. [d] Petronilli *et al.*, 1994. [e] Kinnally *et al.*, 1989. [f] Igbavboa and Pfeiffer, 1991. [g] Szabó and Zoratti, 1992. [h] Haworth and Hunter, 1980. [i] Bernardi *et al.*, 1992. [j] Campo *et al.*, 1995.

during patch-clamp experiments. Similarly, MCC activity (assayed by patch-clamping) is induced by the presence of 10^{-5} M Ca^{2+} in mitochondrial suspensions (Kinnally *et al.*, 1991). The calcium binding site for the reversible activation is presumed to be on the matrix face of the inner membrane since the experiments were carried out on excised patches (Zoratti and Szabó, 1994). However, calcium may be acting at additional sites in mitochondrial suspensions, e.g., by enhancing dynamic contact sites (Sandri *et al.*, 1988) which may be a transient location for MCC (Kinnally *et al.*, 1992). Moran *et al.* (1990) report MCC-like channel activity from preparations enriched with contact sites. MCC can also be induced in electrically silent patches by the application of large-amplitude transmembrane potentials ($> \pm 60$ mV) (e.g., Zorov *et al.*, 1992a; Kinnally *et al.*, 1992).

MCC activity may be modulated by its interaction with other proteins, possibly in contact sites. MCC exists in yeast strains lacking the outer membrane channel VDAC, indicating VDAC is not required for MCC activity. However, the voltage dependence of MCC from VDAC-less yeast mitoplasts is modified outside the voltage range of −40 to 30 mV (Lohret and Kinnally, 1995b). Furthermore, the voltage dependence of MCC from wild-type yeast is modified by reconstitution in a similar manner as shown in Fig. 1. These findings indicate that reconstitution, like VDAC deletion, may disrupt factors responsible in part for MCC's voltage dependence. Note that a shift in V_0 to higher positive potentials and a decrease in gating charge were observed in some patches after reconstitution (unpublished results of Lohret and Kinnally).

PHARMACOLOGY

A considerable number of pharmacological agents have been identified that influence MCC as

Table II. Pharmacological Comparison of MCC and PTP

	Action	MCC (μM)	PTP (μM)
Cyclosporin	Inhibit	0.25[a]	100 pM/mg protein[b]
Dibucaine	Inhibit	500[c]	n.t.[d]
Spermine	Inhibit	n.t.	30[e]
Carboxyatractyloside	Mixed	no effect[p]	Induce
Sulfhydryl reagents			
Dithiothreitol	Inhibit	1000[f]	1000[g]
Menadione	Activate	n.t.	150[g]
Diamide	Activate	n.t.	100[g]
CCCP	Activate	0.1–10[f]	0.003/mg protein[h]
FCCP	Activate	0.1–10[f]	0.08[i]
Metabolic effectors			
Antimycin A	Inhibit	0.5–2[j]	n.t.
	Activate	10[j]	n.t.
Rotenone	No effect	5[j]	n.t.
Amphiphilic cations:			
Amiodarone	Inhibit	0.4–8[k]	5[o]
Quinine	Inhibit	150–700[k]	n.t.
Propranolol	Inhibit	70–700[k]	n.t.
Benzodiazepines			
RO5-4864	Mixed	0.07 inhibit[l]	25 μM activate[h]
PPIX	Activate	0.25[l]	1[m]
	Inhibit	0.05[l]	n.t.
Diazepam	Activate	n.t.	40[m]
Aldipen	Activate	0.015–2[n]	n.t.

[a] Szabó and Zoratti, 1991. [b] Bernardi *et al.*, 1994. [c] Unpublished data of Kholmuhamedov and Kinnally. [d] n.t., not tested. [e] Lapidus and Sokolove, 1994. [f] Campo *et al.*, 1995. [g] Petronilli *et al.*, 1994. [h] Moreno-Sanchez *et al.*, 1991. [i] Igbavboa and Pfeiffer, 1991. [j] Campo *et al.*, 1992. [k] Antonenko *et al.*, 1991. [l] Kinnally *et al.*, 1993. [m] Pastorino *et al.*, 1994. [n] Szabó *et al.*, 1993a. [o] Zorov and Kinnally, unpublished data. [p] Lohret *et al.*, 1996.

shown in Table II. Cyclosporine is perhaps the most notable because it is a high-affinity inhibitor (Szabó and Zoratti, 1991) and because of its clinical applications as an immunosuppressant. It has also been found to reduce ischemia-reperfusion injury (as would occur in stroke or myocardial infarction) in laboratory animals and model systems (Kurokawa *et al.*, 1992; Gogvadze and Richter, 1993). The ability of cyclosporin to suppress injury following ischemia may be linked to the drug's ability to inhibit the mitochondrial calcium-induced permeability transition (see below).

