Properties of a Cyclosporin-insensitive Permeability Transition Pore in Yeast Mitochondria*

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Yeast mitochondria (Saccharomyces cerevisiae) contain a permeability transition pore which is regulated differently than the pore in mammalian mitochondria. In a mannitol medium containing 10 mM Pi and ethanol (oxidizable substrate), yeast mitochondria accumulate large amounts of Ca\(^{2+}\) (>400 nmol/mg of protein) upon the addition of an electrophoretic Ca\(^{2+}\) ionophore (ETH129). Pore opening does not occur following Ca\(^{2+}\) uptake, even though ruthenium red-inhibited rat liver mitochondria undergo rapid pore opening under analogous conditions. However, a pore does arise in yeast mitochondria when Ca\(^{2+}\) and Pi are not present, as monitored by swelling, ultrastructure, and matrix solute release. Pore opening is slow unless a respiratory substrate is provided (ethanol or NADH) but also occurs rapidly in response to ATP (2 ms) when oligomycin is present. Pi and ADP inhibit pore opening (EC\(_{50}\) 1 and 4 ms, respectively), however, cyclosporin A (7 μg/ml), oligomycin (20 μg/ml), or carboxyatractyloside (25 μM) have no effect. The pore arising during respiration is also inhibited by nigericin or uncoupler, indicating that an acidic matrix pH antagonizes the process. Pi also inhibits pore opening by lowering the matrix pH (P/ OH\(^{-}\) antiport). However, inhibition of the ATP-induced pore by Pi, is seen in the presence of mersalyl, suggesting a second mechanism of action. Since pore induction by ATP is not sensitive to carboxyatractyloside, ATP appears to act at an external site and Pi may antagonize the interaction. Isosmotic polyethylene glycol-induced contraction of yeast mitochondria swelled during respiration, or in the presence of ATP, is 50% effective at a solute size of 1.0–1.1 kDa. This suggests that the same pore is induced in both cases and is comparable in size with the permeability transition pore of heart and liver mitochondria.

Mammalian mitochondria (mMt) contain a permeability transition pore (PTP) that, when opened, allows solutes of molecular mass ≤1.5 kDa to equilibrate across the inner membrane (1–3). An elevated matrix Ca\(^{2+}\) concentration in combination with a second agent (e.g. Pi, or an oxidant of matrix space components) is required for pore opening in most cases. Cyclosporin A (CsA) is a potent inhibitor of the PTP in mMt (mPTP) (2) and may function by binding to a mitochondrial cyclophilin that normally regulates or catalyzes pore opening (4, 5). mPTP opening is also inhibited by adenine nucleotides, Mg\(^{2+}\), H\(^{+}\), relatively positive membrane surface potentials, and high transmembrane potentials (1–4, 6–8). The mPTP has been postulated to form from the opening of a unique and latent channel in the inner membrane, by transformation of a normally selective transporter, or as a highly complex structure formed from the adenine nucleotide translocase, the peripheral benzodiazepine receptor, and the outer membrane voltage-dependent anion channel (1–3, 9–11). Whether or not the mPTP opens in cells under physiological conditions is not known, and evidence for and against this possibility has been reported (12–14). Thus, the function of the mPTP is also not known although it has been postulated to have a role in cellular Ca\(^{2+}\) homeostasis, mMt protein import, thermal regulation, and apoptosis, to name some of the possibilities (3, 15–19).

Several studies have sought to determine if a PTP is present in yeast mitochondria (yMt), with conflicting results. Manon and Guerin (20), investigating electrophoretic pathways of ion transport, reported that Ca\(^{2+}\) is without an effect on the coupling state or swelling and concluded that the existence of a PTP in yMt (yPTP) is uncertain. Szabo et al. (21) applied the patch-clamp technique to isolated yMt and observed a channel with multiple conductance levels (100–600 pS) that is not inhibited by CsA, ADP, or H\(^{+}\). These authors also did not observe Ca\(^{2+}\)-related swelling of yMt and were inconclusive regarding the existence of a PTP (21). In contrast, Lohret and Kinnally (15, 22) described a channel with multiple conductance levels, having a peak conductance of 1.5 nS, in patch-clamped yeast mitoplasts. They concluded that this channel represents the yPTP, or the megachannel to use their nomenclature. In addition, Ballarin and Sorgato (23), also using the patch clamp technique on yeast mitoplasts, described a channel of lower conductance (400–800 pS), which remains open in the presence of ATP.

