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Minireview: Characterization of the Yeast Mitochondria Unselective Channel: A Counterpart to the Mammalian Permeability Transition Pore?[1]

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Large and unselective permeabilities through the inner membrane of yeast mitochondria have been observed for more than 20 years, but the characterization of these permeabilities, leading to hypothesize the existence of a large-conductance unselective channel in yeast inner mitochondrial membrane, was done only recently by several groups. This channel has been tentatively identified as a yeast counterpart to the mammalian permeability transition pore, the crucial role of which is now well-documented in physiopathological phenomena, such as Ca^{2+} homeostasis, ischemic damages, or programmed cell death. The aim of this review is to make a point on the known characteristics of this yeast mitochondrial unselective channel (YMUC) and to analyze whether or not it can be considered as a "yeast permeability transition pore."

KEY WORDS: Permeability transition; inner mitochondrial membrane; nucleotides; yeast.

INTRODUCTION

The existence of a transient large-conductance nonselective channel in the inner membrane of mammalian mitochondria is now widely accepted. This system is termed permeability transition pore (PTP) or mitochondrial megachannel (MMC), since its activity can be followed by both biochemical and electrophysiological methods. The groups of Bernardi and Zoratti, on the basis of an extensive description of bioenergetical parameters driving the opening/closure of PTP/MMC, have proposed a model of regulation involving Ca^{2+}, redox state, matricial pH, and transmembrane potential ($\Delta\Psi_m$) (Bernardi *et al.*, 1994; Zoratti and Szabo, 1995 for reviews). The involvement of this system in physiopathological phenomena, such as the energetic collapse following ischemia/reperfusion or the early steps of apoptosis, is under evaluation by many groups. Several investigators also demonstrated a possible participation of mitochondrial Ca^{2+}, via PTP, in the regulation of cellular Ca^{2+} homeostasis and signaling (Ichas *et al.*, 1997).

The structural aspects of PTP are less well-defined. The cyclosporin A sensitivity of PTP has been a powerful tool to identify the possible involvement of (mitochondrial) cyclophilin D in the regulation of pore opening/closure (Tanveer *et al.*, 1996). The peptidyl-prolyl *cis-trans* isomerase activity of this protein led to the hypothesis that PTP activation should be related to structural alterations of one or several membrane proteins normally having another activity.

[1] Abreviations are: ANC, adenine nucleotides carrier; MMC, mitochondrial megachannel; PSC, peptide sensitive channel; PTP, permeability transition pore; VDAC, voltage-dependent anion channel; YMUC, yeast mitochondria unselective channel.

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Potential candidates have been proposed: the fact that two specific inhibitors of the adenine nucleotide carrier (ANC), namely carboxyatractyloside and bongkrekic acid, are able to, respectively, activate and inhibit PTP, led many investigators to consider that ANC is the target of cyclophilin D. A few attempts to test this hypothesis showed that purified and reconstituted ANC was able to transport electrophoretically H^+, K^+, Na^+, and Cl^- under conditions known to open PTP, e.g., mersalyl treatment (Tikhonova *et al.*, 1994). In addition, Ca^{2+} treatment of patches of reconstituted ANC in lipid bilayers behave as a megachannel resembling PTP (Brustovetsky and Klingenberg, 1996).

A more sophisticated version of this hypothesis is the proposal that PTP is supported by mitochondrial contact sites. It is now well documented that contact sites between the outer and inner mitochondrial membranes have both a structural and functional significance. Structurally, they contain the outer membrane porin (VDAC), the inner membrane ANC, *plus* several associated enzymes, depending on the tissue [e.g., hexokinase (HXK) and adenylate kinase (AdK) in hepatocytes; creatine kinase (CrK) in myocytes]. Functionally, this contact should be responsible for metabolic channeling between the mitochondrial matrix and the cytosol (see Brdiczka, 1991 for a review). A recent attempt to test this hypothesis showed that contact sites isolated from liver mitochondria, containing VDAC, ANC, HXK, and cyclophilin D, once reconstituted in liposomes, had the behavior expected from the reconstituted PTP (Beutner *et al.*, 1996, 1998).

A powerful tool to test the hypothesis of the involvement of ANC, VDAC, and cyclophilin in PTP could be the yeast *Saccharomyces cerevisiae*, which has the double advantage to be genetically manipulable and to be a facultative aerobic cell, thus able to grow even with mitochondria inactive for oxidative phosphorylation. Unfortunately, all the attempts to find an "orthodox" PTP in yeast mitochondria failed. However, parallel studies by several groups on the ionic permeability of the inner mitochondrial membrane of yeast, conclude that these exists a large-conductance, unselective channel, which was recently termed a "yeast permeability transition pore" (Jung *et al.*, 1997). The aim of this paper is to review and to analyze the somewhat contradictory data about this system and to answer the question whether or not this "yeast PTP" is a good model to understand some structural aspects of mammalian PTP.

