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Mechanism and Regulation of the Mitochondrial ATP-Mg/P_i Carrier

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The mitochondrial ATP-Mg/P_i carrier functions to modulate the matrix adenine nucleotide pool size (ATP + ADP + AMP). Micromolar Ca^{2+} is required to activate the carrier. Net adenine nucleotide transport occurs as an electroneutral divalent exchange of ATP-Mg^{2-} for HPO_4^{2-}. A steady-state adenine nucleotide pool size is attained when the HPO_4^{2-} and ATP-Mg^{2-} matrix/cytoplasm concentration ratios are the same. This means that ATP-Mg^{2-} can be accumulated against a concentration gradient in proportion to the [HPO_4^{2-}] gradient that is normally maintained by the P_i/OH^- carrier. In liver, changes in matrix adenine nucleotide concentrations that are brought about by the ATP-Mg/P_i carrier can affect the activity of adenine nucleotide-dependent enzymes that are in the mitochondrial compartment. These enzymes in turn contribute to the overall regulation of bioenergetic function, flux through the gluconeogenesis and urea synthesis pathways, and organelle biogenesis. The ATP-Mg/P_i carrier is distinct from other mitochondrial transport systems with respect to kinetics and to substrate and inhibitor sensitivity. It is the only carrier regulated by Ca^{2+}. This carrier is present in kidney and liver mitochondria, but not in heart.

KEY WORDS: Mitochondria; ATP-Mg/P_i carrier; adenine nucleotide; calcium; liver; kidney; transport; phosphate; magnesium.

INTRODUCTION

The mitochondrial ATP-Mg/P_i carrier transports adenine nucleotide in exchange for phosphate across the inner mitochondrial membrane and thus regulates the matrix adenine nucleotide total pool size (ATP + ADP + AMP). The discovery of the ATP-Mg/P_i carrier in liver mitochondria was prompted by the need to explain how changes in the matrix adenine nucleotide content could occur *in vivo* as it does in many physiological situations (reviewed in Aprille, 1988a,b). One of the most dramatic examples of adenine nucleotide pool size regulation, indeed the one that initiated the search for the ATP-Mg/P_i carrier, is of central importance in the newborn mammal's adaptation to independent aerobic life (Aprille, 1986, 1990). In newborn liver the mitochondrial adenine nucleotide content increases 3–4-fold within a few hours after birth, due to net uptake of adenine nucleotide from the cytoplasm; the adenine nucleotide content of the liver as a whole does not change. This was reported by several laboratories in the late 1970s (reviewed in Aprille, 1986). The only adenine nucleotide carrier known at the time was the ADP/ATP translocase; however, the typical one-for-one exchange of ATP and ADP that occurs via ADP/ATP translocase obviously cannot bring about any net increase or net decrease in the total adenine nucleotide content of mitochondria. In the studies that ensued, the properties of net adenine nucleotide transport were found to be so different from the ADP/ATP translocase that a new anion carrier was postulated (Pollak and Sutton, 1980; Aprille and Asimakis, 1980; Austin and Aprille, 1984). The new adenine

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nucleotide carrier was descriptively named the ATP-Mg/P_i carrier because net transport of adenine nucleotide normally occurs as a reversible counter-exchange of ATP-Mg^{2-} for HPO_4^{2-} (Aprille, 1988a).

The counterexchange mechanism had been hypothesized in broad outline by 1984 (Austin and Aprille, 1983; 1984). Studies since then have allowed us to paint a more complete picture of the mechanism, regulation, and physiological importance of this carrier. Several previous reviews have given attention to the metabolic role served by variations in the matrix adenine nucleotide pool size that are regulated by the ATP-Mg/P_i carrier (Aprille, 1986, 1988a,b, 1990). This review will briefly summarize functional aspects of the carrier and then concentrate on what is known about the mechanism, regulation, and distribution of the carrier itself, with emphasis on recent findings.

OVERVIEW OF FUNCTIONAL ASPECTS OF THE ATP-Mg/P_i CARRIER IN CELLULAR CONTEXT

The ATP-Mg/P_i carrier functions to modulate the matrix adenine nucleotide (ATP + ADP + AMP)