The present study was undertaken to clarify whether or not a PTP is present in yMt, and subsequently to investigate its regulation. The results show that a PTP is induced in the isolated organelle by respiration or the presence of external ATP. It is inhibited by ADP, is comparable in size to the homologous structure in mMt, but is Ca\(^{2+}\)-independent and not subject to inhibition by CsA. P, which promotes opening of the mPTP, is an inhibitor of the yPTP through an action exerted via the matrix pH, and through a second action exerted at a site that is probably accessible from the extramitochondrial volume. Possible functions of the yPTP and other implications of these findings are considered.

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1 The abbreviations used are: mMt, mammalian mitochondria; CsA, cyclosporin A; FCCP, carbonyl cyanide-4-trifluoromethoxyphenylhydrazone; mPTP, mammalian permeability transition pore; PTP, permeability transition pore; Pi, inorganic phosphate; PEG, polyethylene glycol; ΔpH, pH gradient; Δψ, membrane potential; TEA\(^{+}\), tetraethylammonium cation; yMt, yeast mitochondria; yPTP, yeast permeability transition pore; BSA, bovine serum albumin; S, siemens.
**EXPERIMENTAL PROCEDURES**

**Yeast Culture and the Isolation of Mitochondria**—The yeast strain YPH250 (MATa, ura3-52, lys2-801, ade2-101, trp1-1, his3-Δ200, leu2-3,112) (ATCC 69519) or W303–1A (MATa, ade2–1, his3–11 & 3–15, trp1–1, leu2–3 & 2–112, ura3–1, can1–100) were grown at pH 5 in 1% yeast extract, 2% bacto-peptone, 0.05% dextrose, 0.01% adenine, and either 3% glycerol or 2% lactate. One 4-liter or two 2-liter baffled flasks were incubated at 25 °C in a 3-ml solution of Ca\(^{2+}\) (20, 21). We used the electrophoretic Ca\(^{2+}\) ionophore ETH129 (34, 35) to determine if high internal Ca\(^{2+}\) loads trigger a permeability transition in yMt. A typical experiment is shown in Fig. 1A. When oxidizing ethanol in a medium containing 10 mM Pi, yMt fail to accumulate significant Ca\(^{2+}\) when 160 nmol/mg of protein is added externally (Fig. 1A, trace a). However, a rapid accumulation occurs upon the addition of ETH129, which continues until a steady state is reached at approximately 10 mM external Ca\(^{2+}\) (trace b). Significant swelling does not occur under these conditions, indicating that the inner membrane remains impermeant to low molecular weight solutes (data not shown, and see Fig. 2). Indeed, Ca\(^{2+}\) is retained for extensive periods (Fig. 1A, trace b) and is released only upon uncoupling (trace c) or when oxygen or the oxidizable substrate is depleted. Similar data are obtained at Ca\(^{2+}\) loads of 400 nmol/mg of protein or higher (data not shown).

**Under comparable conditions, rat liver mMt, oxidizing succinate instead of ethanol (Fig. 1B), do not retain Ca\(^{2+}\) regardless of whether initial uptake occurs **via** the endogenous uniporter (trace a, ruthenium red absent (−RRed)), or via ETH129 (trace b, ruthenium red present (+RRed)). The release of Ca\(^{2+}\) from rat liver mMt under either condition is accompanied by swelling and is inhibited by CsA (data not shown), indicating that the mPTP is responsible. These contrasts indicate that yMt do not contain a PTP that is induced by Ca\(^{2+}\) plus Pi, unlike mMt.

