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The Emerging Picture of Mitochondrial Membrane Channels

Carmen A. Mannella[1,2] and Henry Tedeschi[3]

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Modern textbooks paint essentially the same picture of mitochondrial membrane transport today as they did two decades ago. The outermost of the two membranes that define this organelle is usually referred to as being "freely" permeable to all molecules and ions smaller than 5–10 kDa. In contrast, and in keeping with the tenets of the chemiosmotic hypothesis, the inner mitochondrial membrane is considered impermeable to even the smallest of ions. Entry of metabolites and ions into the matrix may occur only by specific transport proteins or carriers.

True, the textbook picture of mitochondrial transport has been touched up considerably during this period, especially relating to the outer membrane. Until the early 1980's it was unclear whether the amazing permeability of the outer membrane was an artifact of isolation or the result of some remarkable aspect of this membrane's architecture (e.g., DePierre and Ernster, 1977). It is now known that the latter is the case. The mitochondrial surface contains thousands of copies of a pore-forming protein that was first named VDAC (Schein *et al.*, 1976; Colombini, 1979) and later referred to as mitochondrial porin. This protein has been isolated, sequenced, and manipulated by the tools of modern molecular genetics (e.g., Peng *et al.*, 1992). It has also been crystallized in the plane of the outer membrane and its basic structural parameters determined (Mannella, 1990). When incorporated into bilayers, this protein has many of the properties expected of the outer membrane's main permeability pathway—plus a few that are totally unexpected, such as voltage-dependent gating (Colombini, 1979). Considerable progress has also been made in defining the transport systems of the inner mitochondrial membrane (Kramer and Palmieri, 1989). Many of the constitutive polypeptides have been sequenced and are now known to be part of a protein superfamily (Aquila *et al.*, 1987) that may include VDAC/porin as a distantly related member (Mannella, 1990). Still, the inner mitochondrial membrane's role as an energy-transducing membrane is commonly thought to preclude the presence of large, transmembrane channels such as those that occur in the outer membrane.

This issue of *Journal of Bioenergetics and Biomembranes* (Vol. 24, No. 1, February 1992) contains reviews and research reports summarizing the latest findings from several laboratories working in the field of mitochondrial membrane permeability. Most of the papers are based on material presented at the 31st Annual Meeting of the Biophysical Society, San Francisco, California, February 24–28, 1991, and subsequently updated as of June 1991. It is clear from these reports that the field has moved inexorably to a point of changed perspectives toward some of the old questions. Is the outer membrane really "freely" permeable to metabolites and ions? And might large ion channels exist in the inner as well as in the outer mitochondrial membrane? The familiar answers to these questions are in dispute and the traditional picture of mitochondrial membrane permeability may soon need to be reworked.

[1]Wadsworth Center for Laboratories and Research, New York State Department of Health, Empire State Plaza, Albany, New York 12201-0509.

[2]Department of Biomedical Sciences, School of Public Health, University of New York at Albany, Albany, New York 12201-0509.

[3]Department of Biological Sciences, University of New York at Albany, 1400 Washington Avenue, Albany, New York 12222.

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Some reports in this issue describe progress made toward achieving molecular detail in the steadily evolving picture of the main outer membrane channel (Mannella *et al.*, 1992a,b; DePinto and Palmieri, 1992; Peng *et al.*, 1992). New structural and functional data have led to testable models for this channel's basic structure and for the conformational changes that underlie its gating. At the same time, attention is being drawn to a startling new focal point: the regulation of the VDAC/porin channel's conductance. Switching of this channel to lower-conductance substates is greatly facilitated by the binding of certain macromolecular "modulators" (Colombini *et al.*, 1987). The discovery of a modulator-protein activity within the mitochondrion itself (Liu and Colombini, 1992) raises the intriguing possibility that the gating phenomenon may be more than a bilayer curiosity. Recent experimental results (Benz and Brdiczka, 1992) suggest that modulators of VDAC may significantly reduce the outer mitochondrial membrane's permeability to adenine nucleotides. These findings, if confirmed and extended, have tremendous implications for the role of the outer membrane in regulating mitochondrial function. There are also observations of other channels, with permeability properties different from VDAC/porin's, coexisting in the mitochondrial outer membrane (Kinnally *et al.*, 1987; Dihanich *et al.*, 1989; Benz *et al.*, 1990; Chich *et al.*, 1991). It may be that the permeability of this membrane *in situ* is a complex function of the levels of expression of several different channel and modulator proteins.

Other aspects of research into the VDAC/porin channel further increase the complexity of this picture. For example, there is growing evidence for tissue-specific association of this mitochondrial channel with other proteins, such as hexokinase (Nakashima, 1989; Arora *et al.*, 1992) and drug receptors (McEnery, 1992). There is even evidence to suggest that VDAC exists in transmembrane receptor assemblies outside the mitochondrion, e.g., in the plasma membrane (Thinnes, 1992). Attention must now focus on experiments to determine the physiological significance of these new and controversial observations.

In the last five years, the hue and texture of the field of mitochondrial transport has been transformed by the advent of a new technique. Patch-clamping, which has revolutionized the field of ion channels in general, has revealed an unexpected diversity of currents in mitochondrial membranes (Kinnally *et al.*, 1992; Moran and Sorgato, 1992; Szabo and Zoratti, 1992; Zorov *et al.*, 1992). The size of these membrane conductances varies from a few pS, like those of prototypical ion channels, to several nS, even larger than VDAC/porin's conductance. Classifications of these mitochondrial channel activities are presented in this issue (Kinnally *et al.*, 1992; Moran and Sorgato, 1992), based in part on functional properties (size; dependence on voltage, pH, cations, and drugs), and in part on apparent localization. The inescapable conclusion at this juncture, based on experiments with native mitochondrial membranes and with membrane subfractions reconstituted into liposomes, is that many of these conductances are associated with the inner membrane. There is considerable circumstantial evidence (presented in all four patch-clamp papers in this issue) linking two of the conductance classes to the pH- and Ca-induced inner membrane channels or permeability transitions previously inferred from solution studies (Beavis, 1992; Gunter and Pfeiffer, 1990).

The emergence of this diverse set of mitochondrial membrane channel activities has generated a broad spectrum of speculation regarding their nature and physiological roles. Many examples can be found in the papers within this issue. For example, there are implications that certain channels may be involved in mitochondrial regulatory mechanisms that cannot yet be fully deduced from the paucity of available data. Other data suggest that channels may be elements of specialized junctions between the two mitochondrial membranes, hinting at a regulatory role for direct membrane–membrane interactions. Still other lines of investigation point to the involvement of mitochondrial channels in the complex process by which proteins are transported into the organelle (see also Chich *et al.*, 1991).

It is clear from what is known now (only a fraction of which is summarized above) that the old textbook depiction of mitochondrial transport is inadequate at best. The papers in this issue trace the outlines of several new ideas and directions relating to mitochondrial channels, their regulation, and their role in mitochondrial function. The results of experiments now in progress will determine which of these sketches will, in the final picture, be painted in and which painted over.

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[4] Because of the prefatory nature of this paper and the many issues raised, there is no attempt to be comprehensive in the citing of literature. For the most part, references are limited to papers in this issue of *Journal of Bioenergetics and Biomembranes* and to other recent reviews and key first reports.