Journal of Bioenergetics and Biomembranes, Vol. 26, No. 5, 1994

Cation Transport by Mitochondria

Thomas E. Gunter[1]

INTRODUCTION

Mitochondrial cation transport controls a number of vital mitochondrial functions and is also involved in many mitochondrial responses to altered conditions. Mitochondrial matrix free Ca^{2+} concentration ($[Ca^{2+}]_m$) is increasingly recognized as a primary metabolic mediator. Because Mg^{2+} and K^+ function as cofactors in a wide range of vital reactions in the mitochondrial matrix, maintenance of homeostatis of these ions is very important. Furthermore, K^+ also plays a role in control of mitochondrial volume. While the physiological function of the native mitochondrial permeability transition pore (PTP) protein is currently the subject of speculation, it is clearly involved in at least part of the response to hypoxia and ischemia in many cell types, particularly during reperfusion. Thus, mitochondria probably play a role in determining whether irreversible damage to tissue occurs following both heart attack and stroke.

As we learn more about these important mitochondrial cation transport functions and the mechanisms that mediate them, the experimental tools with which we seek to answer questions about the electrophysiological and biochemical characteristics of these mechanisms become increasingly sophisticated. Electrophysiological techniques using patch clamping or mitochondrial membrane components incorporated into planer bilayers can tell us considerably more about the conductance properties and alternative states of the cation transporters than can the older approaches involving measurements of transport kinetics. Isolation and reconstitution of the cation transporters opens the door not only to identifying the specific protein or complex responsible for a given mechanism, but also to accessing the powerful array of tools available through molecular biology. Sequencing these proteins will allow comparison of sequences of these mitochondrial transport systems with what may be related transport systems from the plasma membrane and other cellular membranes. Production of sufficient amounts of these proteins through molecular biological approaches could lead to structural studies utilizing X-ray crystallography or NMR to provide detailed structural information on these transporters.

The purpose of this mini review series is to bring the reader up to date with respect to what is known about the characteristics of these cation transport mechanisms and their physiological functions. It also updates the reader on progress in applying the more sophisticated electrophysiological techniques and reconstitution techniques to studies of these mechanisms.

THE Ca^{2+} TRANSPORT MECHANISMS AND THEIR PHYSIOLOGICAL ROLES

Mitochondria have been known for decades to increase their rate of ADP phosphorylation many fold in response to increases in [ADP] and [P_i], and perhaps in some cases in response to decreases in [ATP]. This "substrate-product feedback" system has been held to be the basis of metabolic control at the mitochondrial level. More recently it has been found, primarily through NMR data, that in at least some important types of tissues (e.g., heart and liver), [ADP] and [P_i] do not increase nor [ATP] decrease as the work load within the cells increases (Balaban, 1990; Balaban and Heineman, 1990; Brosnan *et al.*, 1990; Halow, 1993; Heineman and Balaban, 1990; Katz *et al.*, 1987, 1988, 1989; Koretsky *et al.*, 1989, 1990). Clearly, substrate-product feedback control

[1] Department of Biophysics, University of Rochester Medical School, Rochester, New York 14642.

0145-479X/94/1000-0465$07.00/0

cannot be operating in these cases. Since changes in the cell which require a modification of the metabolic rate are generally signaled to the cell through hormones, neurotransmitters, or growth factors (i.e., through primary messengers), the agent of these changes within the cell cytosol must undoubtedly be a common second messenger. A likely choice of a second messenger is cytosolic $[Ca^{2+}]_c$, already known to be rapidly requestered by mitochondria. Intramitochondrial $[Ca^{2+}]_m$ has been reported to be capable of increasing the rate of oxidative phosphorylation through activation of a series of steps in the metabolic pathway including dehydrogenases coupled to the tricarboxylic acid cycle, electron transport, phosphorylation at the F_1F_0ATPase, and adenine nucleotide transport (Denton and McCormack, 1990; Gunter *et al.*, 1994; Hansford, 1985, 1991). Thus Ca^{2+} activation is viewed as functioning through distributed control (Brand and Murphy, 1987; Brown, 1992; Kacser and Burns, 1973). Clearly, increases in [ADP] and $[P_i]$, perhaps along with decreases in [ATP], can function as a "default mechanism" in metabolic control, with the Ca^{2+}-mediated system superseding the default system in a least several important tissues. A major physiological role of the mitochondrial Ca^{2+} transport mechanisms, i.e., those mechanisms which control $[Ca^{2+}]_m$, is now believed to be control of the rate of production of ATP.

