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Evidence for Three Different Electrophoretic Pathways in Yeast Mitochondria: Ion Specificity and Inhibitor Sensitivity

Stéphen Manon[1] and Martine Guérin[1]

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We identified three electrophoretic pathways by spectrophotometrically following the swelling of isolated yeast mitochondria:

—An anion uniport whose activity could only be detected after depletion of divalent cations from the matrix by treatment with 1,10-phenanthroline. This uniport was inhibited by Mg^{2+} and dicyclohexylcarbodiimide.

—A K^+(Na^+) uniport which was detected only when mitochondria were suspended at low pH and low temperature. This uniport was sensitive to ruthenium red and oleic acid.

—A K^+ selective uniport which was activated by alkaline pH and ATP depletion. This pathway was sensitive to glibenclamide and to various amphiphilic cations.

Similarities and differences between these three electrophoretic pathways and the electrophoretic systems described in mammalian and plant mitochondria are discussed.

KEY WORDS: Transport; mitochondria; ion translocation.

INTRODUCTION

The electrochemical proton gradient generated by the mitochondrial respiratory chain is used to drive ATP synthesis and all the secondary transport processes necessary for mitochondrial metabolism. However, as a consequence of the high transmembrane potential generated by respiration, there is a large driving force for the electrophoretic influx of cations and the electrophoretic efflux of anions. As these processes are usually considered as potentially dangerous for mitochondrial metabolism and therefore believed to be minimized by mitochondria, they have not been recognized as an integral part of mitochondrial bioenergetics.

However, since the first patch-clamp experiment on the inner mitochondrial membrane was reported (Sorgato *et al.*, 1987), new interest has been generated in these processes. The patch-clamp technique facilitated the demonstration of several conductances in mitochondria, mitoplasts, and in inner membrane preparations (for reviews, see Kinnally *et al.*, 1992; Sorgato and Moran, 1993). Apart from the well-characterized outer-membrane voltage-dependent anion channel, three types of conductance can be identified in mammalian mitochondria.

—A 107 pS conductance which was first described by Sorgato *et al.* (1987), which is voltage-dependent and slightly anion-selective.

—A high conductance channel was also identified (Kinnally *et al.*, 1989; Petronilli *et al.*, 1989). This channel, termed mitochondrial megachannel (MMC)[2] due to its conductance higher than 1000 pS (Szabo and Zoratti, 1991) or multi-conductance channel (MCC) (Kinnally *et al.*, 1991) because of its multiple substates, is activated by Ca^{2+} and most probably

[1] Institut de Biochimie Cellulaire du CNRS, Université de Bordeaux II, 1 rue Camille Saint-Saëns, 33077 Bordeaux, France.

[2] Abbreviations: CCCP, *m*-carbonylcyanide *p*-chlorophenylhydrazone; DCCD, dicyclohexylcarbodiimide; TPP^+, tetraphenylphosphonium, IMAC, inner membrane anion channel; LCC, low-conductance channel; MCC, multi-conductance channel; MMC, mitochondrial megachannel; PTP, permeability transition pore.

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corresponds to the cyclosporin A-sensitive permeability transition pore (PTP) described by different laboratories using a variety of effectors (see Gunter and Pfeiffer, 1990 for a review and Bernardi *et al.*, 1992, 1993 and Novgorodov *et al.*, 1992 for recent publications).

—Low conductances (LCC) of less than 50 pS, generally activated by alkaline pH and inhibited by Mg^{2+}, were also observed by many groups (Antonenko *et al.*, 1991; Moran *et al.*, 1992; see Sorgato and Moran, 1993 for review). Interestingly, Inoue *et al.* (1991) described a K^+-selective channel of low conductance (10 pS) which is inhibited by ATP.

In addition, Beavis and Garlid (Beavis and Garlid, 1987; Beavis, 1992) used the classical techniques of following spectrophotometrically the swelling of mitochondria suspensions to demonstrate the existence of an inner membrane anion channel (IMAC) which exhibits pharmacological similarities with LCC (Kinnally *et al.*, 1992).