**RESULTS**

**Ca\(^{2+}\) Uptake by Isolated yMt—**Intramitochondrial Ca\(^{2+}\) has a central role in regulating the mPTP (1, 3); however, yMt lack a high activity Ca\(^{2+}\) uniporter (20, 33). These circumstances complicate the interpretation of earlier studies that reported the absence of an obvious permeability transition in yMt exposed to Ca\(^{2+}\) (20, 21). We used the electrophoretic Ca\(^{2+}\) ionophore ETH129 (34, 35) to determine if high internal Ca\(^{2+}\) loads trigger a permeability transition in yMt. A typical experiment is shown in Fig. 1A. When oxidizing ethanol in a medium containing 10 mM Pi, yMt fail to accumulate significant Ca\(^{2+}\) when 160 nmol/mg of protein is added externally (Fig. 1A, trace a). However, a rapid accumulation occurs upon the addition of ETH129, which continues until a steady state is reached at approximately 10 mM external Ca\(^{2+}\) (trace b). Significant swelling does not occur under these conditions, indicating that the inner membrane remains impermeant to low molecular weight solutes (data not shown, and see Fig. 2). Indeed, Ca\(^{2+}\) is retained for extensive periods (Fig. 1A, trace b) and is released only upon uncoupling (trace c) or when oxygen or the oxidizable substrate is depleted. Similar data are obtained at Ca\(^{2+}\) loads of 400 nmol/mg of protein or higher (data not shown).

Under comparable conditions, rat liver mMt, oxidizing succinate instead of ethanol (Fig. 1B), do not retain Ca\(^{2+}\) regardless of whether initial uptake occurs via the endogenous uniporter (trace a, ruthenium red absent (−RRed)), or via ETH129 (trace b, ruthenium red present (+RRed)). The release of Ca\(^{2+}\) from rat liver mMt under either condition is accompanied by swelling and is inhibited by CsA (data not shown), indicating that the mPTP is responsible. These contrasts indicate that yMt do not contain a PTP that is induced by Ca\(^{2+}\) plus Pi, unlike mMt.

**The capacity of yMt to carry out ETH129 mediated Ca\(^{2+}\) accumulation is dependent upon the medium Pi concentration as shown in Fig. 2. In the presence of 10 mM Pi, Ca\(^{2+}\) is accumulated and retained for over 50 min, but when Pi is reduced to 1 mM significant accumulation is not seen (Fig. 2A). Parallel experiments using safranine to estimate ΔΨ showed that in the presence of 1 mM Pi, yMt respiring on ethanol.
concentration was 25 mM.

panel A

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to enter the matrix space, with swelling arising from an accu-

presence of a CsA-insensitive PTP in yMt that allows mannitol

inner membrane (26, 36). These data are consistent with the

and swelling (panel B), which is an antibiotic that forms large pores in the mMt

experiments revealed that neither exogenous Ca2+ nor ETH129 are required to induce swelling of yMt and that the
depletion of endogenous Ca2+ using ionomycin plus an external
chelator is without effect (data not shown). Thus, yPTP opening
is apparently a Ca2+-independent process. However, respiration
and P, play central roles in regulating the yPTP. Swelling
occurs slowly in the absence of P, and an exogenous respiratory
substrate but accelerates dramatically upon the addition of
ethanol (Fig. 4A). The same result is obtained when exogenous
NADH is used in place of ethanol (data not shown). Antimycin
A prevents the accelerated swelling produced by either substrate
(data not shown) indicating that it is respiration/energi-

Fig. 2. Effect of P, concentration on yMt Ca2+ uptake (panel A)
and swelling (panel B). Conditions were the same as described for
Fig. 1. panel A, except that ethanol was omitted initially, the EGTA
concentration was 25 μM, the protein concentration was 0.35 mg/ml,
and the KH2PO4 concentration was either 1 or 10 mM as indicated.
Additions where indicated were as follows: a, CaCl2 (285 nmol/mg
protein); b, ETH129 (2.4 μM); and c, ethanol (0.5 mM). For panel B, in
addition to the components described above, the medium contained CsA
(1.67 μM) for one of the two experiments conducted at 1 mM P1.
For the experiment conducted at 10 mM P1, alamethicin was added where indi-
cated at 2.6 μg/ml.