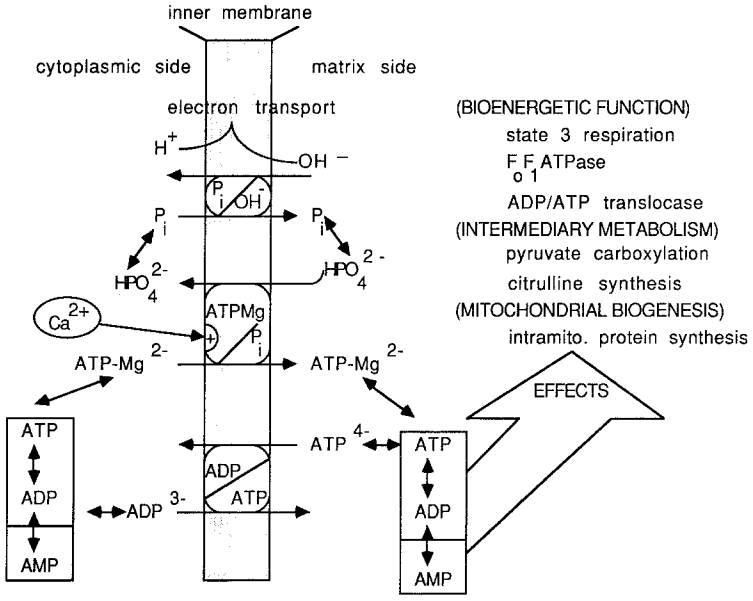

Fig. 1. Key features of the mechanism, regulation, and function of the ATP-Mg/P_i carrier. The diagram is set up arbitrarily to illustrate the events leading to an increase in the matrix adenine nucleotide pool size by exchange of cytoplasmic ATP-Mg^{2-} for matrix HPO_4^{2-}. The ATP-Mg/P_i carrier is reversible, so that exchanges in the opposite direction would account for a regulated decrease in the matrix adenine nucleotide pool size. Starting at the top of the diagram, notice that electron transport establishes ΔpH, which in turn establishes a $[P_i]$ gradient via the P_i/OH carrier. The $[P_i]$ gradient drives the uptake of adenine nucleotide into the matrix against a concentration gradient as external ATP-Mg^{2-} exchanges for matrix HPO_4^{2-} via the ATP-Mg/P_i carrier, whenever a Ca^{2+}-activating signal is present. Notice that the $[P_i]$ gradient is rapidly reset by the continuous activity of the P_i/OH carrier, and that the maximum possible [ATP-Mg^{2-}] gradient will thus be proportional to the steady-state $[P_i]$ gradient. Transported [ATP-Mg^{2-}] is rapidly equilibrated into the ATP + ADP (and AMP) pools (boxes), and the absolute concentration of [ATP-Mg^{2-}] is determined by ATP/ADP ratios as well as by total adenine nucleotide pool size in each compartment. ATP/ADP ratios are established independently by relative rates of ATP synthesis and hydrolysis in each compartment, and the ratio differs on each side of the inner membrane due to the electrogenic activity of ADP/ATP translocase. Specific matrix reactions that are affected by increases/decreases in the matrix nucleotide pool size are listed to the right in the diagram, below the relevant cellular metabolic function in parentheses.

pool size (Fig. 1). The carrier is inactive in the absence of Ca^{2+} and is activated by micromolar Ca^{2+}. The main physiological substrates for transport are ATP-Mg^{2-} and HPO_4^{2-}. Net transport of adenine nucleotide occurs when ATP-Mg^{2-} on one side of the inner membrane exchanges for HPO_4^{2-} on the other side (Fig. 1). Transport is completely reversible and nonproductive exchanges (ATP-Mg^{2-} for ATP-Mg^{2-} and HPO_4^{2-} for HPO_4^{2-}) also occur. It is the numerical sum of ongoing exchanges of ATP-Mg^{2-} for HPO_4^{2-} in both directions that determines any net change in the matrix adenine nucleotide content. For example, net loss occurs when matrix ATP-Mg^{2-} exchanges for external HPO_4^{2-} in excess of the rate that external ATP-Mg^{2-} exchanges for matrix HPO_4^{2-}. ATP-Mg^{2-} and HPO_4^{2-} are competitive substrates, so that a steady-state adenine nucleotide pool size is attained when the ratio of [ATP-Mg^{2-}] to [HPO_4^{2-}] is the same on both sides of the membrane. The exchange of ATP-Mg^{2-} for HPO_4^{2-} on the ATP-Mg/P_i carrier does not measurably alter the transmembrane phosphate concentration gradient in energized mitochondria, because the phosphate gradient is continuously reestablished by the much faster P_i/OH carrier in relation to ΔpH (Joyal and Aprille, 1992). This means that ATP-Mg^{2-} can be taken up against a concentration gradient that is maintained in proportion to the steady-state HPO_4^{2-} concentration gradient across the inner membrane (Fig. 1). When the carrier is active, the direction of any net ATP-Mg^{2-} transport is determined principally by the [ATP-Mg^{2-}] gradient relation to the [HPO_4^{2-}] gradient. The matrix adenine nucleotide pool size is thus indirectly sensitive to energy status via changes in the steady-state ΔpH or ATP/ADP ratios on either side of the membrane, because such changes affect the relative concentrations of HPO_4^{2-} and ATP-Mg^{2-} (Fig. 1).

