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Cation Transport Systems in Mitochondria: Na^+ and K^+ Uniports and Exchangers

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It is now well established that mitochondria contain three antiporters that transport monovalent cations. A latent, allosterically regulated K^+/H^+ antiport appears to serve as a cation-extruding device that helps maintain mitochondrial volume homeostasis. An apparently unregulated Na^+/H^+ antiport keeps matrix $[Na^+]$ low and the Na^+-gradient equal to the H^+-gradient. A Na^+/Ca^{2+} antiport provides a Ca^{2+}-extruding mechanism that permits the mitochondrion to regulate matrix $[Ca^{2+}]$ by balancing Ca^{2+} efflux against influx on the Ca^{2+}-uniport. All three antiports have well-defined physiological roles and their molecular properties and regulatory features are now being determined. Mitochondria also contain monovalent cation uniports, such as the recently described ATP- and glibenclamide-sensitive K^+ channel and ruthenium red-sensitive uniports for Na^+ and K^+. A physiological role of such uniports has not been established and their properties are just beginning to be defined.

INTRODUCTION

The mitochondria in a mammalian cell are exposed to a cytosol that is high in K^+ and low in Na^+. Mitochondria *in situ* must maintain a high negative membrane potential ($\Delta\psi$) in the face of a cytosol $[K^+]$ of about 140 mM. These organelles therefore represent infinite sinks for K^+ uptake, and a mechanism to prevent net K^+ uptake is a necessity (Mitchell, 1966, 1968; Garlid, 1980, 1988b). Mitochondria maintain matrix $[K^+]$ at very nearly the same concentration as cytosol $[K^+]$ as a result of (a) poor electrophoretic permeability to K^+ except when $\Delta\psi$ is elevated and (b) the presence of a latent K^+/H^+ antiport that extrudes excess K^+ (see Brierley, 1976, 1983 or Diwan, 1988 for reviews).

In contrast, mitochondria appear quite permeable to Na^+ as a result of the presence of an apparently unregulated Na^+/H^+ antiport that keeps the electrochemical Na^+-gradient equal to the H^+-gradient (see Brierley and Jung, 1988a or Garlid, 1988a for reviews). The Na^+-gradient in turn supports the efflux of Ca^{2+} on the Na^+/Ca^{2+} antiport. A kinetic balance between this antiport and Ca^{2+} influx on the ruthenium red-sensitive Ca^{2+} uniport appears to maintain matrix $[Ca^{2+}]$ and permits this ion to serve as a metabolic regulator in the matrix compartment (see Gunter and Pfeiffer, 1990 or Crompton, 1990 for reviews of Ca^{2+} cycling in the mitochondrion). Each of these three antiports, the K^+/H^+, Na^+/H^+, and Na^+/Ca^{2+}, therefore has a well-defined physiological role. All three have been extracted from the membrane, reconstituted into liposomes and at least partially purified (Li *et al.*, 1990; Garlid *et al.*, 1991; Li *et al.*, 1992), so there is good progress toward defining the molecular structure and properties of these antiports. The status of work on each of these components in intact mitochondria is summarized in the discussion to follow.

THE Na^+/H^+ ANTIPORT

This antiport transports Na^+ and Li^+ in exchange

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for H^+, but is unaffected by K^+ or cations such as tetramethylammonium$^+$. The efficient respiration-dependent extrusion of Na^+ and osmotic contraction of mitochondria swollen in Na^+ salts is supported by this antiport (Brierley *et al.*, 1977). Nakashima and Garlid (1982) made the important observation that the Na^+-specific antiport is insensitive to quinine and that this property can be used to distinguish this activity from that of the activated K^+/H^+ antiport which also transports Na^+. The exchange of Na^+ for H^+ on this antiport is inhibited by Li^+ and Mn^{2+} but insensitive to DCCD[2] and Mg^{2+}, inhibitors of the K^+/H^+ antiport (see Garlid, 1988a or Brierley and Jung, 1988a for reviews).