The presence of specific electrophoretic cation pathways, different from simple leaks, first proposed by the group of Brierley was definitively established by different methods (Jung *et al.*, 1977; Kapus *et al.*, 1990; Bernardi *et al.*, 1990; Nicolli *et al.*, 1991). It is now widely accepted that a ruthenium red-sensitive potassium uniport, sensitive to Mg^{2+}, exists in the inner membrane of respiring mammalian mitochondria.

More recently, a great interest developed in the existence of an ATP-dependent potassium uniport. Beavis *et al.* (1993) recently published a model of regulation of this uniport, based on different sensitivity to nucleotides. It seems quite obvious that this uniport and the channel described by Inoue *et al.* (1991) should be the same system.

Few attempts to purify the proteins involved in these processes have been made. Diwan's group (Diwan *et al.*, 1988; Costa *et al.*, 1991; Paliwal *et al.*, 1992) published the reconstitution of a partially purified fraction able to induce a 40 pS conductance in proteoliposomes. Subsequently, Garlid's group (Paucek *et al.*, 1992) reported the partial purification of the ATP-sensitive K^+ uniport. It has become evident that full knowledge of the actual role and regulation of these processes requires a molecular approach.

For such an approach, yeast would be an ideal model system. Unfortunately, very little is known about these processes in yeast mitochondria, in contrast to mammalian mitochondria. It is well known, however, that both the function and the structure of yeast mitochondria are very sensitive to monovalent cations (Velours *et al.*, 1977). Moreover, the presence of a spontaneously active K^+/H^+ exchange (Manon and Guérin, 1992) suggests that extensive K^+-cycling occurs during oxidative phosphorylation (Manon and Guérin, unpublished results; see also Dabadie *et al.*, 1986).

In the present paper, we have used the swelling technique to show the existence of three different electrophoretic pathways in yeast mitochondria. One is a chloride transport pathway, exhibiting similarities with the mammalian IMAC. The second is a ruthenium red-sensitive monovalent cations transport pathway. The third is a potassium-specific transport pathway activated by alkaline pH and ATP depletion.

MATERIALS AND METHODS

The wild-type diploid strain Yeast Foam was grown aerobically on a complete medium (1% Yeast Extract, 1% Bacto-peptone, 2% galactose, pH 5.5), and cells were harvested in the mid-exponential growth phase. Mitochondria were isolated from spheroplasts (Guérin *et al.*, 1977) and suspended in a buffer containing 0.6 M mannitol, 2 mM EGTA, 10 mM Tris/maleate (pH 6.8). Sorbitol and mannitol solutions used in isolation buffers were routinely deionized before use. Protein was measured according to Bradford (1976). The swelling was followed by recording the decrease of light diffusion at 520 nm of a suspension of mitochondria. The experiments were done in a 0.3 M KCl, 10 mM Tris/maleate buffer (pH 6.8) at 25°C (except otherwise indicated). The concentration of 0.3 M salt was chosen because it allows a full reversibility of the swelling after suspending preswollen mitochondria in a 0.6 M mannitol solution. This full reversibility was not observed when mitochondria were suspended in 0.2 M KCl (not shown).

RESULTS

Swelling of Mitochondria Suspended in KCl in the Presence of Valinomycin: Chloride Transport

Mammalian mitochondria are able to transport a wide range of inorganic (chloride, thiocyanate, phosphate, nitrate, etc.) and organic (gluconate,