The ATP-Mg/P_i carrier is quite slow compared to the reactions for ATP synthesis and hydrolysis that determine ATP/ADP ratios in the cytoplasm and matrix. Therefore, ATP-Mg^{2-} transported by this mechanism distributes quickly into the ATP + ADP (and eventually also AMP) pools (Fig. 1). The end result of net uptake or loss of ATP-Mg^{2-} is a change in the absolute concentrations of both ATP and ADP without necessarily changing the ATP/ADP ratios in either compartment. Because the mitochondrial compartment occupies a small fraction of total cell volume, large changes in the matrix adenine nucleotide content result in relatively minor changes in the cytoplasmic adenine nucleotide concentrations (Aprille, 1988a).

Changes in the matrix adenine nucleotide concentrations that are brought about by the ATP-Mg/P_i carrier can affect adenine nucleotide-sensitive enzymes localized to the matrix, either through mass action of adenine nucleotides required as enzyme substrates or possibly through allosteric action. The list of reactions shown to be affected by variation of the matrix adenine nucleotide concentrations in liver mitochondria includes (Fig. 1) pyruvate carboxylation, citrulline synthesis, F_0F_1ATPase, state 3 respiration, and ADP/ATP translocase (see Fig. 3 in Aprille, 1988a) and intramitochondrial protein synthesis (unpublished results). Reactions not affected include uncoupled respiration (Asimakis and Aprille 1980a,b) and intramitochondrial RNA synthesis (unpublished results).

In aerobic hepatocytes the steady-state [ATP-Mg^{2-}] gradient across the inner mitochondrial membrane is poised for net uptake of ATP-Mg^{2-} from the cytoplasm into mitochondria in response to a Ca^{2+} signal that activates the carrier (Dransfield and Aprille, 1993). The resulting increase in matrix adenine nucleotide concentration can stimulate the activity of intramitochondrial enzymes, with the potential to contribute thereby to hormone regulation of cellular metabolic activities such as gluconeogenesis (via stimulation of pyruvate carboxylase) and urea synthesis (via stimulation of carbamoyl phosphate synthetase) (Aprille, 1986, 1988a). In contrast, when cytoplasmic [ATP-Mg^{2-}] is far below the K_m for the ATP-Mg/P_i carrier (as might occur in hypoxia), net uptake of adenine nucleotide into the mitochondrial compartment cannot occur and net loss may occur instead if the carrier is activated by Ca^{2+} (Dransfield and Aprille, 1993). Cellular homeostasis may thus be protected during hypoxia because a hormonal call for stepped-up metabolism can be "resisted." Any cytoplasmic ATP that is available (e.g., from glycolysis) can be preserved for activities (e.g., Na/K ATPase, Ca^{2+} ATPase) that are most essential for cell survival during hypoxic episodes. In hepatocytes, the ATP-Mg/P_i carrier thus has a role in prioritizing cellular needs above systemic demands for energy-expensive metabolism.

In general, the ATP-Mg/P_i carrier is envisioned to help match cellular energy demands with energy supply by regulating adenine nucleotide concentrations in the mitochondrial compartment where key metabolic enzymes are permanently sequestered. This mechanism for regulation by compartmentation

has a response time for reversible changes in metabolic flux that is slower than allosteric regulation and faster than regulation by enzyme synthesis/degradation. Modulation of the matrix adenine nucleotide content by the ATP-Mg/P_i carrier thus contributes to the transient ebb and flow of metabolic activity that occurs in response to continuously varying demands. New data suggest that changes in the matrix adenine nucleotide content, as occurs in newborn liver, have the added effect of regulating mitochondrial biogenesis. This effect has the potential to adapt the *capacity* for aerobic energy metabolism to match energy requirements for cellular metabolic activity.