RESPIRATION-INDUCED LARGE AND UNSELECTIVE PERMEABILITY

The first unrecognized evidences for the existence of a large-conductance, unselective ion channel in the inner mitochondrial membrane of yeast were obtained at the same time as the first systematic bioenergetic studies of yeast mitochondria. De Châteaubodeau *et al.* (1974, 1976) studied phosphate transport through the inner mitochondrial membrane of the industrial baker's yeast strain, Yeast Foam (PS194), through the nonenergetic swelling method. Mitochondria were suspended in isoosmotic K-phosphate in the presence of valinomycin, allowing the observation of phosphate transport to the matrix. Besides the characterization of the classical transport system (mersalyl-sensitive phosphate/H^+ cotransporter), they observed that respiration was able to induce a mersalyl-insensitive large-amplitude swelling. The addition of an inhibitor of the respiratory chain (antimycin A) was required to inhibit this swelling and to allow the observation of the mersalyl-sensitive phosphate/H^+ cotransport. A similar respiration-induced large-amplitude swelling was observed when other anions, such as succinate or chloride, replaced phosphate.

The evidence for the existence of a system able to transport relatively large neutral molecules, such as mannitol, through the inner mitochondrial membrane was further obtained. Velours *et al.* (1977) showed that mitochondria isolated from the same industrial yeast strain, suspended in a mannitol-based medium and respiring in the presence of a low concentration of K^+ (10mM), in the absence of phosphate, supported a large-amplitude swelling inducing an irreversible increase of the respiration rate and finally leading to structural damage of both mitochondrial membranes. The phenomenon was prevented by low phosphate concentrations (0.5–1 mM) or higher concentrations of other permeant anions such as arsenate, acetate, or propionate. Remarkably, the efficiency of the protecting effect depended on the pK_a of the acid: the more the protonated form that was present, the more the acid was efficient. These observations led to the conclusion that permeant acids allowed the reentry of protons back to the matrix, thus preventing the formation of a "too high" electrochemical proton gradient, which was supposed to alter the structure of the inner membrane. The requirement of potassium to observe this effect was tentatively explained as an increase of transmembrane ΔpH caused by a phenomenon of K^+/H^+ exchange (without precluding the existence of a

catalyzed phenomenon). At this date, the possibility of the existence of an unselective channel in the inner mitochondrial membrane was hardly considered.

This respiration-induced permeability was reinvestigated further, after the observation of the ATP-induced permeability (see below). Guérin *et al.* (1994), still working on the wild-type industrial strain Yeast Foam, showed that mitochondria suspended in an isoosmotic solution of K-gluconate (or K-acetate) exhibited a respiration-induced large-amplitude swelling, which was further stimulated by the addition of valinomycin. On this basis they proposed, for the first time, the existence of a respiration-induced unselective channel able to transport both K^+ and gluconate. However, this swelling was not inhibited by phosphate (opposite to the ATP-induced swelling; see below), and the authors did not attempt to correlate the existence of this channel and the observations reported above.

Roucou *et al.* (1997b) investigated this correlation and observed that Yeast Foam mitochondria suspended in a mannitol-based medium in the presence of K-chloride, supported a respiration-induced large-amplitude swelling, which was fully inhibited by 0.5 mM phosphate. In the presence of 0.5 mM phosphate, a further addition of valinomycin induced a swelling totally prevented by 5mM phosphate. They, therefore, distinguished the respiration-induced K^+ transport and the respiration-induced chloride transport on the basis of their different phosphate sensitivity: respiration-induced K^+ transport is inhibited by 0.5 mM phosphate, whereas respiration-induced Cl^- transport is inhibited by 5 mM phosphate.

What about the permeability to neutral molecules? We could never induce any swelling of Yeast Foam mitochondria suspended in an isoosmotic solution of mannitol (without K-salts) by adding a respiratory substrate. This observation suggests that the respiration-induced channel of Yeast Foam mitochondria is only able to transport cations and anions. This appears to be surprising since this channel is able to transport gluconate, which is approximately the same size as mannitol (Guérin *et al.*, 1994). Moreover, the first experiments by Velours *et al.* (1977) strongly suggested that mannitol was able to enter the matrix. A possible explanation to these contradictory observations could be that, in Yeast Foam mitochondria, the presence of a K-salt is required to induce the respiration-induced channel (able to transport mannitol, as suggested from the experiments reported by Velours *et al.*, 1977). The most likely hypothesis is that, in addition to matrix alkalization induced by respiration, an additional alkalization by K^+/H^+ exchange (working in the direction of K^+ entry/H^+ exit) is required. It should be noted that, in yeast mitochondria, this exchange is spontaneously active, without any treatments after mitochondria isolation (Manon and Guérin, 1992; Welihinda *et al.*, 1993).

It is only recently that Jung *et al.* (1997) ascertained the existence of a respiration-induced channel able to transport mannitol through the inner membrane of mitochondria isolated from laboratory strains. They observed that this respiration-induced mannitol permeability had the same characteristics as the ATP-induced mannitol permeability (see below). They estimated the average size of transported molecules to about 1100 Da, approximately equal to the value found for mammalian PTP. The other characteristics of this respiration-induced mannitol permeability were identical to the characteristics of the respiration-induced gluconate permeability described by Guérin *et al.* (1994) (apart from the inhibitory effect of phosphate) and of the respiration-induced mitochondria damage described by Velours *et al.* (1977). It is then clear that the three phenomena are caused by the opening of a unique respiration-induced unselective channel, the regulation of which is different in mitochondria isolated from Yeast Foam and in mitochondria isolated from laboratory strains. The differences in phosphate sensitivity will be discussed below.