Metabolic effectors have also been found to influence MCC although the sites of action are not thought to be those related to their disruption of oxidative phosphorylation. For example, antimycin A inhibits MCC at 1–2 μM and activates MCC at 10–50 μM levels (Campo *et al.*, 1992); but only nanomolar levels are needed to block respiration (Slater, 1973). The uncoupler CCCP oxidizes sulfhydryl groups at micromolar concentrations (Kabeck *et al.*, 1974). CCCP and FCCP activate MCC at micromolar concentrations and this activation can be reversed by the reducing agent dithiothreitol (Campo *et al.*, 1995). However, uncoupling of oxidative phosphorylation requires only nanomolar levels of CCCP or FCCP (Heytler and Prichard, 1962).

MCC has been linked to a two-membrane complex associated with the mitochondrial benzodiazepine receptor (mBzR) protein. The isolated mBzR complex is composed minimally of VDAC and the 18-kDa mBzR protein in the outer membrane, and the adenine nucleotide translocator (ANT) in the inner membrane (McEnery, 1992; McEnery *et al.*, 1992). Ligands that bind to outer membrane components of the mBzR complex with nanomolar affinity inhibit MCC activity at similar concentrations, i.e., protoporphorin IX and Ro5-4864, which bind to VDAC (Kinnally *et al.*, 1993; McEnery, 1992). Recall that studies with VDAC-less yeast indicate that VDAC itself is not required for MCC activity (Lohret and Kinnally, 1995b). Furthermore, studies with ANT-less yeast indicate that ANT is not required for MCC activity reconstituted in proteoliposomes. (Lohret *et al.*, 1996). In addition, another mitochondrial channel, mCS, is affected by nanomolar levels of mBzR complex ligands including protoporphorin IX, Ro5-4864, and PK11195 (which binds to the 18-kDa mBzR protein) (Kinnally *et al.*, 1993). Clearly, further studies are needed to clarify whether MCC and/or mCS are functional components of the mBzR complex.

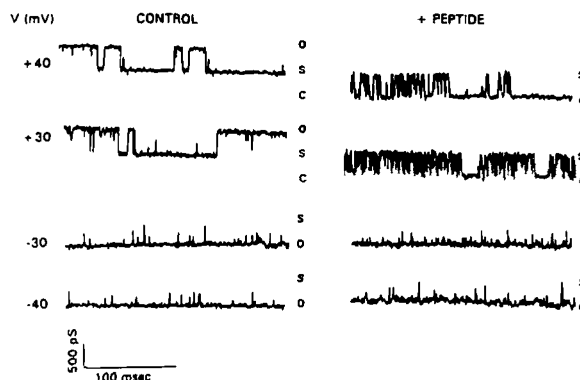

Fig. 3. MCC activity is transiently blocked by targeting peptides. MCC was reconstituted in proteoliposomes enriched in inner membranes from VDAC-less yeast mitochondria. Current traces of MCC at various voltages are shown in the presence and absence of 50 μM targeting peptide from the amino terminus of precursor subunit IV of cytochrome oxidase. O, S, and C correspond to the open, substate, and closed conductance levels. Data from the study by Lohret and Kinnally (1995a).

POSSIBLE PHYSIOLOGICAL ROLE OF MCC

Although MCC was initially characterized several years ago, its physiological role is still a subject of speculation. MCC may function in calcium homeostasis in parts of the cell where the endoplasmic reticulum is sparse, e.g., synapses, and/or it may be involved in the propagation of calcium transients within the cell (Martinez-Serrano and Satrustegui, 1992; Rizzuto *et al.*, 1993). Alternatively, it may be the consequence of a "damaged" transporter since several transporters are known to display "channel-like" behavior under some conditions, e.g., Na^+/K^+ ATPase (Last *et al.*, 1983). At this juncture, one of the more likely roles for MCC is involvement in protein import into mitochondria, a conclusion drawn from the specific effects of targeting peptides on open probability and mean open time of MCC as illustrated in Fig. 3 (Lohret and Kinnally, 1995a).

IS MCC RELATED TO THE OUTER MEMBRANE PEPTIDE-SENSITIVE CHANNEL?

The transient-blockade of MCC by the targeting peptide of cytochrome oxidase subunit IV is similar to that observed for another mitochondrial channel, PSC (Thieffry *et al.*, 1989; Fevre *et al.*, 1994). Szabó

et al., (1995) have speculated that MCC and PSC may represent the same activity, although this seems unlikely since PSC has been localized to the outer membrane (Chich *et al.*, 1991) and MCC is associated with mitoplasts. Alternatively, one could speculate that PSC and MCC might work in tandem at contact sites to provide an intermembrane import pathway for precursor proteins from the site of synthesis in the cytoplasm to the mitochondrial matrix.