STANDARD ASSAYS FOR ATP-Mg/P_i TRANSPORT

Two complementary approaches have been used to study ATP-Mg/P_i transport activity in isolated mitochondria. One approach is to measure the initial velocities (30–90 sec) of the separate unidirectional components (influx and efflux) of transport. The other is to measure net transport (loss or uptake), followed over longer times, usually 10–15 min. The same standard assay conditions are used for both kinds of assays. The medium is 225 sucrose, 10 mM KCl, 2 mM K_2HPO_4-KH_2PO_4, 5 mM $MgCl_2$, 10 mM Tris-Cl, and 5 mM each glutamate and malate; temperature is 30°C (Austin and Aprille, 1984). Ca^{2+} sufficient for maximal activation is present as a contaminant of the $MgCl_2$ reagent; if Mg^{2+} is varied, Ca^{2+} must be added independently (Nosek *et al.*, 1990). For unidirectional influx and efflux, external ATP is 1 mM, which is just sufficient to maintain the steady-state matrix adenine nucleotide content of adult liver mitochondria (11–14 nmol ATP + ADP + AMP per mg mitochondrial protein). To measure unidirectional influx kinetics, the external ATP is ^{14}C trace-labeled and the amount of isotope that is taken by the mitochondria is followed between 30 and 90 sec. To measure efflux kinetics, the matrix adenine nucleotides are uniformly labeled in a preincubation wtih ^{14}C-ADP (Austin and Aprille, 1984), and the amount of isotope remaining in the mitochondria is measured between 30 and 90 sec. For both influx and efflux, 5 μM carboxyatractyloside is added to prevent equilibration of the radiolabeled adenine nucleotides between the matrix and external pools via ADP/ATP translocase.

To measure the rate of net adenine nucleotide transport (uptake or loss), mitochondria are incubated in the same standard medium as for unidirectional flux, except that it is not necessary to include carboxyatractyloside and the external ATP concentration is usually varied. As noted above, 1 mM ATP is steady-state; lower or higher concentrations result, respectively, in net loss from or net uptake into the mitochondria. To assess the net transport, the chemical amounts of ATP, ADP, and AMP are measured enzymatically in neutralized acid extracts of the mitochondria which have been separated from the incubation medium by centrifugation.

The criteria for attributing transport activity to the ATP-Mg/P_i carrier is inhibition by EGTA and no inhibition by carboxyatractyloside. When testing a particular condition for an effect on transport, care must be taken to avoid inadvertent confounding changes in matrix volume, pH, ΔpH, ATP/ADP ratios, or other factors that can affect the concentrations of ATP^{4-}, ADP^{3-}, Mg^{2+}, Ca^{2+}, and HPO_4^{2-} in the matrix or medium.

As an aside, investigators who study isolated liver (or kidney) mitochondria in other contexts (such as oxidative phosphorylation, biogenesis, or intermediary metabolism) should be aware that matrix adenine nucleotides will be rapidly released to the medium via the ATP-Mg/P_i carrier in incubations that contain phosphate when ATP-Mg is not present or in those that contain uncouplers. Loss of matrix adenine nucleotides may have profound effects on the matrix reactions of interest, as noted above in the Introduction (Aprille, 1988a).

KINETIC CHARACTERISTICS, SUBSTRATES, AND INHIBITORS

Compared to most other anion carriers, the activity of the ATP-Mg/P_i carrier is relatively slow in intact liver mitochondria. Kinetic characteristics are summarized in Table I. The specific activity of unidirectional ATP-Mg^{2-} influx and efflux is usually about 1.7–1.8 nmol/min per mg mitochondrial protein in the steady-state under standard assay conditions. The V_{max} for ATP-Mg^{2-} influx and efflux is about 4–5 nmol/min per mg mitochondrial protein. These relatively slow rates in intact mitochondria are probably due to low abundance of this carrier, but it is possible that the rates actually reflect a slow rate constant.

Table I. Properties of ATP-Mg/P_i Transport Activity in Isolated Adult Liver and Kidney Mitochondria[a]

	Liver		Kidney	
	Influx	Efflux	Influx	Efflux
Apparent K_m for ATP-Mg (mM)[b]	2.7	10	1.7	8.6
Apparent V_{max}[c]	3.8[e]	4.7	3.5	3.6
Specific activity[a,d]	1.7	1.8	1.1	1.2
Mersalyl inhibition (%)[f]	68	73	84	92
NEM inhibition(%)[g]	44	60	—	—
$[Ca^{2+}]_{free}$ (μM) for half maximum activation	~1.3	~1.2	~1.1	~1.1

[a] Carboxyatractyloside was present in all assays, and activity was EGTA-sensitive. Standard adenine nucleotide influx and efflux assays were used to measure separate unidirectional transport rates as described in the text. Properties for liver mitochondria are typical values from the data of several references as cited in the text. Properties for kidney mitochondria are average values from Hagen *et al.*, 1993.
[b] Determined in the presence of phosphate, so "actual" K_m for ATP-Mg must be lower.
[c] Units are nmol adenine nucleotide/min/mg mitochondrial protein.
[d] Standard conditions with external ATP at 1 mM, external P_i at 2 mM.
[e] Typical value for recent experiments; one much higher value (8.33 mM) was reported in early work (Austin and Aprille, 1984).
[f] 100 μM for liver, 200 μM for kidney produced maximum inhibition.
[g] 200 μM produced maximum inhibition.