From these experiments, it is possible to conclude that the inner membrane of yeast mitochondria contains a respiration-induced channel, the size of which is comparable to that of mammalian PTP. This system is generally inhibited by phosphate, but also by other permeant anions, which allows us to conclude that it is probably inhibited by matrix acidification. This sensitivity to matrix pH correlates well with the fact that in the strain Yeast Foam the addition of a K salt is required for the opening, possibly via the activation of the K^+/H^+ exchange contributing to matrix alkalization.

ATP-INDUCED LARGE AND UNSELECTIVE PERMEABILITY

ATP-Induced Swelling in Isoosmotic Solutions of K Salts

Mitochondria suspended in isoosmotic solutions of K-salts (acetate, gluconate, chloride, glutamate, etc.) are able to swell following the addition of both valino-

mycin and ATP (Prieto *et al.*, 1992, 1995, 1996; Guérin *et al.*, 1994; Roucou *et al.*, 1997a). The phenomenon can be observed on mitochondria isolated from the wild-type industrial strain Yeast Foam (Guérin *et al.*, 1994), as well as on mitochondria isolated from the laboratory strain W303 (Prieto *et al.*, 1992, 1995, 1996) or from other laboratory strains (Roucou *et al.*, 1997a). A controversy appeared about the nature of this swelling. Prieto *et al.* (1992), having observed this swelling in K-acetate, interpreted this phenomenon as the opening of a H^+-selective permeability, able to compensate for the electrogenicity of K^+ entry via valinomycin (acetic acid being considered to enter under the undissociated form). Guérin *et al.* (1994), having observed this swelling in K-gluconate (as well as in other K-salts solutions), interpreted this phenomenon as the opening of a nonspecific anion channel. In addition, since they also observed, to a lesser extent, an ATP-induced swelling in the absence of valinomycin, they proposed that the swelling was supported by the opening of an unselective channel, able to transport both anions and cations. Their observations were further confirmed by Prieto *et al.* (1995, 1996), who observed the same characteristics in mitochondria isolated from W303. It is now accepted that yeast mitochondria, whatever their origin, support an ATP-induced unselective channel, with somewhat different characteristics, depending on the strain (see Roucou *et al.*, 1997a and below).

This system is induced by ATP but also by other nucleotides triphosphates, such as GTP and CTP (Guerin *et al.*, 1994; Prieto *et al.*, 1996). It is not induced by nonhydrolyzable analogs of ATP (Roucou *et al.*, 1997a), which suggests that ATP hydrolysis is required for opening the channel. More surprisingly, a swelling can be induced by GDP in W303 mitochondria suspended in K-acetate but not in K-gluconate (in the presence of valinomycin), whereas no effect of GDP could be observed on Yeast Foam mitochondria, whatever the salt (Roucou *et al.*, 1997a). This allows us to conclude that a GDP-induced H^+ channel actually exists in W303 mitochondria, but not in Yeast Foam mitochondria. By extrapolating, we proposed that, as GDP, ATP could open a H^+ permeability in laboratory strain but not in Yeast Foam mitochondria.

Another difference between the swellings in K-acetate and K-gluconate is Mg^{2+} sensitivity. This cation fully inhibits the ATP-induced swelling of mitochondria suspended in K-gluconate, but is without effect on the ATP-induced swelling of mitochondria suspended in K-acetate (Roucou *et al.*, 1997a).

The common characteristics of the different types of swelling described above are the sensitivity to phosphate and the sensitivity to decavanadate. The phosphate sensitivity does not reflect the same phenomenon as the phosphate sensitivity of the respiration-induced channel since the effect of phosphate on the ATP-induced channel is not prevented by inhibiting phosphate/H^+ cotransport with mersalyl, demonstrating, that in this case, phosphate acted outside the mitochondrion (Guérin *et al.*, 1994 and see below).

The sensitivity to decavanadate occurred at very low concentrations (10 μM, measured as the monomeric form) (B. Guérin, unpublished results; Roucou *et al.*, 1997a). Decavanadate is a known competitive inhibitor of several nucleotide-binding enzymes, such as adenylate kinase or the phosphofructokinase. It can be hypothesized that it also competes with ATP (or GDP) at the nucleotide-binding sites of the channel. It should be noted that, at this concentration, decavanadate is without effect on ANC and on F_0F_1-ATPase/ATP synthase.

The ATP-induced channel was sensitive to some amphiphilic amines, such as propranolol (Guérin *et al.*, 1994), which, however, was shown to have a general effect on membrane fluidity via a binding to negative phospholipids (Roucou *et al.*, 1995b). On the other hand, another amphiphilic amine, quinine, less efficiently inhibited the ATP-induced channel than other ion-transport systems, such as the K^+/H^+ exchanger (Guérin *et al.*, 1994).

ATP-Induced Stimulation of Respiration

The experiments reported until now were done on mitochondria suspended in the presence of high salt concentrations, the driving force of the transport being the chemical gradient of the transported species. Under these conditions, the channel was mostly anionic, since the observation of a maximal swelling rate required the addition of the K^+ ionophore valinomycin (Guérin *et al.*, 1994). In the presence of a $\Delta\Psi_m$ maintained by the respiratory chain, the transport of the charged species was expected to be altered, since they should be transported according to their electrochemical gradient: cations are expected to be transported more efficiently and rapidly than anions. The following is, therefore, focusing on the characteristics of the ATP-induced channel studied at relatively low salt concentration (opposite to previous experiments)

in the presence of a $\Delta\Psi_m$ maintained by the respiratory chain.