Individual liver mitochondria containing endogenous levels of $[Ca^{2+}]_m$, bound to a cover slip, are shown on the journal cover through fluorescence of intramitochondrial fura-2 (a calcium indicator). The partially resolved mitochondria are seen with the indicator excited at 380 nm and emitting at 510 nm. Because the size of the individual pixels is significantly less than the resolving power of the microscope and camera system, the ratio of the background-corrected total intensity of light excited at 340 nm and emitted at 510 nm to that of light excited at 380 nm and emitted at 510 nm over an intact mitochondrion should be taken as a measure of $[Ca^{2+}]_m$ for that mitochondrion. The inset shows a magnification of the indicated region on the lower left side.

Three of the reviews below address important aspects of this control of cell metabolism by intramitochondrial $[Ca^{2+}]_m$: First, "Transport of Calcium by Mitochondria" by K. K. Gunter and T. E. Gunter describes what is known about the three mechanisms of Ca^{2+} transport found in the mitochondrial inner membrane, i.e., the mitochondrial Ca^{2+} uniporter, the Na^+-dependent Ca^{2+} efflux mechanism, and the Na^+-independent Ca^{2+} efflux mechanism. Second, "Mitochondrial Free Ca^{2+} Concentration in Living Cells" by S-S. Sheu and M-J. Jou reviews the evidence for sufficient uptake of Ca^{2+} from cytosolic Ca^{2+} pulses to activate the intramitochondrial Ca^{2+}-linked metabolic activation steps. Third, "Physiological Role of Mitochondrial Ca^{2+} Transport" by R. G. Hansford reviews the evidence that $[Ca^{2+}]_m$ regulates pyruvate dehydrogenase and the tricarboxylic acid cycle in response to increased tissue energy demands. This review also describes the impact of mitochondrial Ca^{2+} transport on changes in cytosolic free calcium concentration ($[Ca^{2+}]_c$) and proposes that a failure to sufficiently elevate $[Ca^{2+}]_m$ in response to an increase in work load may lead to specific types of cardiomyopathies of metabolic origin.

THE MITOCHONDRIAL Ca^{2+}-INDUCED PERMEABILITY TRANSITION

Mitochondria from all vertebrate species studied have been found to undergo a transition in the permeability of their inner membrane to small ions and molecules, induced by matrix Ca^{2+} and an additional inducing agent (Gunter and Pfeiffer, 1990). Following this permeability transition, mitochondria exchange the small ions and molecules of their matrix with those of the external medium and lose their electrochemical proton gradient, thereby becoming incompetent to mediate oxidative phosphorylation. Induction of this permeability transition causes colloid osmotic swelling of the mitochondria. This highly permeable state can be reversed by removal of Ca^{2+} or by addition of one of a small group of pharmacological agents similar to the immunosuppressant drug, cyclosporin A. Reversal of the permeabilized state can restore the mitochondrion's ability to mediate oxidative phosphorylation. This permeability transition is mediated by a highly conductive pore, usually called the permeability transition pore (PTP), whose open probability is increased by binding of Ca^{2+} and is greatly decreased by binding of cyclosporin A. Many other factors such as pH, membrane potential, and the presence of inducers or inhibitors of the PTP influence the open probability of this pore. It is not currently known whether the pore *per se* has a physiological function or only opens under pathological conditions. Regardless of the importance of the

possible physiological function of the PTP, it has been found to occur under many pathological conditions as evidenced by swollen mitochondria and by the protective or ameliorating effects of cyclosporin A. Current views of this permeability transition pore are presented in a discussion of recent research on the PTP by P. Bernardi, K. M. Broekemeier, and D. R. Pfeiffer in "Recent Progress on Regulation of the Mitochondrial Permeability Transition Pore: A Cyclosporin-Sensitive Pore in the Inner Mitochondrial Membrane."