The apparent K_m values for influx of ATP-Mg^{2-} and HPO_4^{2-} are in the millimolar range. The K_m values are consistent with the role of this carrier in regulating subcellular compartmentation of adenine nucleotides, because ATP-Mg^{2-} and HPO_4^{2-} are in the millimolar concentration range in the cytoplasm and mitochondrial matrix. The K_m values for ATP-Mg^{2-} in Table I were determined under standard conditions, with 2 mM HPO_4^{2-} present in the external medium and a typical matrix [HPO_4^{2-}] of about 8 mM. Since HPO_4^{2-} and ATP-Mg^{2-} are competitive, the actual K_m for ATP-Mg^{2-} transport must be somewhat lower, but certainly still in the millimolar range. HPO_4^{2-} transport on the ATP-Mg/P_i carrier cannot be measured directly because inhibitors such as NEM and mersalyl that inhibit the P_i/OH carrier also inhibit this carrier. However, indirect methods such as varying the concentration of phosphate to induce adenine nucleotide efflux, and using phosphate to compete with ATP-Mg^{2-} influx, suggest that the K_m for HPO_4^{2-} is about half the K_m for ATP-Mg^{2-}.

When external [ATP-Mg^{2-}] is higher or lower than 1 mM, net uptake or loss of matrix adenine nucleotide occurs at a rate that is dependent on the direction and magnitude of the transmembrane [ATP-Mg^{2-}] gradient (Austin and Aprille, 1983; Joyal and Aprille, 1992). For example, in the complete absence of external ATP-Mg^{2-}, matrix adenine nucleotides are released by exchange with HPO_4^{2-} at an initial rate of about 0.5–1 nmol/min per mg mitochondrial protein, resulting in complete depletion after 10–12 min. Conversely, if the external ATP-Mg^{2-} is set at 4 mM, net uptake occurs and the matrix adenine nucleotide pool size nearly doubles after about 10–12 min. Net changes of these magnitudes and with similar time courses are elicited by a Ca^{2+}signal in intact cells under particular conditions (Dransfield and Aprille, 1993).

Mg^{2+} is required in stoichiometric amounts for ATP transport on this carrier. Atomic absorption spectrophotometry shows that the matrix Mg content increases in proportion to net ATP uptake (Aprille *et al.*, 1993). There is also indirect evidence for the transport of Mg^{2+} along with ATP^{4-} (as ATP-Mg^{2-}) (Nosek and Aprille, 1992; Joyal and Aprille, 1992). Ca^{2+} and Mn^{2+} can substitute for Mg^{2+} in this role, but since the divalent cation requirement is stoichiometric with ATP in the mM range, Mg^{2+} must be the operative cation in cells (Nosek and Aprille, 1992). Mg^{2+} is not required for HPO_4^{2-} transport (Nosek and Aprille, 1992; Joyal and Aprille, 1992).

Besides ATP-Mg^{2-} and HPO_4^{2-} the only other transport substrates known at this time are $HADP^{2-}$ (Nosek and Aprille, 1992; Joyal and Aprille, 1992) and PP_i-Mg^{2-} (unpublished results). Mg^{2+} is not required at all for $HADP^{2-}$ transport. $HADP^{2-}$ competes poorly with ATP-Mg^{2-} (Nosek and Aprille, 1992), so *in vivo*, ATP-Mg^{2-} and HPO_4^{2-} probably are the physiologically relevant transport species under most circumstances (Dransfield and Aprille, 1993). ATP is not hydrolyzed to ADP by the transport event, at least not in the direction of efflux (Nosek and Aprille, 1992).

The hypothesis that anion transport by the ATP-

Mg/P_i carrier is divalent and electroneutral was proven just recently by experiments that compared the equilibrium distribution ratios of monovalent and divalent phosphate and divalent ATP-Mg in the steady-state (Joyal and Aprille, 1992). An electroneutral exchange of ATP-Mg^{2-} for HPO_4^{2-} predicts a distribution ratio that is similar for these two substrates and that is twice ΔpH; this result is obtained under every condition tested. Most other types of exchanges are explicitly ruled out, including electrogenic exchange of ATP-Mg^{2-} for $H_2PO_4^{1-}$, electroneutral exchanges such as $HATPMg^{1-}$ for $H_2PO_4^{1-}$ or two $H_2PO_4^{1-}$ for one ATP-Mg^{2-}, and proton-compensated HPO_4^{2-} exchange for ATP-Mg^{2-}. An electroneutral divalent exchange is also supported by the equilibrium distribution of $HADP^{2-}$, determined under special conditions where ADP is the only adenine nucleotide substrate available for transport (Joyal and Aprille, 1992). Transport of divalent phosphate is consistent with data showing that externally added monofluorophosphate (divalent analogue) but not difluorophosphate (monovalent analogue) stimulates adenine nucleotide efflux from the matrix (Nosek and Aprille, 1992).