develop a small ΔΨ which decays to no measurable value
within 60 s (detection limit ~80 mV). In contrast, a large ΔΨ
is generated and maintained when the medium contains 10 mM P1
(data not shown). The fact that ΔΨ is not maintained makes
the absence of Ca2+ uptake in the medium containing 1 mM P1,
but it is not clear immediately why yMt depolarize under that
condition. CsA does not substitute for the high P1 concentra-
tion, suggesting that depolarization and the failure to accumu-
late Ca2+ at 1 mM P1 is unrelated to a yPTP (Fig. 2A). Never-
theless, parallel swelling and related experiments yield the
opposite conclusion, as follows.

While no swelling is associated with Ca2+ accumulation at
10 mM P1, a CsA-insensitive swelling is apparent at 1 mM P1
(Fig. 2B). The magnitude of swelling, expressed as ΔA460
is relatively small, compared with mPTP-dependent swelling of
mMt (e.g. Ref. 26). However, it is comparable with the swelling
induced by alamethicin (compare Fig. 2B, trace a with traces b
and c), which is an antibiotic that forms large pores in the mMt
inner membrane (26, 36). These data are consistent with the
presence of a CsA-insensitive PTP in yMt that allows mannitol
to enter the matrix space, with swelling arising from an accu-
mulation of mannitol/water driven by the Donnan potential
and the oncotic pressure differential. Fig. 3 confirms this in-
terpretation by showing that the swelling seen in Fig. 2B
reflects ultrastructural changes characteristic of the perme-
ability transition in mMt (32, 37, 38). In addition, marked
release of matrix nucleotides is also seen, as expected if swell-
ing results from the opening of a large and solute-nonselective
pore (e.g. Ref. 8). We interpret these findings to indicate that
yMt contain an endogenous PTP.

Respiration and ATP Induce Opening of the yPTP—Addi-
tional experiments revealed that neither exogenous Ca2+ nor
ETH129 are required to induce swelling of yMt and that the
depletion of endogenous Ca2+ using ionomycin plus an external
chelator is without effect (data not shown). Thus, yPTP opening
is apparently a Ca2+-independent process. However, respiration
and P, play central roles in regulating the yPTP. Swelling
occurs slowly in the absence of P, and an exogenous respiratory
substrate but accelerates dramatically upon the addition of
ethanol (Fig. 4A). The same result is obtained when exogenous
NADH is used in place of ethanol (data not shown). Antimycin
A prevents the accelerated swelling produced by either substrate
(data not shown) indicating that it is respiration/energi-

PEG-induced Contraction of Swollen yMt—The size of the
mPTP has been estimated by observing the ability of different
size PEG to inhibit swelling (26) or to induce contraction of
previously swollen mMt, (e.g. Ref. 29). We used the latter
approach to further examine the solute size-exclusion prop-
erties of the yPTP. Ethanol-supported respiration or exogenous
ATP was used to open the yPTP initially. After swelling
reached completion, PEG of various molecular weights were
added under isoosmotic conditions to determine their effective-
ness in causing contraction. Small PEG (0.4 and 0.6 kDa)
readily penetrate through the open yPTP since little or no
contraction is observed upon their addition (Fig. 6A). Contraction
is seen with larger PEG, indicating that they are excluded
or pass through the yPTP at a much slower rate. Although the
rate of contraction declines for PEG larger than 1.5 kDa (Fig.
6A), the extent of contraction that is ultimately attained is
similar for PEG of 2 kDa and larger. Plotting the maximum
extent of contraction versus PEG size indicates a half-maximal
effect at ~1.1 kDa when yMt have swollen during respiration
on ethanol (Fig. 6B) or in response to ATP (data not shown).
This value is virtually identical to that reported for the PEG-
induced contraction of beef heart mMt following mPTP opening
induced by Ca2+ (29). It is somewhat greater than the values
obtained by examining PEG-dependent inhibition of swelling in
rat liver mMt when mPTP opening is induced by Ca2+ plus
phosphate, or by mastoparan. In these cases, a half-maximal
effect is obtained at 0.65 and 0.95 kDa PEG, respectively (26).