It is difficult to directly compare MCC and PSC since they have been characterized in different systems: MCC in mitoplasts in 0.15 M KCl and PSC (predominantly) after reconstitution in tip-dip experiments in 0.15 M NaCl (Fèvre *et al.*, 1994; Thieffry *et al.*, 1992). As shown in Table III, both channels have a conductance of over 1 nS and predominant transitions of ~500 pS, and both are slightly cation selective. Although the V_0 for the two channels are similar, there are also important differences in their reported voltage dependences, i.e., mammalian MCC is open at matrix negative potentials and PSC is reported open at cytoplasmic negative potentials, and the gating charges for the two channels also are different (Kinnally *et al.*, 1993; Chich *et al.*, 1991). Since some or all of the differences between PSC and MCC may be induced during reconstitution (Fig. 1), there is currently insufficient data to establish whether the two activities represent the same entity.

MCC IS SIMILAR TO OTHER MEGA-CHANNELS

Channels are integral players in inter- and intracellular signaling as well as in a myriad of other functions. It is perhaps not surprising that MCC shares many characteristics with other intracellular channels (see Table IV). Many of these channels, like MCC, have multiple conductance levels and relatively poor ion selectivity (due likely to large pore diameters). While MCC has the largest reported conductance for an intracellular channel, its size is approached by channels found in bacterial cell membranes (Martinac *et al.*, 1987; Simon and Blobel, 1992; Szabó *et al.*, 1993).

Most intracellular channels, including MCC, are subject to regulation by physiological effectors and exhibit voltage-dependent behavior. Many of these channels are activated and/or inactivated by calcium, a common intracellular messenger. The activation of MCC by micromolar calcium and its inhibition by millimolar calcium (Kinnally *et al.*, 1991) are similar to the effects of calcium on the ryanodine receptor/calcium-release channel of the sarcoplasmic reticulum (Coronado *et al.*, 1994). In addition, both MCC and the ryanodine receptor are thought to interact with the immunophilins, cyclophilin and FK506-binding proteins, respectively (Szabo and Zoratti, 1992; Cameron *et al.*, 1995).

Like mitochondrial MCC and PSC, several other megachannels are sensitive to targeting peptides and/or puromycin (which causes premature release of nascent polypeptides from ribosomes), including a 500-pS channel in the endoplasmic reticulum and a 500-pS channel in the *E. coli* cell membrane (Lohret and Kinnally, 1995a; Fevre *et al.*, 1994; Simon and Blobel, 1991, 1992). A signal peptide added to the cytoplasmic (but not periplasmic) side of the *E. coli* inner membrane in bilayers elicits a dramatic increase in conduc-

Table III. Comparison of MCC and PSC Activities[a]

	MCC[a]	PSC[b,c]
Membrane localization	Inner/contact sites	Outer
Conductance levels	Several	Four
Peak conductance (pS)	1000–1500[d]	900[e]
Predominant transition (pS)	300–600[d]	450–500[d] 220–330[e]
Ion selectivity	P_{K^+/Cl^-} = 3–6	P_{Na^+/Cl^-} = 3–4.5
Gating charge: mammalian	−5.4 ± 1.0	1.8
yeast	−4.7 ± 1.1	1.77
V_0 (mV): mammalian	23 ± 3	−21.5
yeast	5.3 ± 8	0
Voltage for high open probability	Matrix negative	Cytoplasm negative
Calcium	Activation	No effect

[a] Lohret and Kinnally, 1995. [b] Fèvre *et al.*, 1994. [c] Thieffry *et al.*, 1992. [d] 0.15 M KCl. [e] 0.15 M NaCl.

Table IV. Characteristics of Selected Mega-channels

Organelle	Size (pS)[a]	Selectivity	Ca^{2+} dependent	Voltage dependent	Conductance levels
Mitochondria					
inner membrane					
MMC[1]	1300 (500)[b]	Cation	Yes	Yes	Multiple
outer membrane					
VDAC[3]	650 (300)[b]	Anion	No	Yes	Multiple
PSC[2]	1250 (500)[b]	Cation	n.r.[c]	Yes	Three
Chloroplasts[4]	525	Anion	n.r.	Yes	Multiple
Endoplasmic reticulum					
Protein conducting	500[5]	Anion	n.r.	No	n.r.
Anion channel	380[6]	Anion	n.r.	Yes	Two
Ca^{2+} release[7d]	400	Cation	Yes	Yes	Multiple
IP_3 receptor[8]	120	Cation	Yes	Yes	Three
Nucleus	300[9]	Cation	Yes	No	Multiple
	500[10]	Anion	No	Yes	Multiple
Nodulin 26[11]	465	Anion	Yes	Yes	Multiple
Bacterial membranes					
E. coli					
Protein conducting[12]	500	n.r.	n.r.	Yes	Three
Stretch[13]	700	Anion	n.r.	Yes	≳two
S. faecalis[14]	900	None	n.r.	Yes	Multiple