ATP-Mg/P_i carrier activity is inhibited by sulfhydryl reagents (Table I) in a concentration-dependent manner. Complete inhibition of both influx and efflux is observed when mersalyl is 200 μM. The maximum inhibition with NEM is only 60%, even at 500 μM. At submaximal concentrations, inhibition by NEM and mersalyl is not additive. When mersalyl is added first, subsequent addition of NEM reduces the inhibition expected from mersalyl alone. These data suggest that both sulfhydryl reagents act at the same site with greater potency for mersalyl. Mersalyl is impermeant and therefore the site is probably on the outer face of the inner membrane. The equivalent inhibition of influx and efflux by sulfhydryl reagents is consistent with a coupled transport mechanism.

Several reagents that are specific for certain amino acid functional groups have been tested in preliminary experiments for an effect on transport activity. The tyrosine-specific reagent N-acetylimidazole has no effect, whereas an arginine-specific reagent (phenylgloxal) and a lysine-specific reagent (pyridoxal-5-phosphate) do inhibit transport (influx and/or efflux) by 75–85% at 1 mM and by 100% at 2 mM (Joyal and Aprille, unpublished results). These results suggest but do not prove that arginine and/or lysine may be functionally important at the active site for transport.

Table II lists a series of specific inhibitors that have no significant effect on the ATP-Mg/P_i carrier at concentrations that inhibit other mitochondrial carriers. Substrates for other anion carriers that did not stimulate nor inhibit adenine nucleotide efflux when added to the external medium as counterions, include pyruvate, succinate, oxaloacetate, phosphoenolpyruvate, glutamate, malate, and citrate. The ATP-Mg/P_i carrier thus appears to be distinct from other mitochondrial carriers. Definitive proof of a novel transport protein will require its purification and reconstitution, a goal that remains elusive (Nosek, 1991). One major obstacle to purification is the lack of a specific labeling reagent that can be proved to affect (and therefore bind) the ATP-Mg/P_i carrier. Several ATP analogues were tested as inhibitors of ATP influx under standard assay conditions. Those that did not inhibit at all include α,β-methyleneadenosine 5′-triphosphate, β,γ-methyleneadenosine 5′-triphosphate, $1,N^6$-ethenoadenosine 5′-triphosphate, and 5′-adenylylimidodiphosphate. One analogue, 5′-fluorosulfonylbenzoyladenosine (FSBA),

Table II. Inhibitors of Other Transport Systems That Do Not Significantly Inhibit ATP-Mg/P_i Activity[a]

Inhibitor	Transport system	Maximum concentration tested
Carboxyatractyloside	ADP/ATP translocase	10 μM
Atractyloside	ADP/ATP translocase	100 μM
Benzene 1,2,3-tricarboxylate	Tricarboxylate carrier	20 mM
n-Butylmalonate	Dicarboxylate carrier	20 mM
α-Cyano-4-hydroxycinnamate	Monocarboxylate carrier	0.5 mM
Quinine	K^+ or Mg^{2+} uptake	100 μM
Cyclosporin	Transition pore	10 μM
Ruthenium red	Ca^{2+} uniporter	1 μM

[a] Each inhibitor was tested in a standard ATP-Mg^{2-} influx assay and each inhibitor was tested in at least three different preparations of mitochondria. The inhibitor concentrations are in the range needed to block the transport system for which each is specific (Nosek, 1991).

did inhibit but only weakly (16% at 1 mM). With no labeling agent available, a reconstituted transport assay must be used to follow the carrier protein through purification steps; so far, reconstituted ATP-Mg/P_i carrier activity that is EGTA-sensitive has not been reproducibly measurable (Nosek, 1991).