It is not apparent why the rate of contraction decreases
progressively with PEG larger than 1.5 kDa, but the same
effect is seen with mMt (31). A reduced rate indicates that the
membrane has become less permeable to internal solutes. Thus, fewer PTPs may be open at a given time when the larger PEGs are present. Since pretreatment in the present experiments was the same for PEG of all sizes, the same number of yPTPs should be open initially. Accordingly, the larger PEG may reduce the yPTP open probability through a physical-chemical mechanism (39). Alternatively, the reduced rate of contraction might result from a decreased penetration of the

![Ultrastructural changes and matrix nucleotide depletion accompanying swelling of yMT. Panels A and B show the ultrastructure of yMT following preparation and ethanol-induced swelling, respectively (bar shown in panel A = 1 μm). Panel C shows the matrix nucleotide profile, obtained by high performance liquid chromatography, under the same two conditions.](image)

**FIG. 3.** Ultrastructural changes and matrix nucleotide depletion accompanying swelling of yMT. Panels A and B show the ultrastructure of yMT following preparation and ethanol-induced swelling, respectively (bar shown in panel A = 1 μm). Panel C shows the matrix nucleotide profile, obtained by high performance liquid chromatography, under the same two conditions.

![Ethanol oxidation and ATP induce the permeability transition in yMt. Panel A, the medium contained 230 mM mannitol, 70 mM sucrose, 10 mM HEPES (Na⁺), pH 7.3, 25 μM EGTA (TEA⁻), 0.5 mg/ml BSA, and 10 μg/ml of oligomycin. Where indicated, 10 mM KH₂PO₄ was also present. yMt (0.86 mg of protein/ml) were added at 30 s, followed at 90 s by no addition (control), 2 mM ATP (Na⁺), or 0.5 mM ethanol, as indicated in the figure. Panel B, data were obtained from experiments analogous to those shown in panel A except that the Pᵢ concentration was adjusted to the values indicated on the x axis. The y axis values were calculated from the extents of swelling at 360 s, taking the absorbance value obtained with 10 mM Pᵢ and uncoupler present, but ATP or ethanol absent, to represent 100% inhibition. The absorbance values obtained at 360 s, with ATP or ethanol present and Pᵢ absent, represented no inhibition. ○, swelling induced by 2 mM ATP (Na⁺); ●, swelling induced by respiration (0.5 mM ethanol).](image)

**FIG. 4.** Ethanol oxidation and ATP induce the permeability transition in yMt. Panel A, the medium contained 230 mM mannitol, 70 mM sucrose, 10 mM HEPES (Na⁺), pH 7.3, 25 μM EGTA (TEA⁻), 0.5 mg/ml BSA, and 10 μg/ml of oligomycin. Where indicated, 10 mM KH₂PO₄ was also present. yMt (0.86 mg of protein/ml) were added at 30 s, followed at 90 s by no addition (control), 2 mM ATP (Na⁺), or 0.5 mM ethanol, as indicated in the figure. Panel B, data were obtained from experiments analogous to those shown in panel A except that the Pᵢ concentration was adjusted to the values indicated on the x axis. The y axis values were calculated from the extents of swelling at 360 s, taking the absorbance value obtained with 10 mM Pᵢ and uncoupler present, but ATP or ethanol absent, to represent 100% inhibition. The absorbance values obtained at 360 s, with ATP or ethanol present and Pᵢ absent, represented no inhibition. ○, swelling induced by 2 mM ATP (Na⁺); ●, swelling induced by respiration (0.5 mM ethanol).
larger PEG through channels in the outer membrane since the relationship between swelling and outer membrane rupture has not been determined for yMt.

The pH dependence of contraction induced by 1.5 kDa PEG in the absence of Pi is shown in Fig. 7. Although ethanol-induced swelling is relatively independent of pH (Fig. 5), the rate of subsequent PEG-induced contraction displays a significant dependence (initial rate), increasing as the pH becomes more basic (Fig. 7). This is also true for contraction following spontaneous swelling (ethanol and ATP absent), however, contraction of ATP-swollen yMt is relatively pH independent (Fig. 6). The modest effect of pH on contraction in the presence of ATP is in contrast to ATP-induced swelling, which is pH-dependent (Fig. 5). At the highest pH examined (7.75), contraction was rapid regardless of how an open PTP was initially attained.