Prieto *et al.* (1992) first observed that ATP induced a stimulation of the respiration of W303 mitochondria suspended in a mannitol-based medium (without salt) and at low phosphate concentration (0.5 mM). This supported the hypothesis of the opening an ATP-induced H^+ permeability. Roucou *et al.* (1995a) did not observe this effect on Yeast Foam mitochondria suspended in a mannitol-based medium, but observed a stimulation in the presence of KCl and then concluded the existence of an ATP-induced K^+ permeability. In the same paper, the authors also presented evidence that this ATP-induced K^+ permeability was induced when F_0F_1-ATPase, instead of respiratory chain, was creating a $\Delta\Psi_m$. These authors further investigated this phenomenon on mitochondria isolated from different strains and observed that, as for the ATP-induced swelling, an ATP-induced H^+ permeability exists in the mitochondrial membrane from laboratory strains, whereas an ATP-induced K^+ channel (also able to transport other cations, but at a lower rate) exists in the mitochondrial membrane of Yeast Foam mitochondria (Roucou *et al.*, 1997a).

The ATP-induced stimulation of the respiration exhibited the same characteristics as the ATP-induced swelling: induction by GTP, CTP, and GDP, absence of induction by nonhydrolyzable ATP analogs, and inhibition by high phosphate (>3mM) and by low decavanadate concentrations. A doubt remained about the localization of the effect of ATP, because of an ambiguous effect of carboxyatractyloside: this ANC inhibitor fully prevented the stimulation of Yeast Foam mitochondria respiring on ethanol (Roucou *et al.*, 1995a), whereas it only partially prevented the stimulation of Yeast Foam mitochondria respiring on NADH (Roucou *et al.*, 1997a). Further, it had no effect on W303 mitochondria, whatever the respiratory substrate (Prieto *et al.*, 1992, 1995). However, it was found that the other inhibitor of the adenine nucleotide carrier, bongkrekic acid, had no effect on the stimulation of Yeast Foam mitochondria respiring on ethanol or NADH. Moreover, a W303-based mutant strain deleted of the three genes of the adenine nucleotide carrier still supported an ATP-induced stimulation of the respiration (Roucou *et al.*, 1997a). Thus, it is now clear that ATP acts outside the matrix. The unexpected effect of carboxyatractyloside on Yeast Foam mitochondria still remains to be explained.

ATP-Induced Swelling in Mannitol-Based Medium

All the studies by Rial's and our group were done on mitochondria suspended in isotonic media (400 to 600 mOsm). Under these conditions, no ATP-induced swelling could be seen on mitochondria suspended in a mannitol-based medium. In the study by Jung *et al.* (1997), the authors worked on mitochondria from laboratory strains suspended in a slightly hypotonic medium (0.3 M mannitol). Under these conditions, they could observe a large-amplitude swelling induced by ATP and inhibited by phosphate. They did not assay for all of the characteristics reported above (sensitivity to decavanadate and Mg^{2+}, induction by other nucleotides, lack of induction by nonhydrolyzable ATP analogs, etc.), but it is very likely that this system should be the same system as the ATP-induced unselective channel described by Rial's and Guérin's groups. Recent experiments in our laboratory showed that ATP also induced a swelling of mitochondria isolated from Yeast Foam and suspended in hypotonic mannitol solutions (S. Manon, unpublished data).

Electrophysiology of the Inner Membrane

The first attempt to do an electrophysiological study of the inner membrane of yeast mitochondria was done by Szabo *et al.* (1995). These authors presented evidence of a large conductance channel, mostly cationic, and exhibiting several substates. This channel had strong similarities with the peptide-sensitive channel (PSC), previously characterized by the Henry group, which was first localized in the outer mitochondrial membrane (Fèvre *et al.*, 1990) and was further identified as a possible component of the protein-import machinery (Thieffry *et al.*, 1992; Juin *et al.*, 1995). It is very likely that the mitoplast preparation by Szabo *et al.* (1995) still contained enough outer membrane to allow the depiction of this channel.

Two other studies were done by the Kinnally group (Lohret and Kinnally, 1995; Lohret *et al.*, 1996, 1997) and Sorgato group (Ballarin and Sorgato, 1995). The large-conductance channel reported by the Kinnally group was considered an equivalent of PTP (Lohret and Kinnally, 1995) but the main characteristics of PTP, namely, Ca^{2+} induction and cyclosporin A sensitivity, had not been assayed. This conductance is active in mitochondria isolated from a strain devoid of adenine nucleotide carrier (Lohret *et al.*, 1996).

Tim23, a component of the protein-import machinery, is required for the activity of this channel, suggesting that it corresponds to the electric activity of this machinery (Lohret *et al.*, 1997). However, the characteristics of this channel are different from the characteristics of PSC (Juin *et al.*, 1995). The two channels might possibly correspond to the import machinery through the outer and the inner membrane, respectively.

The channel observed by Ballarin and Sorgato (1995) is more relevant to the observations by Rial's and Guérin's groups, since the effect of ATP was assayed. A large-conductance anionic channel was depicted, the opening probability of which was markedly increased by ATP. This conductance probably reflects the electrical activity of the ATP-induced unselective channel observed by swelling experiments. A disagreement was raised by the fact that the site of action of ATP was localized inside the mitochondrion, but the orientation of mitochondrial membranes was not clearly defined.