REGULATION OF ATP-Mg/P_i CARRIER ACTIVITY BY CALCIUM

Haynes *et al.* (1986) were the first to report that micromolar Ca^{2+} was required for net adenine nucleotide transport. Regulation of the ATP-Mg/P_i carrier by calcium has now been studied extensively in isolated liver mitochondria and in hepatocytes (Nosek *et al.*, 1990; Dransfield and Aprille, 1993). In isolated mitochondria ATP-Mg/P_i carrier activity increases as a linear function of $[Ca^{2+}]_{free}$ between 0 and 2 μM. Influx and efflux are stimulated to exactly the same extent by Ca^{2+}, a result that is consistent with a coupled transport process. Ca^{2+} stimulates activity even when ruthenium red is added, suggesting that external (or cytoplasmic) Ca^{2+} is sufficient to regulate transport activity. Ca^{2+} seems to activate transport by lowering the apparent K_m for ATP-Mg^{2-}; V_{max} is not affected, and since influx and efflux are stimulated equally, Ca^{2+} influences the rate but not the direction of any net transport that results. Calmodulin antagonists inhibit activation by Ca^{2+}, suggesting that Ca^{2+} may stimulate ATP-Mg/P_i carrier activity via a specific Ca^{2+} binding site, either on the carrier itself or perhaps secondarily via interaction with another Ca^{2+} binding protein.

In well-energized hepatocytes, net adenine nucleotide uptake into the mitochondrial compartment occurs in response to an increase in cytoplasmic $[Ca^{2+}]_{free}$. This response is seen when cells are treated with glucagon, vasopressin, A23187 (a Ca^{2+} ionophore), or thapsigargin (an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase) (Dransfield and Aprille, 1993). None of these agents cause mitochondrial volume to increase, nor do they cause any measurable change in cytoplasmic or mitochondrial ATP or phosphate concentrations. It seems then that the matrix/cytoplasm [ATP-Mg^{2-}] gradient is lower than the [HPO_4^{2-}] gradient in normal cells, and this implies further that the ATP-Mg/P_i carrier is not active in the absence of a positive Ca^{2+} signal (Aprille, 1988a; Dransfield and Aprille, 1993).

The *in vivo* injection of hormones that elevate cytoplasmic $[Ca^{2+}]_{free}$ causes an immediate increase in the mitochondrial adenine nucleotide content in liver. In newborns, postnatal tissue oxygenation promotes a rapid increase in cytoplasmic ATP concentration through oxidative phosphorylation and the electrogenic mechanism of ADP/ATP translocase. This sets up an [ATP-Mg^{2-}] gradient that favors net uptake of adenine nucleotide into the matrix. Normal postnatal hormone secretions elicit a Ca^{2+} signal that activates the ATP-Mg/P_i carrier. This scenario explains the dramatic increase in matrix adenine nucleotide content that occurs soon after birth (Aprille, 1988a).

The ATP-Mg/P_i carrier is the only mitochondrial transport system that is regulated by Ca^{2+}. The increase in matrix adenine nucleotides that follows Ca^{2+} activation of the carrier will stimulate matrix reactions that enhance metabolic flux in the cell, as noted above. This effect of adenine nucleotides is apparently coordinated with a Ca^{2+}-mediated increase in aerobic energy metabolism because certain Krebs cycle dehydrogenases are stimulated by an increase in matrix $[Ca^{2+}]_{free}$ (McCormack *et al.*, 1990).

DISTRIBUTION OF THE ATP-Mg/P_i CARRIER AND OTHER MECHANISMS FOR NET ADENINE NUCLEOTIDE TRANSPORT IN MITOCHONDRIA

The characteristics of the mitochondrial ATP-Mg/P_i carrier described so far in this review are derived entirely from studies in liver cells and mitochondria. Recently, net adenine nucleotide transport has been studied in kidney mitochondria (Hagen *et al.*, 1993). Kidney is especially interesting in this context, because mitochondrial adenine nucleotides are released to the cytoplasm during ischemia, and bioenergetic function is impaired as a result. Upon organ reperfusion, the mitochondrial adenine nucleotide content and bioenergetic function are restored. The mechanism(s) responsible for the changes in the subcellular distribution of adenine nucleotides are thus obviously important for protection and recovery of transplanted kidneys.

Kidney cortex mitochondria have net adenine nucleotide transport activity that is EGTA-sensitive and carboxyatractyloside-insensitive. The kinetics and mechanistic features of this activity are virtually identical to the activity of the ATP-Mg/P_i carrier in liver, and so it is assumed that the same carrier exists in both tissues (Table I). However, there is another mechanism

for net adenine nucleotide transport in kidney mitochondria that is not present in liver (Hagen *et al.*, 1993). This other mechanism exchanges ATP for phosphate as does the ATP-Mg/P_i carrier, but its inhibitor sensitivity is markedly different; it is *not* inhibited by EGTA, it *is* inhibited by carboxyatractyloside, and it is only partially inhibited by mersalyl (Hagen *et al.*, 1993). Net adenine nucleotide transport via the carboxyatractyloside-sensitive mechanism in kidney may represent a novel function of the ADP/ATP translocase. This interpretation invokes different isoforms of the translocase in kidney and liver, because carboxyatractyloside-sensitive exchange of ATP and phosphate cannot be demonstrated in liver mitochondria. An alternative possibility is the existence of another, as yet unidentified, carboxyatractyloside-sensitive carrier protein in kidney mitochondria.