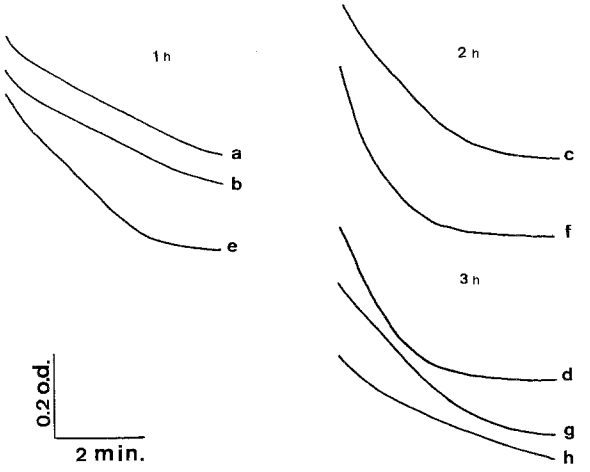

Fig. 1. Induction of valinomycin-induced swelling by divalent cation depletion and inhibition by Mg^{2+}. Mitochondria (0.33 mg/ml) were suspended at 25°C in 0.3 M KCl and 10 mM Tris/maleate buffer (pH 7.8) with addition of 0.83 μg/ml valinomycin except in (a). Before the experiment, mitochondria stored in the isolation buffer were kept at 4°C for 1 h (a,b,e), 2 h (c,f), or 3 h (d,g,h). Additions: 1.5 mM 1,10-phenanthroline (e,f), 1 mM $MgCl_2$ (g), or 10 mM $MgCl_2$ (h).

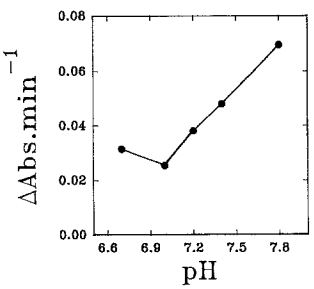

Fig. 2. Effect of pH on the rate of the valinomycin-induced swelling. Mitochondria (0.17 mg/ml) were suspended at 25°C in 0.3 M KCl and 10 mM Tris/maleate buffer adjusted at different pH with Tris in the presence or in the absence of valimomycin 0.83 μg/ml. Data represent the difference between the rates of the valinomycin-induced swelling minus the spontaneous swelling.

dicarboxylates, tricarboxylates, etc.) anions through the IMAC (Beavis, 1992). Although we investigated the transport of other inorganic anions, we will discuss only the transport of chloride.

As shown in Fig. 1 (traces a,b), the potassium ionophore valinomycin did not stimulate the slow swelling of freshly isolated mitochondria suspended in KCl, even at alkaline pH, in accordance with previous observations by Arselin de Châteaubodeau *et al.* (1976). Mitochondria kept for increasing periods of time up to 2 h at 4°C in the isolation buffer displayed, however, a valinomycin-induced swelling which increased progressively with the incubation time of mitochondria at 4°C (Fig. 1, traces c and d). This swelling was not due to any unspecific permeability transition appearing with time as storage at 4°C did not modify the initial absorbance of the mitochondria suspension (not shown). Since it is well documented that the valinomycin-induced swelling of mammalian mitochondria is inhibited by Mg^{2+} (Beavis and Garlid, 1987), we investigated whether a depletion of matricial divalent cations could induce swelling. Indeed, addition of the permeant chelator 1,10-phenanthroline stimulated the valinomycin-induced swelling of freshly isolated mitochondria (Fig. 1, traces e and f), whereas the impermeant chelators EDTA and EGTA were ineffective (not shown). Conversely, addition of Mg^{2+} to mitochondria kept at 4°C for 2 h inhibited the valinomycin-induced swelling (Fig. 1, traces g,h). These results indicated that Mg^{2+} could be an intrinsic inhibitor of electrophoretic anion transport through the inner mitochondrial membrane of yeast, similar to that described in mammalian mitochondria (Beavis and Garlid, 1987).

We have investigated the effect of several positive and negative effectors acting on the mammalian IMAC on the valinomycin-induced swelling of yeast mitochondria.

Protons: Similarly as in mammalian mitochondria, the swelling was clearly stimulated by alkaline pH, although a significant swelling was still observed at neutral pH and lower (Fig. 2).