It may be noted that, in the same study, the authors depicted an anionic channel of lower conductance which was inhibited by ATP (Ballarin and Sorgato, 1995). The correspondance with any channel evidenced by swelling experiments is not clear. Manon and Guérin (1993) observed a swelling of ATP-depleted mitochondria suspended in KCl at alkaline pH (7.8) but failed to observe any further inhibition by ATP. In addition, this swelling did not occur in the presence of NaCl or LiCl, suggesting that it was caused by the opening of a K^+-specific conducting pathway, whereas the conductance evidenced by Ballarin and Sorgato were clearly, although slightly, anionic.

Conclusion

After several years of debate, it can be proposed that the inner mitochondrial membrane of yeast contains an ATP-induced unselective channel. Under non-energetic conditions, this system is mostly anionic (as also shown by electrophysiology experiments), but still able to transport cations (although less efficiently than anions), as well as protons (in laboratory strains). Under slightly hypotonic conditions, this system can function as a pore able to transport neutral molecules. Under energetic conditions (in the presence of a transmembrane potential maintained by the respiratory chain), this system is able to switch to a cation channel.

The site of action of ATP seems to be outside mitochondria. GTP and CTP are almost as efficient as ATP, whereas nonhydrolyzable ATP analogs are not [and, in fact, inhibit the effect of ATP (Roucou *et al.*, 1997a)]. GDP is also able to open the channel but it then works in a different way from the ATP-induced channel, since it is still able to transport cations (in respiring mitochondria) and protons (in mitochondria isolated from laboratory strains), but not gluconate. The effect of GDP on mannitol transport and its ability to open an anionic conductance (measurable by electrophysiology) were not assayed.

Under every condition, decavanadate was a potent inhibitor of this channel, and is likely to compete with nucleotides at their binding site.

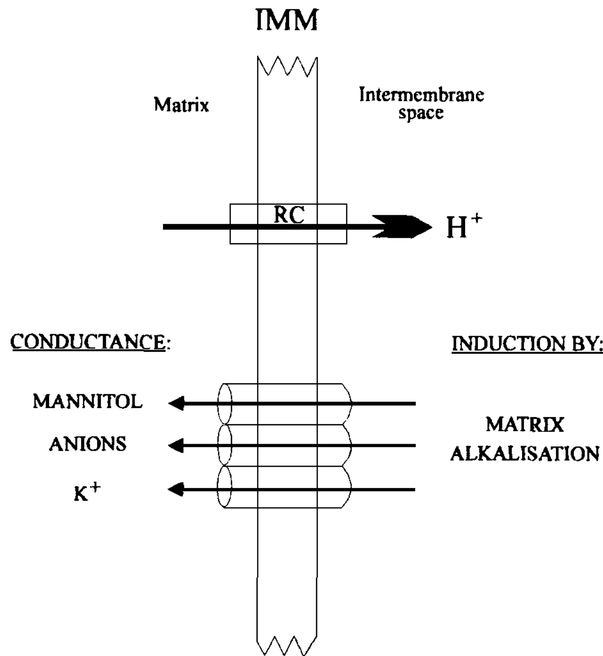
ARE THE RESPIRATION-INDUCED AND THE ATP-INDUCED UNSELECTIVE CHANNELS THE SAME SYSTEM?

The obvious common point between these two permeabilities is their absence of selectivity. Depending on the experimental condition, they can transport cations, anions, protons, and neutral molecules. Jung *et al.* (1997) clearly demonstrated the similitudes between the respiration and ATP-induced mannitol transport.

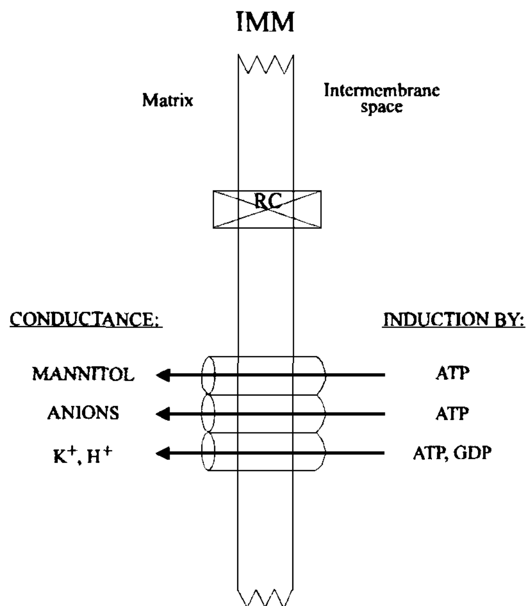
Roucou *et al.* (1997b) distinguished the respiration-induced unselective permeability and the ATP-induced K^+ permeability on the basis of the inhibitory phosphate concentration: the former was inhibited by 0.5 mM phosphate whereas the later was inhibited by 5 mM phosphate. In addition, they observed, as it had also been done by Velours *et al.* (1977), that the respiration-induced permeability was fully inhibited by other permeant anions, such as acetate or propionate, whereas the ATP-induced permeability was only partially inhibited by these permeant acids. Figure 1 summarizes the three different modes of induction of the channel with the corresponding selectivity for cations, protons, anions, and neutral molecules.