**Actions of P_i on the yPTP Are Related to Matrix pH**—The effects of medium pH on the swelling and contraction of yMt raise the possibility that the inhibitory action of P_i results from acidification of the matrix space. As in mMt, P_i is transported into yMt via a P_i/OH antiport, resulting in matrix acidification (40). To determine if a decreased matrix pH plays a role in the inhibitory action of P_i, the actions of nigericin or an uncoupler (FCCP) on yPTP opening were examined. Nigericin causes matrix acidification by exchanging internal K^+ for external H^+.

**Fig. 5. Effect of medium pH on PTP-dependent swelling of yMt.** Conditions were the same as described in the legend to Fig. 4 except for the medium pH, which was adjusted to 6.80, 7.25, or 7.75 as indicated in the figure. yMt (0.5 mg of protein/ml) were added at 30 s followed by, at the arrows, no addition (control), 2 mM ATP (Na^+), or 0.5 mM ethanol, as indicated in the figure. Alamethicin was added at 3 μg/ml, shortly after ethanol during one of the experiments conducted at pH 6.80, as also shown in the figure.

**Fig. 6. Solute size exclusion properties of the yPTP.** The medium contained 300 mM mannitol, 10 mM HEPES (TEA^+), pH 7.35, 0.2 mM EGTA (TEA^+), 0.5 mM BSA, and 17 μg of oligomycin/mg of protein. Panel A, yMt (1.9 mg/ml) were induced to swell initially by the addition of 0.5 mM ethanol (see Fig. 3A). At 480 s, PEG of the indicated average molecular mass (in kDa) were added, and contraction was observed. The volumes and PEG concentrations were adjusted to keep the osmotic pressure constant at 300 mosM, with 30 mosM derived from PEG, as further described under “Experimental Procedures.” Measurements were extended beyond the time period shown in panel A, until contraction reached the maximum value. Panel B, the maximum values are plotted as a function of the PEG size and fit to an expression for a sigmoidal curve (solid line), illustrating that a half-maximal effect (C_50) is obtained at ~1.1 kDa PEG.
to those of uncoupler in all cases (data not shown). Mersalyl, an inhibitor of P/OH_2 and Pi/dicarboxylate antiport in yMt (41, 42), does not prevent inhibition of ATP-induced swelling by Pi but diminishes the inhibition that is seen when swelling is promoted by respiration or occurs under the spontaneous condition (data not shown).

These results support a role for matrix acidification, via Pi/OH_2 antiport activity, in the mechanism by which Pi antagonizes yPTP opening during respiration or under the spontaneous condition. However, the failure of mersalyl to diminish the inhibitory effect of Pi when the yPTP is induced by ATP suggests that an external site is also be involved.

Other Effectors of Swelling and Contraction—Several agents that influence the mPTP were tested on yMt by monitoring swelling and subsequent PEG-induced contraction. As indicated above, CsA (up to 7 μg/ml) does not inhibit the swelling of yMt induced by ethanol or ATP or PEG-induced contraction in the presence of either agent. Neither ATP- nor respiration-induced swelling is affected by oligomycin (20 μg/mg of protein), which inhibits the yMt ATP synthase (43) and antagonizes induction of the mPTP by Ca^{2+} plus Pi (1).

Carboxyatractyloside (25 μM), an inhibitor of the adenine nucleotide translocase (44) that favors opening of the mPTP (1, 8, 45), has little or no effect on ATP-induced swelling of yMt but inhibits ethanol-induced swelling by 10–15% (Fig. 8, A and B). DCCD (50 nmol/mg of protein), which can inhibit or activate the mPTP depending upon conditions (46, 47), decreased swelling and contraction after a relatively long preincubation period (60 min) but not following a shorter period (25 min). Mg^{2+} (2 mM), which favors the closed form of the mPTP (1, 8, 45), prevents the spontaneous swelling of yMt but not ATP-induced swelling (data not shown).