Inhibitors of the K^+/H^+ exchange: The amphiphilic amines propranolol and quinine and the hydrophobic carboxyl-reagent DCCD have been previously reported to inhibit the antiport in rat-liver (Nakashima and Garlid, 1982) and yeast (Manon and Guérin, 1992) mitochondria. Furthermore, they have also been found to inhibit mammalian IMAC (Beavis and Garlid, 1987). We observed that, in yeast mitochondria, propranolol and DCCD fully inhibited the valinomycin-induced swelling, at the same concentrations as for the K^+/H^+ antiport (Fig. 3, traces b,g). At the concentration which inhibits the K^+/H^+ exchange, quinine promoted only a partial inhibition of the swelling (Fig. 3, trace d). The divalent cation Zn^{2+}, which has been found to inhibit K^+/H^+ exchange both in yeast and rat-liver mitochondria (Manon and Guérin, 1992), also inhibited chloride transport (not shown).

Triorganotins: In rat-liver mitochondria, the IMAC and the ATP synthetase/ATPase activities are inhibited by nearly the same concentrations of

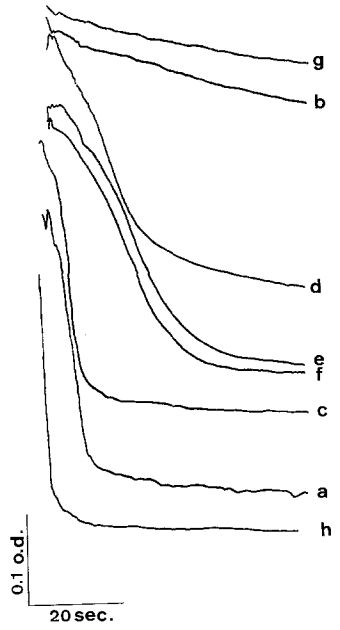

Fig. 3. Effect of inhibitors on the valinomycin-induced swelling. Mitochondria (0.33 mg/ml) were suspended at 25°C in 0.3 M KCl and 10 mM Tris/maleate buffer (pH 7.8) in the presence of 0.83 μg/ml valinomycin and 1.2 mM 1,10-phenanthroline. (a) No addition; (b) 0.5 mM propranolol; (c) 25 μM amiodarone; (d) 0.5 mM quinine; (e) 5 μM triphenyltin; (f) 20 μM triethyltin; (g) mitochondria were preincubated for 15 min with 50 nmol DCCD/mg; (h) mitochondria were preincubated for 4 min with 50 nmol mersalyl/mg.

triorganotins (Powers and Beavis, 1991). We tested two of these compounds, triethyltin and triphenyltin, at twice the concentration inhibiting the ATPase activity, on chloride transport. To obtain the maximal transport of chloride, mitochondria were kept at 4°C for 3 h. As shown in Fig. 3 (traces e,f), both compounds only partially inhibited the valinomycin-induced swelling. The inhibitory effect was not increased by raising the triorganotins concentration (not shown). Oligomycin, a well-known inhibitor of ATP synthetase/ATPase, which also inhibits mammalian IMAC (Beavis and Garlid, 1987), had also only a partial inhibitory effect on the swelling of yeast mitochondria (not shown).

Amiodarone: This antiarrythmic drug has been found to be the most efficient inhibitor of both IMAC (Beavis, 1992) and LCC (Kinnally *et al.*, 1992). It was without effect, however, in yeast mitochondria, even when used at 50 times the IC_{50} of mammalian IMAC.

Mersalyl: Depending on the pH, thiol reagents have been found to have a complex effect on mammalian IMAC (Beavis, 1992): at very alkaline pH (8.4), they inhibited the activity of IMAC, whereas at neutral or slightly alkaline pH, low concentrations stimulated the activity and higher concentrations inhibited it. In yeast mitochondria, and at all the pH tested (6.7–7.8), 50 nmol mersalyl per mg protein, i.e., two times the concentration promoting the maximal inhibition in mammalian mitochondria, stimulated the swelling (Fig. 3, trace h).

Swelling of Mitochondria in KCl in the Absence of Valinomycin: Potassium Transport

When suspended in KCl in the absence of valinomycin, at pH 7.0 and 25°C, yeast mitochondria swelled very slowly. This swelling was insensitive to all the inhibitors described above but was strongly stimulated by the protonophore CCCP (Manon and Guérin, 1992), which suggested that it could be due to electrophoretic leaks of potassium and chloride.