The entry of Na^+ into intact mitochondria on the Na^+/H^+ antiport can be followed by passive osmotic swelling in Na^+ acetate (Mitchell and Moyle, 1969; Brierley *et al*, 1978) or by $^{22}Na^+$ exchange (Nath and Garlid, 1988). The Na^+-dependent extrusion of matrix H^+ can be monitored with a glass electrode (Crompton and Heid, 1978; Kapus *et al.*, 1988) or by the fluorescence of matrix sequestered BCECF (Brierley *et al.*, 1989; Kapus *et al.*, 1989). These studies show good agreement that the K_m for Na^+ uptake at neutral external pH is near 30 mM with a V_{max} at 25°C over 160 ng-ion · mg^{-1} · min^{-1} (Table I). The uptake of Li^+ under similar conditions shows a K_m of 1–2 mM and much lower V_{max}. Li^+ is a competitive inhibitor of Na^+ uptake with a K_i of from 0.7 to 1.6 mM (Nath and Garlid, 1988; Kapus *et al.*, 1988). The uptake of Na^+ is also inhibited competitively by external $[H^+]$ (Kapus *et al.*, 1988; Brierley *et al.*, 1989). The K_m for Na^+ decreases with increasing external pH to an extrapolated limit of 4.6 mM at an external $[H^+]$ of zero (Brierley *et al.*, 1989). These studies indicate that the antiport moves Na^+ inward with low affinity, but high capacity, and that is has a common external binding site for Na^+, Li^+, and H^+.

All of the studies summarized in Table I followed the uptake of Na^+ and extrusion of H^+ by intact mitochondria. The opposite reaction, Na^+ extrusion driven by ΔpH, is presumed to be the relevant physiological activity for the Na^+/H^+ antiport. Rosen and Futai (1980) used quinacrine fluorescence to follow Na^+ entry into submitochondrial particles, the equivalent of Na^+ efflux from intact mitochondria, and found a K_m of 26.5 mM for Na^+ and 1 mM for Li^+ at pH 8.0. These results are compatible with the antiport being symmetrical in its interaction with Na^+. In this regard Garlid *et al.* (1991) have reconstituted Na^+/H^+ activity using detergent extracts of SMP and have partially purified a fraction that shows Na^+/H^+ exchange in both directions in liposomes. The K_m for Na^+ uptake was about 30 mM and the reaction was inhibited by *cis* $[Li^+]$ with an I_{50} near 1 mM. However, the corresponding value for *trans* $[Li^+]$ was 24 mM. They suggest that the high affinity of Li^+ for the transporter makes it a poor substrate and that the Li^+-loaded antiporter may turn over slowly (Garlid *et al.*, 1991).

Table I. Kinetic parameters for Na^+ influx on the Na^+/H^+ antiport

Method	pH_0	K_m (mM)	V_{max} (ng-ion min^{-1} mg^{-1})	Reference
Osmotic swelling	7.4	30	400	Nath and Garlid (1988)
$^{22}Na^+/Na^+$ exchange	7.4	31	170	Nath and Garlid (1988)
H^+ efflux (electrode)	6.9	24	270	Kapus *et al.* (1988)
H^+ efflux (BCECF)	7.6	32	160	Brierley *et al.* (1989)
H^+ efflux (BCECF)	8.0	14	160	Brierley *et al.* (1989)

The interaction of the Na^+/H^+ antiport with $[H^+]$ is complex. Matrix H^+ serves as a transported co-substrate during Na^+ influx whereas external $[H^+]$ competes with Na^+ for binding to a common site. In addition, ΔpH provides a major component of the driving force for exchange even when a $[Na^+]$ gradient is imposed experimentally. Kapus *et al.* (1988) showed that Na^+-dependent H^+ extrusion is inhibited by external $[H^+]$ with a Hill coefficient of 1.07 and K_i between 30 and 60 nM (pH_0 7.22–7.5). Our study (Brierley *et al.*, 1989) evaluated the K_i as about 3 nM (pH_0 8.6), and this discrepancy has not been resolved.

Brierley *et al.* (1989) also found a hyperbolic dependence of Na^+ entry on matrix $[H^+]$ when pH_0 was maintained constant and matrix $[H^+]$ manipulated by varying a Donnan distribution. Under these conditions the K_m was constant over the range of external pH from 7.5 to 8.3 and was equivalent to a matrix pH of 6.8, or 1.6×10^{-7} M $[H^+]$. The V_{max}

[2] Abbreviations used: BCECF, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; SBFI, sodium-binding benzofuran isophthalate from Molecular Probes, Inc., Eugene, Oregon; SMP, submitochondrial particles; DCCD, dicyclohexylcarbodiimide; PBFI, potassium-binding fluorescent indicator (Molecular Probes); pH_0, extramitochondrial pH.

increased in this protocol as the inhibitory external $[H^+]$ was decreased.