Besides ATP-Mg/P_i activity, the only other mechanism for net transport of adenine nucleotides in liver mitochondria is an exchange of adenine nucleotide for inorganic pyrophosphate that can occur via the ADP/ATP translocase (Asimakis and Aprille, 1980a,b). However, the K_m for pyrophosphate is so high relative to the K_m for adenine nucleotides, that the physiological significance of this activity is questionable (Krämer, 1985). Pyrophosphate is a competitive substrate for the ATP-Mg/P_i carrier (unpublished results). So in the event that pyrophosphate concentration was elevated to the millimolar range, pyrophosphate would be more likely to promote net adenine nucleotide transport via the ATP-Mg/P_i carrier than transport by ADP/ATP translocase.

Newborn liver mitochondria exhibit ATP-Mg/P_i activity that has lower specific activity (and V_{max}) than adult mitochondria. Other characteristics of carrier activity, such as inhibitor sensitivity, apparent K_m, substrate specificity, Mg^{2+} requirements, and Ca^{2+} regulation are the same in newborn and adult mitochondria. The specific activity of the ATP-Mg/P_i carrier in newborns develops to adult values within a few days (Joyal and Aprille, unpublished results).

It is interesting that certain hepatoma cell lines have low mitochondrial adenine nucleotide contents (Barbour and Chan, 1983), just as fetal liver does (Aprille, 1986, 1988a, 1990). The possibility that low ATP-Mg/P_i transport activity, and consequently low matrix adenine nucleotide contents, may contribute to the potential for fast growth rates, especially under the relatively hypoxic conditions that often prevail in tumors, deserves further investigation.

Net adenine nucleotide transport has been studied in mitochondria from other cell types including corn shoots, cauliflower, and heart (for discussion see Nosek and Aprille, 1992 and Hagen *et al.*, 1993). The activities described in those other types have some features in common with the ATP-Mg/P_i carrier; for example, they involve an exchange of adenine nucleotide for phosphate or pyrophosphate. However, there are significant differences as well, and liver and kidney are so far the only type of mitochondria that exhibit the prototype ATP-Mg/P_i carrier activity. Net adenine nucleotide transport in heart mitochondria may involve separate mechanisms for net uptake and net loss, neither of which is typical of ATP-Mg/P_i carrier activity (Asimakis and Conti, 1985; Wilson and Asimakis, 1987; Asimakis *et al.*, 1990). Net adenine nucleotide loss in heart mitochondria occurs by exchange for external phosphate (or pyrophosphate) and is inhibited by carboxyatractyloside, features that are reminiscent of the second mechanism for net transport in kidney mitochondria (see above). Net efflux from heart mitochondria is also partially inhibited by phenylsuccinate or 2-*n*-butylmalonate, which invokes participation of the dicarboxylate carrier (Wilson and Asimakis, 1987; Asimakis and Conti, 1985).

FUTURE WORK

A high priority for future work is to purify the carrier protein, and to use liposome-reconstituted activity to further characterize the mechanism and regulation of transport. The mode by which Ca^{2+} regulates the carrier is of particular interest. It will be important to eventually investigate the molecular biology of this carrier in relation to other adenine nucleotide and phosphate carriers. The molecular biology relating to the tissue-specific distribution of the ATP-Mg/P_i carrier and other systems for net adenine nucleotide transport will be another fruitful area of study.

Preliminary work suggests that the ATP-Mg/P_i carrier has a role in regulating mitochondrial biogenesis, via an effect of the matrix adenine nucleotide pool size on intramitochondrial protein synthesis. This ties mitochondrial biogenesis to oxygen tension (the adenine nucleotide pool size is depleted in hypoxia) and to hormone signals (via activation of the carrier by Ca^{2+}). These regulatory interactions also suggest a model for the development of periportal-perivenous differences in hepatocyte function

(Aprille, 1988b), and as noted above, for the unusual metabolic features of certain tumor cells.

And finally, returning full circle to where the study of this carrier began, we are now prepared to understand more fully the key role that this carrier plays in the newborn's adaptation to independent aerobic metabolism.

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