Ruthenium Red-Sensitive Monovalent Cation Transport

In order to limit these possible leaks, we decreased the pH and the temperature to 6.7 and 15°C, respectively. Under these conditions, the remaining swelling became sensitive to ruthenium red, as reported for mammalian mitochondria (Kapus *et al.*, 1990). Unexpectedly, it was also inhibited by oleate (Fig. 4, traces a–c). The same ruthenium red/oleate-sensitive swelling was observed when potassium was replaced by sodium, lithium, or rubidium (not shown). Mitochondria isolated with buffers in which the concentration of bovine serum albumin (which binds fatty acids) was decreased 10-fold did not exhibit any oleate-sensitive or ruthenium red-sensitive swelling (not shown). This observation suggests that fatty acid may be intrinsic inhibitors of this uniport.

The fact that the swelling at 25°C (Fig. 4, traces d,e) or at pH 7.3 (Fig. 4, traces f,g) was insensitive to ruthenium red and oleate indicated that, under these conditions, K^+ leaks masked this uniport, suggesting that its activity was very low.

ATP-Sensitive K^+-Transport

In order to test whether a more active electrophoretic pathway for monovalent cations could be induced by alkaline pH, we suspended freshly isolated mitochondria at pH 7.8. Under these conditions, mitochondria still swelled very slowly (Fig. 5, trace a). The addition of 1,10-phenanthroline, which opens the chloride influx pathway, did not

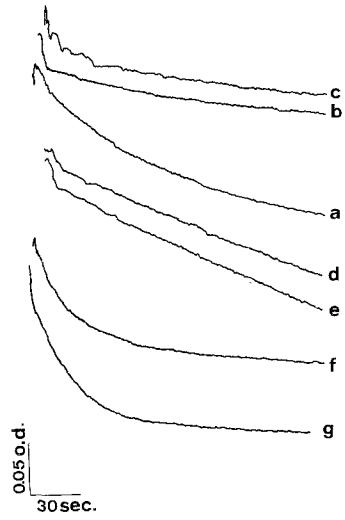

Fig. 4. Swelling in KCl at neutral pH. After their isolation, mitochondria were kept at 4°C for 4 h before measurements (it was verified that the Cl^--influx pathway was fully active). They were suspended in 0.3 M KCl and 10 mM Tris/maleate buffer under different conditions. (a) pH 6.7, $T = 15$°C, no addition; (b) same as (a) +1.1 μM ruthenium red; (c) same as (a) +20 μM oleate (sodium salt); (d) pH 6.7, $T = 25$°C, no addition; (e) same as (d) +1.1 μM ruthenium red; (f) pH 7.3, $T = 15$°C, no addition; (g) same as (f) +1.1 μM ruthenium red.