Kapus *et al.* (1989) also used BCECF fluorescence to follow changes in matrix $[H^+]$ and varied matrix pH by altering the rate of succinate respiration. They found that the rate of Na^+ influx decreased to zero when matrix pH was 7.4 or higher and suggested that the antiporter could be modulated by internal pH in the same way as the plasmalemmal antiporter. In our study the rate of passive Na^+ influx also fell off markedly when matrix pH was increased above 7.2 in three different protocols (Brierley *et al.*, 1989). It is possible that matrix pH regulates this reaction as suggested by Kapus *et al.* (1989), but it seems more likely that the combination of matrix $[H^+]$ decreasing to far below its K_m and external $[H^+]$ being set well above the K_i would conspire to limit Na^+ influx under their conditions.

Crompton and Heid (1978) measured Na^+ and H^+ distribution in rat heart mitochondria under conditions in which the matrix was acid. They concluded that the activity of the Na^+/H^+ antiport was high relative to other potential pathways for Na^+ flux and that Na^+ distribution is effectively determined by the equilibrium of this exchanger. Their conclusion that the $[Na^+]$ gradient is maintained equal to the electrochemical $[H^+]$ gradient was confirmed under more physiological conditions by continuously monitoring matrix $[Na^+]$ with the fluorescent probe SBFI (Jung *et al.*, 1992). The sequestered probe is readily calibrated using ionophores and shows that respiring mitochondria maintain a steep $[Na^+]$ gradient with $Na^+_{out} > Na^+_{matrix}$. The $[Na^+]$ gradient was nearly equivalent to the $[H^+]$ gradient over a wide range of external $[Na^+]$ for both respiring and nonrespiring mitochondria and changed rapidly in response to changes in matrix pH (Jung *et al.*, 1992). This probe also offers the possibility of measuring the kinetics of Na^+ efflux from the matrix and has been used in phospholipid vesicles to follow the activity of the reconstituted antiport (Garlid *et al.*, 1991).

The mitochondrial Na^+/H^+ antiport is not inhibited by amiloride but is sensitive to a number of amiloride analogues. Kapus *et al.* (1988) found 50% inhibition of Na^+ uptake when external $[Na^+]$ was 15 mM at external pH 7.0 by 60 μM 5-(*N*-4-chlorobenzyl)-*N*-(2′,4′-dimethyl)benzamil and by 60 μM 3′,5′-bis(trifluoromethyl)benzamil. The inhibition by these reagents was competitive with Na^+ and presumed to result from interaction with the external Na^+ site. A number of other analogues were much less effective. In contrast, Brierley *et al.* (1989) found a strong inhibition by benzamil, 4′-fluorobenzamil, N^5,N^5-hexamethleneamiloride and N^5-ethyl-N^5-isopropylamiloride with IC_{50} values from 60 to 100 μM. These measurements were made at elevated pH_0, and the effectiveness of these reagents decreased with declining pH_0. The pH-dependence of these inhibitors is compatible with their accumulation as neutral amines from the alkaline medium and protonation to the reactive form in the more acid matrix. Such concentrating effects complicate interpretation as to whether benzamil binds symmetrically to the mitochondrial antiporter (Brierley *et al.*, 1989).

The mitochondrial Na^+/H^+ antiport shares many properties with the corresponding antiport in the plasma membrane, but differs in other respects. Both antiports have a common external binding site for Na^+, Li^+, and H^+ and both are sensitive to amiloride analogues. The mitochondrial antiport may be less sensitive to these inhibitors and shows a different effectiveness profile. These differences may not be as significant as they first appear, however, because the response to these reagents is highly dependent on assay conditions. The mitochondrial Na^+/H^+ antiport does not seem to be regulated (Garlid, 1988a, but see Kapus *et al.*, 1989), whereas that of the plasma membrane responds to a number of regulators (Fliegel and Frohlich, 1993; Mahnensmith and Aronson, 1984, for example). Garlid (1988a) has proposed that the Na^+-specific antiporters are representatives of a class of proteins that share transport properties, but differ in their regulation by virtue of different regulatory subunits. Progress toward purification of the mitochondrial antiport protein and characterizing its molecular properties (Garlid *et al.*, 1991) should clarify this issue in the near future.