It is very likely that both types of permeabilities are caused by the same system with two different modes of regulation. The lack of matrix alkalization is enough to inhibit the respiration-induced permeability, whereas phosphate is required by itself (and not only via the matrix acidifying capacity of its transport) to inhibit the ATP-induced permeability. The difference can be evidenced not only on the basis of phosphate concentration but also on the localization of the action

A. RESPIRATION-INDUCED PERMEABILITIES



B. NUCLEOTIDE-INDUCED PERMEABILITIES IN NON-RESPIRING MITOCHONDRIA



C. NUCLEOTIDE-INDUCED PERMEABILITIES IN RESPIRING MITOCHONDRIA

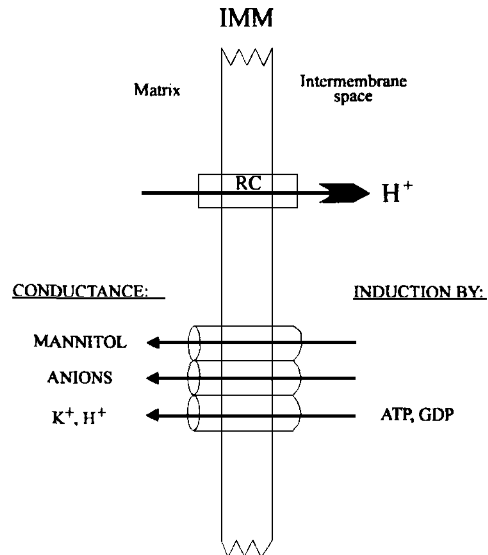
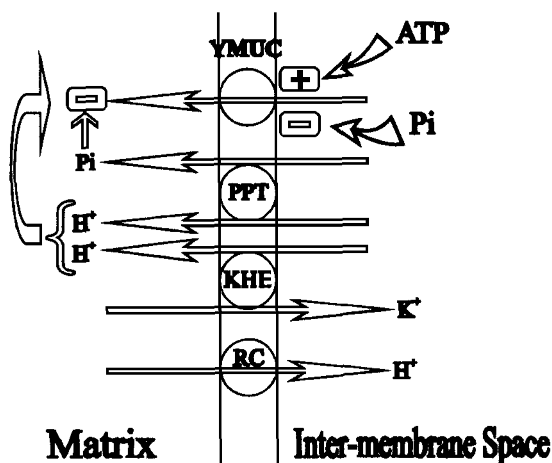
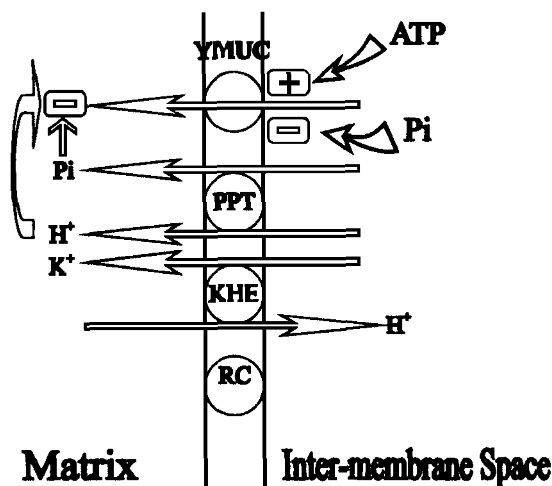


Fig. 1. Representation of the different modes of induction of the YMUC. (A) In the presence of a respiratory substrate and in the absence of phosphate, YMUC is activated by matrix alkalization. In Yeast Foam mitochondria, K^+ would be required to increase matrix alkalization via K^+/H^+ exchange. The cationic pathway is inhibited by low phosphate concentrations (0.5–1 mM); higher phosphate concentrations (5–10 mM) are required to inhibit the anionic and neutral (mannitol) pathways. (B) In the absence of a respiratory substrate, nucleotides activate YMUC, leading to mitochondria swelling in the presence of a salt. Under slightly hypotonic conditions, swelling also occurs without the presence of a salt, because YMUC transports mannitol. Protons may be transported only in mitochondria isolated from laboratory strains. Mg^{2+} specifically inhibits anion transport. Decavanadate and high phosphate concentrations prevent this induction by nucleotides. (C) In the presence of a respiratory substrate and low phosphate concentrations (0.5–1 mM), nucleotides are required to activate the cationic pathway: the electrophoretic uptake of positive charges collapses $\Delta\Psi_m$ and stimulates the respiratory chain. The anionic and neutral pathways are activated by matrix alkalization as shown in (A). Under hypotonic conditions, mannitol is transported by YMUC. Protons may be transported into mitochondria isolated from laboratory strains. Decavanadate and high phosphate concentrations (5–10 mM) prevent the induction by nucleotides. IMM, inner mitochondrial membrane; RC, respiratory chain.