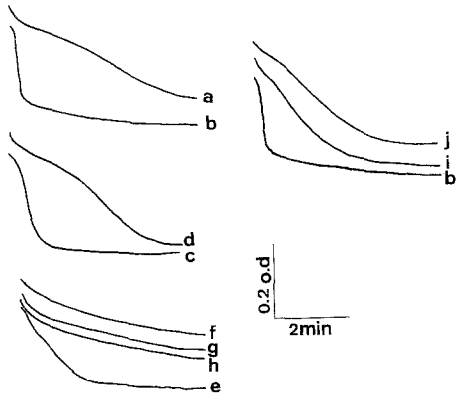

Fig. 5. Swelling in KCl at alkaline pH. Mitochondria were preincubated or not under different conditions (see below) before being suspended (0.33 mg/ml) in 0.3 M KCl and 10 mM Tris/maleate buffer (pH 7.8) at 25°C. (a) Freshly isolated mitochondria without preincubation; (b) mitochondria kept at 4°C for 3 h; (c) freshly isolated mitochondria (7.6 mg/ml) were preincubated for 3 min at 25°C in a 0.6 M mannitol, 7.6 mM $MgCl_2$, and 10 mM Tris/maleate buffer (pH 6.7) with addition of 24 mM glucose anf 15.3 units/ml yeast hexokinase; (d) same as (c) but to the preincubation medium was added oligomycin (5 μg/mg protein) and atractyloside (250 nmol/mg protein); (e–h) mitochondria kept at 4°C for 3 h were suspended in 0.3 M KCl, LiCl, NaCl, and RbCl, respectively, and 10 mM Tris/maleate (pH 7.8) (this experiment was done on a different mitochondria preparation from the other traces in the figure; it was ascertained that ATP-depleted mitochondria obtained as in trace (c) did not swell when suspended in NaCl); (i) same as (b) with 50μM glibenclamide; (j) same as (b) with 20 μM tetraphenylphosphonium.

increase the swelling (not shown; in some experiments, it even inhibited it).When mitochondria were kept for 3–4 h at 4°C, a spontaneous and rapid swelling was observed (Fig. 5, trace b). Similarly as for the valinomycin-induced swelling, we searched for the factor responsible for this time-dependent permeability transition.

It was observed that storage of mitochondria at room temperature instead of 4°C reduced the time necessary for the induction to 15–20 min instead of several hours. One possible explanation for such a time-dependent phenomenon could be the involvement of slow hydrolysis of matrix ATP. The effect of ATP depletion on the swelling of mitochondria at alkaline pH was therefore tested by incubating mitochondria at 25°C in the presence of glucose, hexokinase, and Mg^{2+}. Under these conditions, a spontaneous swelling appeared after 3–4 min of preincubation (Fig. 5, trace c). Addition of oligomycin and atractyloside during the preincubation (in order to inhibit both the ATPase and the ATP/ADP translocator) prevented the induction of this spontaneous swelling (Fig. 5, trace d). Addition of ATP to mitochondria kept at 20°C for 20 min did not, however, prevent the swelling (not shown), which indicates that ATP only arrested swelling when present on the matrix side of the inner membrane.

This ATP-sensitive uniport exhibited some particular features:

(i) It was specific for potassium since no swelling occurred when mitochondria were suspended in NaCl, LiCl, or RbCl (Fig. 5, traces e–h).

(ii) It was active only at alkaline pH (Fig. 6).

(iii) It was inhibited by glibenclamide (Fig. 5, trace i), a potent inhibitor of ATP-dependent potassium channels (Davis *et al.*, 1991).

(iv) Interestingly, it was also sensitive to a group of amphiphilic cations described previously as unusual inhibitors of ATP synthesis both in mammalian (Higuti *et al.*, 1980) and in yeast mitochondria (Manon and Guérin, 1993), including tetraphenylphosphonium (Fig. 5, trace j), triphenyltetrazolium, and ethidium (not shown).

In our assay, which involved both K^+ and Cl^- influxes, the inhibitors of the latter (Mg^{2+}, propranolol, and DCCD) could not be tested. We verified,

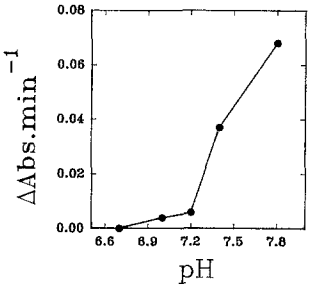

Fig. 6. Effect of pH on the swelling in KCl. Mitochondria (0.33 mg/ml) were suspended in 0.3 M KCl or 0.3 M NaCl, and 10 mM malate (buffered at different pH with Tris). Data represent the rate of swelling in KCl minus the rate of swelling in NaCl.

however, that quinine, amiodarone, and ruthenium red had no effect on swelling at alkaline pH (not shown). Conversely, glibenclamide and tetraphenylphosphonium did not alter the swelling induced by valinomycin (not shown). Furthermore, it was verified that ATP depletion and inhibitors of the swelling in KCl did not change the swelling rate of mitochondria suspended in potassium (or sodium) acetate, showing that the $K^+(Na^+)/H^+$ exchanger was not involved in the observations reported above.