THE Na^+/Ca^+ ANTIPORT

The Na^+/Ca^{2+} antiport accepts Na^+, Li^+, Ca^{2+}, and Sr^{+2} as substrates, is activated by K^+, and has an external regulatory site for Ca^{2+} (see Crompton, 1985 or Brierley and Jung, 1988a for reviews). Transport on this antiport can be inhibited by a number of reagents, among them diltiazem and other Ca^{2+} antagonists and the benzodiazepines. The reaction is also strongly inhibited in heart mitochondria by Ba^{2+} reacting at an external site (Lukacs and Fonyo, 1986). This is of interest because Ba^{2+} inhibits the reaction in liver mitochondria at much higher concen-

trations and appears to react on the matrix side of the antiport (Lukacs and Fonyo, 1985). This suggests that more than one form of the antiport may be present in different tissues.

The role of the mitochondrial Na^+/Ca^{2+} antiport in the regulation of respiration and oxidative phosphorylation has been explored recently (Cox and Matlib, 1993). Activation of the antiport by increasing $[Na^+]$ resulted in decreased matrix $[Ca^{2+}]$ in heart mitochondria and inhibited the rate of NADH production and of phosphorylation. These Na^+-dependent changes were prevented by inhibitors of Na^+/Ca^{2+} antiport. It seems clear that this antiport plays a major role in regulating mitochondrial $[Ca^{2+}]$ and Ca^{2+}-dependent reactions in the matrix. The concept that this antiport must work in concert with the Na^+/H^+ antiport (which provides the Na^+ gradient) is strengthened by the observation that Na^+/H^+ antiport is lacking in plant and yeast mitochondria that do not appear to utilize matrix $[Ca^{2+}]$ as a regulatory ion (Jung an Brierley, 1979; Welihinda *et al.*, 1993). This raises the question as to why liver mitochondria contain a highly active Na^+/H^+ antiport, but show little Ca^{2+} extrusion by the Na^+/Ca^{2+} antiport pathway.

A study from this laboratory (Baysal *et al.*, 1991) has established that the Na^+/Ca^{2+} antiport can also be regulated by matrix $[H^+]$. Heart mitochondria respiring in a KCl medium maintain a large ΔpH and show optimum Na^+-dependent Ca^{2+} efflux only at acid external pH. At neutral external pH the Na^+/Ca^{2+} exchange rate is increased markedly by addition of nigericin, an ionophore that decreases ΔpH to a very low value. When matrix pH was evaluated using cSNARF-1 fluorescence it was found that Na^+/Ca^{2+} antiport shows an optimum activity at pH 7.5–7.6 (Baysal *et al.*, 1991). It appears that the Na^+/Ca^{2+} antiport have regulatory sites on its matrix aspect that respond to $[H^+]$. This brings up the possibility that changes in matrix pH may contribute to the regulation of matrix $[Ca^{2+}]$ and to reactions that respond to this cation.

Early studies of Na^+-dependent Ca^{2+} extrusion (Crompton *et al.*, 1977; Hayat and Crompton, 1982) suggested that the mitochondrial Na^+/Ca^{2+} antiport might promote an electrogenic exchange of three Na^+ for one Ca^{2+} as is seen with the corresponding antiport found in the sarcolemma. However, a thermodynamic analysis (Brand, 1985) concluded that the mitochondrial antiport promoted an electroneutral exchange, and there has been a consensus that the stoichiometry is $2Na^+/Ca^{2+}$ (see Crompton, 1990). A recent isolation and reconstitution study supports this electroneutral stoichiometry (Li *et al.*, 1992). However, the study of Baysal *et al.*, (1991) showed that high rates of Na^+/Ca^{2+} antiport activity are seen in the presence of nigericin and under these conditions ΔpH and the Na^+ gradient (ΔpNa) are very low with elevated $\Delta\Psi$.