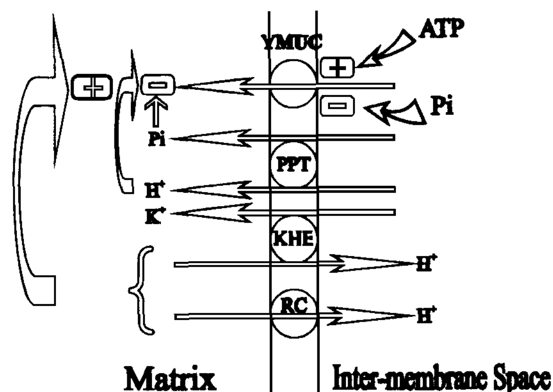
of phosphate. Mersalyl did prevent the inhibition by phosphate of the respiration-induced unselective permeability (Velours *et al.*, 1977; Jung *et al.*, 1997) and of the respiration-induced K^+ transport (Roucou *et al.*, 1997b). On the other hand, it did not prevent the inhibition by phosphate of the ATP-induced gluconate trans-



A. Mitochondria respiring in a low-salt medium



B. Non-respiring mitochondria suspended in a high-salt medium



C. Mitochondria respiring in a high-salt medium

Fig. 2. Schematic representation of the inhibitory effects of phosphate and protons on the different states of YMUC. (A) Mitochondria respiring in a low-salt medium (<60 mM) exhibit an ATP-induced channel activity, which is very efficiently inhibited by phosphate because of the additional inhibitory effects of external and internal $[P_i]$ and internal $[H^+]$. $[H^+]$ are back-transported into the matrix via both the phosphate/H^+ cotransporter (PPT) and the K^+/H^+ exchanger (KHE). (B) Mitochondria suspended in a high-salt medium (>200 mM) in the absence of any activity of the respiratory chain exhibit an ATP-induced channel activity, which is moderately inhibited by phosphate, because the K^+/H^+ exchanger, working now in the reverse direction, no longer participates in matrix acidification. The phosphate/H^+ cotransporter is now the only system allowing this acidification. (C) In a high-salt medium and in the presence of respiration, the addition of the alkalinizing effects of the respiratory chain and the K^+/H^+ exchanger overcomes the capacity of acidification of the phosphate/H^+ cotransporter; the only inhibitor of YMUC is now P_i, which is much less efficient than the combination of P_i and H^+.

port (Guérin *et al.*, 1994), of the ATP-induced mannitol transport (Jung *et. al.*, 1997), and of the ATP-induced K^+ transport (Roucou *et al.*, 1997b). It may be noted that, in only one type of induction, namely the respiration-induced swelling in an isoosmotic solution of salt, phosphate was without any inhibitory effect (Guérin *et al.*, 1994). This correlates with the fact that De Châteaubodeau *et al.* (1974, 1976) presented evidence of a mersalyl-insensitive phosphate transport which, most probably, was very likely caused by the same system. This remains an unresolved problem. It can be hypothesized that the added alkalinizing effects of the respiration and of the K^+/H^+ exchanger overcame the capacity of matrix acidification by the phosphate/H^+ cotransporter. The hypothetical effects of phosphate/H^+ cotransport and K^+/H^+ exchange on YMUC activity under different conditions are schematized in Fig. 2.

Guérin *et al.* (1994) discussed the possibility of a regulation of this system by ΔG_p and Roucou *et al.* (1997a), on the basis of the different characteristics of mitochondria oxidizing different substrates, suggested a regulation by either ΔG_{ox} or the electron flow rate through the respiratory chain. These two aspects still remain a matter of investigation.

IS THE YEAST MITOCHONDRIA UNSELECTIVE CHANNEL RELEVANT TO YEAST BIOENERGETICS?

Most of the work on yeast mitochondria bioenergetics was done on Yeast Foam mitochondria in a mannitol-based medium and at high (5 mM) phosphate concentration. Under these conditions, YMUC is inactive and it is then clear that its existence is irrelevant to the interpretations of the experiments done under these conditions (e.g., ATP regulation of cytochrome-c oxidase (Rigoulet *et al.*, 1987) or stoichiometry changes of the respiratory chain (Ouhabi *et al.*, 1991; Fitton *et al.*, 1994)].

On the other hand, a study was done on the effect of KCl on Yeast Foam mitochondria bioenergetics (Manon *et al.*, 1995). It was found that K^+ stimulated oxidative phosphorylation by the way of K^+ cycling (electrophoretic K^+ entry and electroneutral K^+/H^+ exchange) and a further stimulation of phosphate/H^+ cotransport, a point of high kinetic control of ATP synthesis in yeast mitochondria (Mazat *et al.*, 1986; Beauvoit *et al.*, 1989).

Similar work was done at low phosphate concentration (Manon and Guérin, 1997). It was found that K^+ addition induced an uncoupling of oxidative phosphorylation, which was prevented by decavanadate. No effect of decavanadate could be seen at high phosphate concentration in the absence or in the presence of K^+, or at low phosphate concentration in the absence of K^+. This clearly showed that, under conditions where it is able to work (low phosphate concentration and presence of a salt in Yeast Foam mitochondria), YMUC is able to uncouple oxidative phosphorylation. Do these conditions have any physiological significance? The average phosphate concentration in yeast is 1–2 mM (Beauvoit *et al.*, 1991), not much above the noninhibitory concentration on YMUC. Local variations of this concentration may occur but, until now, we did not obtain clear evidence for such variations to occur or for an activity of YMUC under peculiar conditions, although an effect of ATP on $\Delta\Psi_m$ monitored *in situ* was recently shown (Manon and Guérin, 1998).

IS YMUC THE YEAST EQUIVALENT OF MAMMALIAN PTP?

This is an exciting question about this system. The structural aspects of mammalian PTP still remain a matter of debate. It is obvious that if an equivalent would exist in yeast, it should be a powerful tool for understanding the structure of the system.