DISCUSSION

Since the maintenance of the high transmembrane potential required for ATP synthesis is dependent on the integrity of the inner membrane, the volume of mitochondria has to be controlled by regulating the net salt flux across this membrane. As postulated earlier (Mitchell, 1966), the electroneutal K^+/H^+ exchange must play a central role in this process, since it allows an efflux of potassium without any collapsing of the transmembrane potential. The possible role of the IMAC has also been discussed in terms of matrix volume regulation (Beavis and Garlid, 1987).

In contrast, the potential role of electrophoretic pathways catalyzed by specific proteins looks *a priori* less clear. Although numerous hypotheses have been proposed (see the discussion by Sorgato and Moran, 1993), it is clear that the definitive understanding of the role and the physiology of these processes requires knowledge of their molecular basis.

In Table I, we have summarized the probable mammalian and plant counterparts of the three pathways described in this paper. Additionally, we compared the inhibitor sensitivity of the four (the three uniports and the antiport) yeast systems to their probable mammalian counterparts (Table II).

High-conductance channels: Since we did not use patch-clamp experiments, we obviously could not investigate the existence of a yeast counterpart of the 107 pS conductance and of the MCC. However, the latter is probably the same system as the PTP induced by Ca^{2+} and a variety of other agents

Table I. Mammalian, Yeast, and Plant Mitochondria Ion Transport Systems

Yeast	Mammalian	Plant
$K^+(Na^+)/H^+$ antiport [1]	$K^+(Na^+)/H^+$ antiport [2,3]	K^+/H^+ antiport [17]
Not found[a]	Na^+/H^+ antiport [2,4]	
Anion uniport	IMAC [5,6] LCC (?)	Plant IMAC [16]
Neutral pH $K^+(Na^+)$ uniport	K^+uniport [7,8] Ca^{2+} uniport (?)	
Alkaline pH K^+ uniport	10 pS K^+ channel [9] K^+ uniport [10,11]	Alakaline pH K^+ uniport [17]
Not investigated	107 pS channel [12]	
Not investigated[b]	MCC, MMC, PTP [13–15]	

[a] No quinine-insensitive swelling could be detected in sodium acetate.
[b] Ca^{2+} does not induce any swelling nor uncoupling of yeast mitochondria.
[1] Manon and Guérin, 1992; [2] Nahashima and Garlid, 1982; [3] Li *et al.*,1990; [4] Garlid *et al.*, 1991; [5] Beavis and Garlid, 1987; [6] Beavis, 1992; [7] Kapus *et al.*, 1990; [8] Nicolli *et al.*, 1991; [9] Inoue *et al.*, 1991; [10] Paucek *et al.*, 1992; [11] Beavis *et al.*, 1993; [12] Sorgato *et al.*, 1987; [13] Kinnally *et al.*, 1989; [14] Petronilli *et al.*, 1989; [15] Gunter and Pfeiffer, 1990 (review); [16] Beavis and Vercesi, 1992; [17] Hanson, 1985 (review).

Table II. Inhibitor Sensitivity of Ion Transport Systems in Yeast and Mammalian Mitochondria

	K^+/H^+ antiport		Anion uniport		K^+ uniport, neutral pH		K^+ uniport, alkaline pH	
	M	Y	M	Y	M	Y	M	Y
H^+ (pH < 7.0)	+/–	+/–	+/–	+/–			+	+
Mg^{2+}	+	–[a]	+	+			+	
Zn^{2+}	+	+		+				
DCCD	+	+	+	+			+	
Quinine	+	+	+	+/–			+	—
Quinidine	–	+						
Propranolol	+	+	+	+				
Amiodarone			+	–				–
Mersalyl			+[b]	–[c]			+	
Triorganotins			+	+/–				
Ruthenium red					+	+		–
Oleate						+		
ATP							+	+
Glibenclamide					–		+[d]	+
TPP^{+}[e]					–			+

M = mammals, Y = yeast; (+) = full inhibition, (+/–) = partial inhibition, (–) = no inhibition.