[a] Estimated for 150 mM KCl from reported values assuming linearity. [b] Predominant transition size is in parentheses. [c] Not reported. [d] Ryanodine receptor. [1] Kinnally *et al.*, 1992. [2] Fevre *et al.*, 1994. [3] Colombini, 1986. [4] Fuks and Homble, 1995. [5] Simon and Blobel, 1991. [6] Morier and Sauve, 1994. [7] Coronado *et al.*, 1994. [8] Mak and Foskett, 1994. [9] Mazzanti *et al*, 1991. [10] Tabares *et al.*, 1991. [11] Weaver *et al.*, 1994. [12] Simon and Blobel, 1992. [13] Martinac *et al.*, 1987. [14] Szabó *et al.*, 1993.

tance at positive potentials. At low salt, the *E. coli* channels gate open and closed (as the signal peptide reversibly binds) while at high salt they remain open regardless of the voltage (Simon and Blobel, 1992). In the endoplasmic reticulum, channels opened by puromycin-induced release of nascent polypeptides reclose at high salt, presumably because of dissociation of the ribosome from the channel. The puromycin-revealed channels are not voltage dependent and no gating was reported (Simon and Blobel, 1991). Protein-conducting channels are also thought to exist in other organelles, e.g., chloroplasts, but electrophysiological studies with peptides have not yet been reported.

IS MCC RELATED TO THE PERMEABILITY TRANSITION PORE?

A permeability transition in the inner mitochondrial membrane that can be induced by Ca^{2+} has been the subject of several reviews (e.g., Bernardi *et al.*, 1994, Gunter *et al.*, 1994; Gunter and Pfeiffer, 1990). For the most part, the transition requires Ca^{2+} accumulation in the matrix and is facilitated by the presence of an inducing agent, many of which are chemically unrelated, suggesting an indirect and complex process. The permeability transition permits the passage across the inner membrane of rather large molecules such as pyridine nucleotides (e.g., Gazzotti, 1975) and even matrix proteins (Igbavboa *et al.*, 1989). It has been proposed that the permeability increase results from the activation of a channel or pore, i.e., a permeability transition pore (PTP) (e.g., Haworth and Hunter, 1980). The process can be inhibited or reversed by a variety of conditions including the chelation of Ca^{2+} or the presence of cyclosporin, ADP, Mg^{2+}, reduced pyridine nucleotides, or low pH (Bernardi *et al.*, 1992). There is evidence that the channels act cooperatively (Crompton and Costi, 1988) and may be formed by assembly involving cross-linking by disulfide bridges (Fagian *et al.*, 1990). The possible physiological role of this channel is not clear but it has been implicated in ischemia-reperfusion injury (Pastorino *et al.*, 1993, 1994; Godvadze and Richter, 1993; Kurokawa *et al.*, 1992) and the intracellular propagation of calcium transients (Ichas *et al.*, 1994; Evtodienko *et al.*, 1994).

Related studies of MCC activity using electrophysiological techniques (Szabó and Zoratti, 1991,

1992; Szabó *et al.*, 1992) and PTP using mitochondrial suspensions (Bernardi *et al.*, 1992) suggest that the two may be equivalent. As shown in Table I, MCC and PTP are subject to regulation by the same physiological effectors including divalent cations, ADP, and pH. Furthermore, the pharmacology studies of PTP and MCC indicate that they are affected by the same agents at similar concentrations, as shown in Table II and exemplified by cyclosporin, and both are implicated in cell death due to ischemia-reperfusion injury. While the pharmacology and physiological effector studies support the identity of MCC and PTP, there are inconsistencies that need further attention; e.g., the effects of Ro5-4864 on PTP and MCC do not agree and MCC activity is not affected by the PTP-inducer carboxyatractyloside (Lohret *et al.*, 1996) as shown in Table II.

FUTURE DIRECTIONS

While MCC has been extensively characterized using electrophysiological techniques, considerably more information is needed to determine the molecular identity and function of this megachannel. It appears likely that MCC resides (at least transiently) in contact sites, making its interactions with VDAC, mBzR, and other outer membrane components an important arena of study. Of equal importance will be the delineation of the putative role of MCC in protein import and its relationship to PSC. Finally, understanding how MCC functions within the context of mitochondrial energy transduction will require a full description of the channel's physiological and pharmalogical effectors, including its role in ischemia-reperfusion injury and its association with the mitochondrial permeability transition pore.

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