ADP favors the closed form of the mPTP (1, 8, 45) and was found to inhibit both ethanol- and ATP-induced swelling of yMt, with a half-maximal effect obtained at ~4 mM (Fig. 9). Carboxyatractyloside (25 μM) does not alter the inhibitory action of ADP when yPTP opening is induced by ATP (Fig. 9B) but enhances the effectiveness when opening is induced by respiration (Fig. 9A). In addition, ADP (4 mM) is as effective as FCCP at inhibiting the spontaneous swelling that occurs in the absence of ethanol or ATP (Fig. 9A). The rate of PEG-induced contraction of ethanol-swollen yMt is somewhat inhibited by
ADP, but there is little effect on the contraction of yMt that have swollen in response to ATP (Fig. 9D).

**DISCUSSION**

**yMt Contain a PTP**—The present study demonstrates that a permeability transition can be induced in yMt, as indicated by swelling, PEG-induced contraction, ultrastructural changes, and the release of matrix space solutes of substantial size (nucleotides). Thus, these data resolve the controversy regarding the existence of a yPTP. The yPTP opens when yMt are allowed to respire, or are incubated in the presence of ATP, when Pi is absent (Fig. 4). The magnitude of the swelling response that follows is 20–25% of that typically seen with rat liver mMt under analogous conditions but coincides with other reports on the optical properties of swollen yMt (48). Reduced swelling is not due to a failure of a PTP to open in a large fraction of yMt as shown by ultrastructural changes, the marked release of matrix nucleotides (Fig. 3), and by the swelling response to alamethicin, which is the same as that produced by opening the yPTP (Fig. 2). We attribute the reduced swelling to ultrastructural differences between yMt and mMt. The former have relatively few cristae (e.g. Ref. 43 and Fig. 3), which limits the increase in matrix space volume, and the resultant change in $A_{540}$, which is possible when the yPTP is opened.

The absence of a Ca$^{2+}$ requirement and insensitivity to CsA appear to have been factors causing uncertainty in previous studies that sought to determine if yMt contain a PTP. These properties are analogous to the mPTP induced by mastoparan, which is also Ca$^{2+}$-independent and CsA-insensitive under some conditions (26). Caution is warranted in such cases when ascribing swelling to a PTP because smaller channels could be responsible. Accordingly in this study, as with mastoparan (26), the solute size exclusion method was also used to characterize the PTP. The data obtained further support the existence of a yPTP, comparable in size with the mPTP and of the same size when induced by ethanol oxidation or ATP. ADP was added shortly before PEG following the completion of swelling, which occurred under the same conditions described for panels A and B, traces a.

**Regulation of the yPTP**—The present results show that at a minimum, the yPTP is regulated by adenine nucleotides, pH, and Pi. Regarding nucleotides, the site at which ATP acts to promote opening of the yPTP is directly accessible from the external volume, based upon the failure of carboxyatractyloside to alter the potency of ADP as an inhibitor for the mPTP (8). The inhibition of ATP-dependent yPTP opening by ADP can be explained as a competitive binding interaction at the putative allosteric site, with ADP being an ineffective promoter. This interpretation is supported by the failure of carboxyatractyloside to alter the potency of ADP as an inhibitor of yPTP opening induced by respiration (Fig. 9B). However, since ADP also inhibits yPTP opening induced by respiration (Fig. 9A), it is probable that ADP is an authentic inhibitor, as opposed to having a neutral effect when associated with the external site. In the case of yPTP induction by respiration, carboxyatractyloside enhances the inhibitory action of ADP (Fig. 9A). Thus, further investigation may show that adenine nucleotides influence the yPTP through both internal and external sites, as with the mPTP (8).