[a] Mg^{2+} promoted a partial inhibition but at concentrations 50 times higher than those reported on mammalian mitochondria (Manon and Guérin, 1992).

[b] Depending on the pH (Beavis, 1992).

[c] A stimulation was observed at all pH.

[d] Inoue *et al.* (1991) reported an inhibition of the channel, whereas Beavis *et al.* (1993) found only 10% inhibition of the uniport. However, they found a full inhibition on the reconstituted system (Paucek *et al.*, 1992).

[e] Li *et al.* (1992) reported that TPP^+ was an inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger.

(Kinnally *et al.*, 1992). Ca^{2+} is without any effect on swelling and coupling state of yeast mitochondria (Manon and Guérin, unpublished results). We cannot, however, give any definitive answer about the existence of this pore in yeast mitochondria since we did not systematically investigate all the possibilities for its induction. Moreover, considering the growing hypothesis that this pore could be a pathway for the translocation of macromolecules (see the discussion by Sorgato and Moran, 1993), a counterpart of this pathway may also exist in yeast mitochondria.

K^+/H^+ antiport: It was previously reported that yeast mitochondria supported an active $K^+(Na^+)/H^+$ exchange system which, in contrast to the mammalian one, was only slightly sensitive to Mg^{2+} (Manon and Guérin, 1992). This antiport was, however, sensitive to quinine, propranolol, Zn^{2+}, and DCCD, similarly as the system described and purified from mammalian mitochondria by the group of Garlid (Li *et al.*, 1990). A difference between mammalian and yeast mitochondria is the existence of a quinine-insensitive Na^+/H^+ exchange in mammalian mitochondria (Nakashima and Garlid, 1982; Garlid *et al.*, 1991) which does not seem to exist in yeast mitochondria (Manon and Guérin, unpublished results). The existence of such an active K^+/H^+ exchange was also postulated in plant mitochondria (see Hanson, 1985 for a review) which maintain a low pH gradient as yeast mitochondria (Beauvoit *et al.*, 1989).

Cl^- channel: In yeast mitochondria, the system allowing chloride influx was latent until removal of matrix divalent cations. Such a characteristic was observed for the mammalian IMAC (Beavis and Garlid, 1987) but not the plant counterpart (Beavis and Vercesi, 1992). All three systems are inhibited by protons. The yeast system is sensitive to propranolol and DCCD as the mammalian one. However, it is insensitive to mercurials, and amiodarone and poorly sensitive to triorganotins and quinine. The plant system is insensitive to mercurials and DCCD, but inhibited by triorganotins and some nucleotide analogs.

Ruthenium red-sensitive cation uniport: The ruthenium red sensitivity is a characteristic of the cation uniport described by Kapus *et al.* (1990) and further by Nicolli *et al.* (1991) in mammalian mitochondria. The low level of activity of this system in yeast mitochondria is of special interest considering the

view developed by Kapus *et al.* (1991) that this system may be a Ca^{2+} uniport of which the specificity should be altered by Mg^{2+} depletion. Indeed, Ca^{2+} had been shown to be very poorly transported in yeast mitochondria (Carafoli *et al.*, 1970).

ATP-sensitive K+-uniport: Inoue *et al.* (1991) first reported the existence of a low-conductance channel, specific for potassium, and inhibited by ATP and by glibenclamide, in mammalian mitochondria. Beavis *et al.* (1993) described recently the regulation of a K^+-uniport by nucleotides. Paucek *et al.* (1992) reported the partial purification of this ATP-sensitive K^+-selective uniport. It seems likely that the channel described by Inoue *et al.* (1991), the uniport described by Beavis *et al.* (1993), and the yeast uniport described herein are similar systems. However, Kinnally *et al.* (1992) discussed the possibility that, in mammalian mitochondria, this channel may be the same as the LCC which in turn presents strong pharmacological similarities with the IMAC. From data reported herein, the ATP-dependent K^+-uniport and the anion channel are clearly distinct systems.

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