Gunter *et al.* (1991) used a null-point approach to show that the Na^+-independent efflux of Ca^{2+} from liver mitochondria could not be a passive $2H^+/Ca^{2+}$ exchange. In collaboration with the Gunters we used cSNARF-1 to monitor matrix pH and fura-2 to follow matrix $[Ca^{2+}]$ simultaneously in a similar analysis of nNa^+/Ca^{2+} antiport in heart mitochondria (Baysal *et al.*, 1994). Net Ca^{2+} flux across the inner membrane of respiring heart mitochondria was evaluated under conditions in which virtually all Ca^{2+} movement was across the Na^+/Ca^{2+} antiport. If this antiport promotes a passive electroneutral exchange of Ca^{2+} for $2Na^+$, the Ca^{2+} gradient should be equal to the square of the Na^+ gradient at equilibrium. Because the mitochondrial Na^+/H^+ antiport equilibrates the Na^+ and H^+ gradients, the Ca^{2+} gradient should also equal the square of the H^+ gradient. In a series of more than 20 determinations at different matrix $[Ca^{2+}]$, different ΔpH, and varying membrane potential ($\Delta\psi$), it was found that Ca^{2+} is transported out of the mitochondrion against gradients from 15- to 100-fold greater than the value predicted for passive electroneutral exchange (Baysal *et al.*, 1994). It was concluded that the observed gradients are too large to be sustained by $2Na^+/Ca^{2+}$ exchange, but are compatible with the electrogenic $3Na^+/Ca^{2+}$ antiport.

These observations caused us to re-examine the report of Brand (1985) and the experimental basis for the conclusion that the nNa^+/Ca^{2+} antiport is electroneutral. Brand (1985) followed external $[Ca^{2+}]$ with a Ca^{2+}-electrode and allowed heart mitochondria respiring in the presence of ruthenium red to extrude Ca^{2+} on the nNa^+/Ca^{2+} antiport to a steady state. A large ΔpH (almost 1 pH unit, acid matrix) and a corresponding ΔpNa^+ were measured after nigericin addition, and a low matrix volume and high matrix $[Ca^{2+}]$ (500 μM) were calculated. Under these conditions addition of the exogeneous electroneutral exchanger A23187 should have produced a measurable change in external $[Ca^{2+}]$ if $n = 3$ and none if $n = 2$ for the nNa^+/Ca^{2+} antiport. No displacement was seen, and it was concluded that the antiport was electroneutral (Brand, 1985).

Our reassessment of this protocol (Jung *et al.*, in preparation) using fluorescent probes to monitor matrix pH and $[Ca^{2+}]$ reveals that (a) there is essentially no ΔpH (or ΔpNa^+) after addition of nigericin to respiring heart mitochondria suspended in a KCl medium, (b) the steady-state matrix $[Ca^{2+}]$ is considerably lower than external $[Ca^{2+}]$ and this gradient depends on $\Delta\psi$ and (c) in contrast to Brand's finding, dissipation of $\Delta\psi$ with an uncoupler or addition of BrA23187 (as an exogenous electroneutral exchanger) results in Ca^{2+} influx and an equilibration of the $[Ca^{2+}]$ gradient. These results are compatible with an electrogenic antiport of at least three Na^+ for one Ca^{2+}, but not with electroneutral antiport. It seems clear that the $\Delta\psi$ component of protonmotive force can be utilized to extrude Ca^{2+} in a Na^+-dependent reaction when ΔpH is zero or nearly so (Jung *et al.*, in preparation). It is also apparent that the methodology available to Brand in 1985 was inadequate to deal with the question posed and produced misleading results. It is not clear why the reconstituted activity (Li *et al.*, 1992) appears to be electroneutral.

A recent study of the modulation of the sarcolemma Na^+/Ca^{2+} antiport by pH (Khananshvili and Weil-Maslansky, 1994) concluded that at low pH when the transporter is protonated, the exchange of Na^+ for Ca^{2+} was nearly voltage insensitive whereas it became voltage-responsive at higher pH. It seems possible that the mitochondrial antiport may show a similar ability to promote either electroneutral or electrophoretic exchange as a function of its regulatory state, but this remains to be demonstrated. At present the evidence appears to favor an electrophoretic exchange of at least $3Na^+/Ca^{2+}$ on the mitochondrial antiport. There would seem to be advantages to such a stoichiometry for Ca^{2+} extrusion because mitochondria *in situ* appear to maintain most of their protonmotive force as $\Delta\psi$ with only a small ΔpH (see the discussion in Baysal *et al.*, 1994).