The absence of selectivity is evident between PTP and YMUC. Both systems are able to transport all types of molecules and only discriminate on the size of solutes. Jung *et al.* (1997) evaluated the average size of transported molecules to be around 1100 Da, with a maximal size of about 2500 Da, which is in good agreement with values for PTP. Another similarity between both channels is their anionic nature when measured by electrophysiology (Ballarin and Sorgato, 1995), which confirmed the conclusion of swelling experiments (Guérin *et al.*, 1994). In addition, the different selectivity under different conditions (for instance, the GDP instead of the ATP induction; see above) of YMUC suggests that it displays different substates, as also shown for PTP. The mechanisms of transport of molecules by PTP and by YMUC seem, therefore, to be very similar, but their regulations appear to be completely different.

- Ca^{2+}, at physiological concentrations, has definitely no effect on YMUC, even when it is forced to accumulate within mitochondria (Jung *et al.*, 1997). It is certainly not an inducer and it is not an inhibitor, even at relatively high concentrations (1 mM).
- Cyclosporin A, the specific inhibitor of PTP, has no effect on YMUC (Jung *et al.*, 1997). This is of great significance since yeast mitochondria contain a cyclophilin (encoded by the *CYP3* gene), which is known to be involved in the folding of nuclear-encoded mitochondrial proteins (Matoushek *et al.*, 1995).
- Phosphate is a coinductor (with Ca^{2+}) of PTP; it is an inhibitor of YMUC.
- Carboxyatractyloside was shown to induce the opening of PTP, whereas it has no effect (and even inhibits, in Yeast Foam) on YMUC.
- We assayed a large range of pharmacological molecules known to induce or inhibit PTP (e.g., benzodiazepines and pro-oxidants)—none activated or inhibited YMUC (S. Manon, unpublished results).
- The yeast channel is active in strains devoid of VDAC, or of adenine nucleotide carrier, the two main putative components of PTP. This was observed by Roucou *et al.* (1997a) on the ATP-induced K^+ transport and also on the ATP-induced swelling in KCl (X Roucou, unpublished data). This is of special importance. As

mammalian mitochondria, yeast mitochondria display contact sites that essentially contain VDAC and ANC. Considering the hypothesis that contact sites are actually the structure underlying PTP, it is expected that VDAC and ANC should be part of a yeast counterpart to PTP.

From these characteristics, it is clear that the regulations of mammalian PTP and of YMUC are different. However, their functional significance, based on the fact that their opening generally drives to a dramatic collapse of mitochondrial physiology and, therefore, of ATP supply to the cell, can be discussed. The physiological significance of YMUC is not known and the hypotheses, which could be proposed, remain speculative. It is clear, however, that YMUC cannot be involved in cellular Ca^{2+} regulation, which has no evident role in yeast [see the discussion by Welihinda et al. (1993)], or in the other putative pathophysiological roles of mammalian PTP (see below). A possible direct role of YMUC in bioenergetic phenomena, such as matricial volume regulation or uncoupling between cofactor reoxidation and ATP synthesis has been proposed (Guérin *et al.*, 1994; Prieto *et al.*, 1995; Roucou *et al.*, 1997b), but this aspect has not been obviously demonstrated for mammalian PTP. Considering the actual knowledge about this system, an assimilation of YMUC to a counterpart of mammalian PTP remains highly speculative.

A major interest of mammalian PTP, which almost justifies, by itself, all the work done on this system, is its possible involvement in the early steps of apoptosis (Petit *et al.*, 1996; Zamzami *et al.*, 1996, 1997). Although yeast does not support any spontaneous apoptotic phenomenon (but see Madeo *et al.*, 1997), a cellular death can be induced by the expression of proapoptotic mammalian genes such as *bax* (Sato *et al.*, 1994; Hanada *et al.*, 1995; Zha *et al.*, 1996). Moreover, mitochondria seem to be involved in this death (Greenhalf *et al.*, 1996; Zha *et al.*, 1996) and a phenomenon of *bax*-induced cytochrome *c* release has been observed in yeast (Manon *et al.*, 1997) and in mammalian cells (Yang *et al.*, 1997; Kluck *et al.*, 1997; Reed, 1997 for a review). Some investigators tentatively explained the release of cytochrome *c* by the large-amplitude swelling of mitochondria consecutive to the opening of PTP, which should lead to alterations of the outer-membrane structure (Skulachev, 1996; Kantrow and Piantadosi, 1997; Scarlett and Murphy, 1997), anti-apoptotic proteins having the opposite effect (Vander-Heiden *et al.*, 1997). Recent observations in our laboratory showed that *bax*-induced cytochrome *c* release in yeast occurred without any alterations of the inner membrane permeability, including YMUC activity (Priault *et al.*, in press).

CONCLUSION

The inner membrane of yeast mitochondria contains an unselective channel (YMUC) which can be opened by respiration or by ATP (NTP) or GDP under specific conditions. Depending on these conditions, YMUC can transport anions, cations, protons, and neutral molecules. The existence of this channel is clearly irrelevant of previously characterized aspects of yeast mitochondria bioenergetics, which were studied under conditions when it is not activated. However, under the conditions when it can be opened, YMUC dramatically affects oxidative phosphorylation and may lead to a total collapse of mitochondrial physiology. The relevance of these observations to *in vivo* situations is far from being established. Even if YMUC presents some functional analogies with mammalian PTP, its regulation is so different that it can hardly be considered as a model for understanding PTP.

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