MITOCHONDRIAL TRANSPORTERS OF Na^+, K^+, AND Mg^{2+}

K^+ and Mg^{2+} are found in significantly higher concentrations in the cell cytosol than in serum or interstitial fluids, while Na^+ is found in much higher concentrations in serum than within cells. The higher cytosolic concentrations of K^+ and Mg^{2+} (140 and 1–2 mM, respectively) cause problems for mitochondrial transport of these ions which are not experienced with Na^+ or Ca^{2+}. Specifically, a very active uniporter for either K^+ or Mg^{2+}, like that which exists for Ca^{2+}, would be disastrous. If active uniporters which transported these ions at physiological concentrations existed at a mitochondrial membrane potential of 150 mV, and if extramitochondrial concentrations of these ions could be maintained in the cytosol, then the intramitochondrial free concentrations of these ions would equilibrate with the extramitochondrial concentrations at internal concentrations of around 48 M for K^+ and over 100 M for Mg^{2+}, respectively. This is clearly far from viable or realistic, for it would imply that mitochondria in cell types containing high numbers of mitochondria (muscle, nerve, liver, etc.) would stockpile more of these ions that exist in the cytosol. Nevertheless, many intramitochondrial reactions like those of the cytosol require K^+ or Mg^{2+}. Therefore, the mitochondrial membrane has evolved so as to have low permeability to these ions. The Mg^{2+} and K^+ transporters control these ions without sequestering them in much higher concentrations than are found in the cytosol. In the process, K^+ transport is also used as part of the mechanism controlling mitochondrial volume, a parameter which is thought by some to also be linked to control of metabolic rate. There is evidence that mitochondrial Mg^{2+} may also be an important regulatory component of the cell and that it may be partially controlled through the action of hormones. However, until recently the available methods for study of mitochondrial transport of Mg^{2+} have been expensive, inadequate, or inconvenient. Recently, new probes used with fluorescence spectroscopy or with NMR have offered attractive techniques for studying the transport of this ion. The mitochondrial transport of Na^+, K^+ and Mg^{2+} is described by G. P. Brierley, K. Baysal, and D. W. Jung in "Na^+ and K^+ Uniporters and Exchangers", and by D. E. Jung and G. P. Brierley in "Mg^{2+} Transport by Mitochondria" in this mini review series. Jung and Brierley also discuss the new techniques now available for study of Mg^{2+} transport and some of the results obtained using these new techniques.

Na^+, whose concentration in the cell cytosol (around 5–10 mM) is much lower than that of K^+, can be more easily used as an aid in controlling the concentration of other ions, such as Ca^{2+}. Na^+ is known to be transported across the mitochondrial inner membrane via two important exchangers: the rapid Na^+/H^+ antiporter and the Na^+/Ca^{2+} antiporter, which jointly play a role in control of mitochondrial matrix [Ca^{2+}]. There is also evidence for a ruthenium red-sensitive uniporter for Na^+, although many properties as well as the physiological role for this mechanism are unclear. Transport of this ion is also discussed in the review by Brierley *et al.*

STUDIES OF RECONSTITUTION OF MITOCHONDRIAL CATION TRANSPORTS

Isolation of the mitochondrial proteins and protein complexes responsible for transport of cations across the inner membrane is a daunting task. The primary problem is that this membrane is loaded with protein (perhaps as high as 80%). Each component of the electron transport chain is present, for example, in concentrations of the order of 1 nmol/mg protein. The cation transporters of interest are generally present in far lower concentration. The mitochondrial Ca^{2+} uniporter, for example, has been estimated to be present in amounts between 0.01 and 0.001 nmol/mg protein. It is likely then that without some process of selection for a transporter of interest, an ordinary polyacrylamide gel (PAGE) pattern of the protein bands from this membrane would not show a band for the transporter when the more common components of the membrane showed dark bands. Furthermore, all of these transporters are of

necessity membrane proteins. This brings its own set of problems. In many cases it is difficult to isolate a membrane protein from the membrane phospholipids, even with the gentlest detergent, without denaturing the protein. Even in the presence of these difficulties, however, there has been some success in isolating, identifying, and reconstituting these mitochondrial cation transport proteins. Considerable work has been done on purifying the mitochondrial Ca^{2+} uniporter (Gunter and Pfeiffer, 1990; Mironova *et al.*, 1994; Saris *et al.*, 1993). Recently, successful reconstitutions of both the Na^+/H^+ exchanger (Garlid *et al.*, 1991) and of the Na^+/Ca^{2+} exchanger (Li *et al.*, 1992) have been reported. This recent work and more is discussed in this series in the review "Mitochondrial Cation Transporters: A Progress Report" by K. D. Garlid.