THE K^+/H^+ ANTIPORT

This exchanger is latent in mitochondria as isolated and can be unmasked by osmotic swelling (Garlid, 1980) or by depletion of mitochondrial Mg^{2+} (see Garlid, 1988b or Brierley and Jung, 1988b for reviews). It is characterized by its ability to transport Li^+, Na^+, Rb^+, and Cs^+, in addition to K^+ in exchange for H^+ and its sensitivity to quinine (Nakashima and Garlid, 1982), timolol, and other similar amines. There are indications that Mg^{2+} efflux from mitochondria may also be supported by this antiport (Diwan, 1988; Brierley *et al.*, 1988). The K^+/H^+ exchange promoted by this antiport is strongly inhibited by DCCD when the inhibitor is reacted with Mg^{2+}-depleted mitochondria under hypotonic conditions (Martin *et al.*, 1986). It is of interest that DCCD only partially ihibits $^{42}K^+/K^+$ exchange promoted by this antiport under conditions that completely block K^+/H^+ exchange (Brierley *et al.*, 1984). It appears that DCCD selectively blocks binding sites or a subunit for H^+ transport on the antiporter that does not participate in $^{42}K^+$ or $^{86}Rb^+$ exchange (Garlid *et al.*, 1986; 1986; Kakar *et al.*, 1989; Jezek *et al.*, 1990). The reaction with DCCD is irreversible and has been used to estimate that rat liver mitochondria contain about 8 pmol mg^{-1} of the antiporter with a turnover of 700 sec^{-1} (Martin *et al.*, 1986). Kaker *et al.*, (1989) used ^{14}C-DCCD to track the 82-kDa antiport protein during detergent extraction and reconstitution into liposomes. This approach was extended by Li *et al.* (1990) to the production of antibodies and purification of the reconstitutively active unmodified protein to apparent homogeneity. The properties of this high-turnover antiport are discussed in detail in Li *et al.* (1990).

The activity of the K^+/H^+ antiport can be followed using osmotic swelling or $^{42}K^+/K^+$ exchange to measure K^+ influx and by osmotic contraction of mitochondria swollen in K^+ salts to follow K^+ extrusion. The movement of K^+ into or out of proteoliposomes is followed conveniently using the fluorescent probe PBFI (Jezek *et al.*, 1990). The K^+-dependent ejection of H^+ from mitochondria can be followed with a glass electrode (Beavis and Garlid, 1990) or by using a fluorescent pH indicator such as BCECF to monitor changes in matrix $[H^+]$ (Brierley and Jung, 1990). The efflux of K^+ from Mg^{2+}-depleted mitochondria is stimulated at alkaline pH (Bernardi and Azzone, 1983). Studies of H^+ movement promoted by the K^+/H^+ antiport indicate that unlike the Na^+/H^+ antiport, external $[H^+]$ does not compete with K^+ for binding to the K^+/H^+ exchanger. Beavis and Garlid (1990) showed that the K^+/H^+ antiport is regulated by matrix $[H^+]$ as well as by matrix $[Mg^{2+}]$. At constant external pH the rate of swelling of mitochondria increases as matrix pH is increased. BCECF fluorescence shows a ΔpH of about 0.4 units (matrix acid) in the acetate medium and a further acidification when Mg^{2+} is depleted by $Mg^{2+}/2H^+$

exchange on A23187 (Brierley *et al.*, 1991). These changes in matrix pH are sufficient to inhibit K^+/H^+ antiport unless steps are taken to increase matrix pH to above 7.0. The optimum matrix pH varies with external pH in these protocols (Brierley *et al.*, 1991), a result that suggests that the rate of K^+/H^+ antiport is also influenced by $[H^+]$ at the external surface or by the ΔpH maintained.