ELECTROPHYSIOLOGICAL STUDIES OF MITOCHONDRIAL INNER MEMBRANE CHANNELS

There are severe limitations on how thoroughly the properties of mitochondrial membrane transporters can be analyzed in the intact mitochondrial membrane system. As discussed above, the mitochondrial inner membrane contains a very high concentration of protein, with those involved in electron transport present in much higher concentrations than those which transport cations such as Ca^{2+}. When working with intact mitochondria, we have very limited control of the concentration of the transporter, of the membrane potential, and of the free concentration of transported ion on at least one side of the transport membrane. Much more control is available when the same transport system can be studied using patch clamp techniques or can be integrated into a planer lipid bilayer under conditions where control of substrate concentration can be maintained on both sides of the membrane. Under these conditions, a wide range of electrophysiological techniques can be used to analyze the transport systems. However, because the conditions and techniques of study are very different under conditions where patch clamp or planar lipid bilayer techniques can be used compared to those *in vivo* or those employed in the older kinetics studies using intact mitochondria, and because the new conditions could lead to the introduction of additional artifacts, it is often difficult to identify unequivocally the transporters studied by electrophysiological methods with their counterparts in the intact mitochondrion. The review by M. Zoratti and I. Szabo "Electrophysiology of the Inner Mitochondrial Membrane" describes the results of these new electrophysiological studies as well as the similarities and differences in the transporters studied there and those in intact mitochondria.

ACKNOWLEDGMENTS

The author thanks Dr. Karlene Gunter for help in preparing the manuscript and Dr. Karlene Gunter, Dr. Claire Gavin, and Dr. Michael Zuscik for reading and criticizing the manuscript. He also thanks Ms. Genevieve Sparagna for preparing the photograph of mitochondria used on the journal cover. This work was supported by National Institutes of General Medical Sciences Grant GM 35550.

REFERENCES

Balaban, R. S. (1990). *Am. J. Physio.* **258,** C377–C389.
Balaban, R. S., and Heineman, F. W. (1990). *J. Mol. Cell. Biochem.* **89,** 191–197.
Brand, M. D., and Murphy, M. P. (1987). *Biol. Rev. Cambridge Philos. Soc.* **62,** 141–193.
Brosnan, M. J., Chen, L., Van Dyke, T. A., and Koretsky, A. P. (1990). *J. Biol. Chem.* **265,** 20849–20855.
Brown, G. C. (1992). *Biochem. J.* **284,** 1–13.
Denton, R. M., and McCormack, J. G. (1990). *Annu. Rev. Physiol.* **52,** 451–466.
Garlid, K. D., Shariat-Madar, Z., Nath, S., and Jezek, P. (1991). *J. Biol. Chem.* **266,** 6518–6523.
Gunter, T. E., and Pfeiffer, D. R. (1990). *Am. J. Physiol.* **258,** C755–C786.
Gunter, T.E., Gunter, K.K., Shey, S.-S., and Gavin, C.E. (1994) *Am. J. Physiol.* **267,** C313–C339.
Halow, J. M. (1993). "The role of ADP levels in regulation of oxidative phosphorylation in transgenic mouse liver expressing creatine kinase", Ph.D. Thesis, Carnegie Mellon University, Pittsburgh, Pennsylvania.
Hansford, R. G. (1985). *Rev. Physiol. Biochem. Pharmacol.* **102,** 1–72.
Hansford, R. G. (1991). *J. Bionenerg. Biomembr.* **23,** 823–854.
Heineman, F. W., and Balaban, R. S. (1990). *Annu. Rev. Physiol.* **52,** 523–542.
Kacser, H., and Burns, J. A. (1973). In *Rate Control of Biological Processes,* Symposia of the Society for Experimental Biology No. XXVII (Davis, D. D., ed.), University Press, Cambridge, pp. 65–104.
Katz, L. A., Koretsky, A. P., and Balaban, R. S. (1987). *FEBS Lett.* **221,** 270–276.
Katz, L. A., Koretsky, A. P., and Balaban, R. S. (1988). *Am. J. Physiol.* **255,** H185–H188.
Katz, L. A., Swain, J. A., Portman, M. A., and Balaban, R. S. (1989). *Am. J. Physiol.* **256,** H265–H274.

Koretsky, A. P., Katz, L. A., and Balaban, R. S. (1989). *J. Mol. Cell. Cardiol.* **21 Suppl. I,** 59–66.
Koretsky, A. P., Brosnan, M. J., Chen, L., Chen, J., and Van Dyke, T. (1990). *Proc. Natl. Acad. Sci. USA* **87,** 3112–3116.
Li, W., Shariat-Madar, Z., Powers, M., Sun, X., Lane, R. D., and Garlid, K. D. (1992). *J. Biol. Chem.* **267,** 17983–17989.
Mironova, G. D., Baumann, M., Kolomytkin, O., Krasichkova, Z., Berdimuratov, A., Sirota, T., Virtanen, I., and Saris, N.-E. L. (1994). *J. Bioenerg. Biomembr.* **26,** 231–238.
Saris, N.-E. L., Sirota, T. V., Virtanen, I., Niva, K. Penttilä, T., Dolgahova, L. P., and Mironova, G. D. (1993). *J. Bioenerg. Biomembr.* **25,** 305–312.