The sensitivity of the K^+/H^+ antiport to $[Mg^{2+}]$ led Garlid to the "carrier-brake" hypothesis for mitochondrial volume regulation (Garlid, 1980, 1988b). This calls for negative regulation of the 82-kDa carrier by interaction of matrix Mg^{2+} and H^+ with an allosteric domain. A K_i of the antiport of 200–400 μM $[Mg^{2+}]$ and 50–90 μM $[Ca^{2+}]$ has been reported when a ΔpH of 0.3–0.4 (acid matrix) is assumed (Garlid, 1988b). Estimates of matrix $[Mg^{2+}]$ using the fluorescent probe furaptra range from 0.5 mM (Jung *et al.*, 1990) to from 0.8 to 1.5 mM (Rutter *et al.*, 1990). A recent reassessment of the apparent K_d for Mg-furaptra sequestered in the mitochondrial matrix has caused us to revise our value for matrix $[Mg^{2+}]$ upward to 0.7–0.9 mM (Jung *et al.*, in preparation). Matrix $[Mg^{2+}]$ can be increased or decreased by either transport reactions or by ligand availability (Jung *et al.*, 1990; Rutter *et al.*, 1990). Matrix $[Mg^{2+}]$ is decreased reversibly to 40% of the initial value by extensive hypotonic swelling of heart mitochondria, and this would put the value near 300 μM (Jung *et al.*, 1990). This level of matrix $[Mg^{2+}]$ depletion would permit at least a partial activation of K^+/H^+ antiport as a result of extensive swelling.

In the past we have questioned whether the conditions required to demonstrate K^+/H^+ antiport in isolated mitochondria, i.e., elevated pH, hyptonic conditions, and extensive depletion of Mg^{2+}, would ever be met in mitochondria *in vivo* (Brierley *et al.*, 1984; Jung and Brierley, 1986; Brierley and Jung, 1988b). In addition, it is clear that the conditions used to activate K^+/H^+ antiport in intact mitochondria also activate an anion channel (Garlid and Beavis, 1986) and one or more uniport pathways for monovalent cations (see the discussion to follow). The relationship of these components to each other and their physiological roles remain to be clarified. However, there can now be no doubt that mitochondria contain an 82-kDa K^+/H^+ antiport protein of well-defined properties and that such an antiport is a physiological necessity for mitochondria *in situ* (Garlid, 1988b). It seems likely that as more is learned about the molecular properties of this antiport and its regulatory features, some of the apparent inconsistencies may be resolved.

MONOVALENT CATION UNIPORTS

A rationale for the presence of monovalent cation uniports in the mitochondrial membrane is difficult to visualize. It has been known for some time that pathways are present for the electrophoretic uptake of Na^+ and K^+ (see Brierley, 1978, 1983 for reviews). Such uniports permit rapid uptake of these and other cations when $\Delta\psi$ is elevated. Because of the opening of such pathways would be fatal for chemiosmotic mitochondria under most circumstances, we suggested that such uniports could be voltage-gated or otherwise regulated to remain closed except under unusual conditions. Brown and Brand (1986) showed that cation permeability increased in a nonohmic way with increasing $\Delta\psi$, and the possible presence of nonspecific leak pathways for cations was raised. However, the sensitivity of Na^+ and Li^+ uniport to very low levels of $[Mg^{2+}]$ (Brierley *et al.*, 1978) and K^+ and Na^+ uniport at elevated pH to low levels of DCCD (Jung *et al.*, 1980) suggested that specific proteins could be involved. Garlid *et al.* (1989) have presented criteria for distinguishing diffusive leak pathways from specific uniport by analysis of the plots of flux vs $\Delta\psi$. The recent demonstration that mitochondria contain an ATP- and glibenclamide-sensitive K^+ channel (Inoue *et al.*, 1991) and the finding that low levels of ruthenium red block both Na^+ and K^+ uniport activity (Kapus *et al.*, 1990) give strong support to the concept that specific pathways for these cation movements can be opened in the membrane. The physiological role of such uniports remains to be clarified, however.

Diwan *et al.* (1988, 1990) have partially purified a fraction from a quinine affinity column that promotes uniport of K^+ and Tl^+ when reconstituted in liposomes and is labeled by ^{14}C-DCCD under conditions in which DCCD inhibits K^+ influx into mitochondria. Patch clamp analysis has shown the presence of two classes of ion channels in these fractions, a 40- and 140-pS conductance, in high K^+ media. A 57-kDa protein from this fraction appears responsible for the 40-pS conductance (Paliwal *et al.*, 1992). This protein shows little selectivity for K^+ over Na^+.

Inoue *et al.* (1991) used the patch clamp to detect a K^+ channel in mitochondrial membranes that is inhibited by ATP and by glibenclamide, an inhibitor

of ATP-sensitive channels in the plasma membrane. A purified membrane fraction has been reconstituted that promotes electrophoretic K^+ movement in liposomes and channel activity in planar bilayers (Paucek *et al.*, 1992). This 30-pS channel is selective for K^+ over Na^+, has a K_m of 32 mM [K^+], and does not appear to be voltage regulated. It is inhibited by ATP and ADP in the presence of divalent cations (K_i values of 43 μM ATP and about 280 μM ADP). The K_i for Mg^{2+} in the presence of high ATP was found to be 80 μM (Paucek *et al.*, 1992). The channel is inhibited by glibenclamide in the absence of Mg^{2+} with a K_i of 65 nM and by DCCD. Paucek *et al.* (1992) conclude that the properties of this mitochondrial K^+ channel are remarkably like those of ATP-sensitive channels in the plasma membrane of cells from heart, skeletal muscle, and pancreatic β-cells and that the mitochondrial and plasmalemmal K_{ATP} channels may belong to the same gene family.

Beavis *et al.* (1993) have conducted an extensive study of K^+ uniport in intact mitochondria and find the activity to have little pH dependence, but to be highly sensitive to adenine nucleotides. The uptake of K^+ was increased by *N*-ethylmaleimide, only marginal effects were seen with glibenclamide, and the effects of nucleotide analogues, such as Cibacron Blue, suggest that there are at least three different regulatory sites for such reagents. It is proposed that endogenous compounds may react with regulatory sites on the uniport to permit K^+ flux in the presence of endogenous levels of adenine nucleotides (Beavis *et al.*, 1993).

A latent K^+ uniport in mitochondria appears to be unmasked by treatment with both A23187 and EDTA (Wehrle *et al.*, 1976; Bernardi *et al.*, 1989). This treatment depletes matrix divalent cations but also removes surface bound Mg^{2+}. Bernardi *et al.* (1989) suggest that Mg^{2+} depletion opens several ionic conductance pathways that contribute to the regulation of mitochondrial volume. A Na^+ and Li^+ uniport pathway is also opened in mitochondria treated with EDTA (Brierley *et al.*, 1978; Bernardi *et al.*, 1990). This pathway is inhibited competitively by [Mg^{2+}] in the nanomolar range and shows properties consistent with a gated pore in that it is active only when $\Delta\psi$ exceeds 150 mV (Bernardi *et al.*, 1990). This pathway would almost certainly remain closed in the presence of endogenous [Mg^{2+}] unless there are as yet undiscovered regulatory features of the channel that might overcome this inhibition. A very rapid K^+ uniport flux is also unmasked by removal of surface Mg^{2+} (Nicolli *et al.*, 1991).

Both the Na^+ and the K^+ uniport that are induced by the depletion of surface Mg^{2+} are inhibited by low levels (40 nM) of ruthenium red (Kapus *et al.*, 1990). These authors raise the possibility that monovalent cation flux can be mediated by alterations to the ruthenium red-sensitive Ca^{2+} uniport, a proposal advanced earlier by Jung and Brierley (1984). Ruthenium red reacts with a number of proteins and Mg^{2+} removal may uncover previously unreactive sites for its binding. However, the low levels of both Mg^{2+} and ruthenium red required to block monovalent cation uniport activity (Nicolli *et al.*, 1991; Kapus *et al.*, 1990) suggest that specific proteins may be involved in monovalent cation conductance as opposed to a generalized diffusive leak of cations. The presence of the ATP- and glibenclamide-sensitive K^+ uniport in yeast mitochondria (Manon and Guerin, 1993) suggests that this component is widespread.

The physiological role of such uniports is not yet clear, nor are the regulatory mechanisms that control their activity. As we have discussed before (Brierley and Jung, 1988b), it is possible that some of the harsher conditions used to "unmask" such transport components are actually modifying transporters that normally play quite different roles in unmodified mitochondria. There is precedence for alterations of isolated transporters that change the nature of their activity (Stappen and Kramer, 1993, for example).

It has been suggested that regulated mitochondrial cation uniports may be present in mitochondria in order to control substrate uptake, Halestrap (1989) and Halestrap *et al.* (1986) have presented a specific model of how this might occur in response to increased matrix [Ca^{2+}], for example. Other possibilities that have been advanced are that the uniports support volume increases during mitochondrial biogenesis or that they contribute to thermogenesis by futile K^+ cycling when conditions warrant (Paucek *et al.*, 1992). It is also possible that cation uniport pathways are present to prevent dielectric breakdown under conditions that elevate $\Delta\psi$ to very high levels.

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