The influence of respiration and the actions of Pi and pH on the yPTP appear to be closely related, with matrix space pH representing the actual regulatory parameter. Both swelling and contraction data support this interpretation. As regards swelling, the acceleration that is seen as the medium pH rises (Fig. 5) could result from an action exerted on either side of the membrane. However, proton pumping during respiration increases matrix pH while the external pH is unaffected, and respiration promotes opening of the yPTP (Fig. 4A).
tion, nigericin, uncoupler, and external Pi inhibit yPTP opening induced by respiration, and these agents have in common the action of reducing the rise in matrix pH that results from respiration.

The rate of contraction also increases as the pH rises (Fig. 7), however, the relative rates in the presence of ethanol versus ATP are reversed at lower pH, compared with what is seen during swelling (Fig. 5). This difference also supports a central importance of matrix pH in regulating the yPTP, as follows. With ATP acting at an external site to cause opening through a mechanism that is relatively pH-insensitive, it is expected that the site would remain occupied after swelling is complete. In that case, PEG-induced contraction should proceed readily, as observed (Fig. 7), since the yPTP would remain open. No effect of uncoupler on the contraction rate would be expected, and none is seen (Fig. 8B). With ethanol promoting opening through an increase in matrix pH, a reduced open probability is expected after the PTP opens initially since the matrix pH would fall toward the medium value. A reduced and medium pH-dependent rate of contraction would then be expected and is observed (Fig. 7). With the yPTP subject to a reduced open probability and with respiration continuing, uncoupler might further inhibit contraction since it would reduce any transient recovery of an elevated matrix pH during periods when the yPTP was closed. This effect of uncoupler is also observed (Fig. 7B).

Thus, all aspects of the present data are consistent with respiration, medium pH, and Pi regulating the yPTP via changes in matrix pH. However, as noted under “Results,” the inhibition of ATP-dependent yPTP opening by Pi, when mersalyl is present suggests an external site of action for Pi, and further investigation of this point will be necessary.

Attempts to quantitate the relationship between yPTP opening and matrix pH by fluorescent indicator methods were unsuccessful because neither BCECF or cSNARF-1 was loaded into the matrix of yMt when presented as membrane permeant esters. However, previous reports show that Pi has protective effects on yMt under numerous conditions. For example, Verlours et al. used electron microscopy to show that yMt oxidizing external NADH swell and are otherwise altered when Pi is absent (48). Although unrecognized at the time, the ultrastructural changes were reflecting the permeability transition. The same study showed that ultrastructural changes are not seen in medium containing 10 mM Pi, and attributed that effect to a decreased matrix pH (48). The acetate distribution method was used subsequently to show that a decrease in matrix pH is seen when Pi is added to respiring yMt (49). Thus, the change in matrix pH that is required to induce or inhibit opening of the yPTP is small, on the order of ± 0.5 units when the external pH is near neutrality.

When regulation of the yPTP by nucleotides, Pi, and pH is compared with the effects of these parameters on the mPTP, both similarities and differences are apparent. There are no reports of ATP favoring the open form of the mPTP, although ADP favors the closed form (8) in common with the yPTP. Basic pH has the same effect on the PTP from both sources although, with the mMt, other factors are apparently overriding since there is no requirement to reduce ΔpH to maintain a closed PTP in respiring mMt. Indeed, Pi favors opening of the mPTP although it is not clear if this is a direct effect or related to other regulatory parameters.

Of several other agents known to influence the mPTP by direct or indirect mechanisms, none were found to effect the yPTP in an analogous way. These include Ca²⁺, Mg²⁺, oligomycin, carboxyatractysloside, DCCD, and CsA. This suggests that regulation of the yPTP is greatly simplified compared with the mPTP. The lack of inhibition by CsA is of particular interest because with mMt it is thought that the potent inhibitory action of CsA reflects its binding to a matrix cyclophilin (5, 50, 51). This in turn has lead to the proposal that cyclophilin is a structural component of the mPTP (51) or catalyzes mPTP opening via its peptide bond isomerase activity (4, 8). Yeast contain at least five cyclophilins, one of which (cpr3p) is localized in yMt (52). This cyclophilin has been shown to mediate the folding of imported proteins in CsA-sensitive fashion (53).

Thus, the failure of CsA to inhibit opening of the yPTP is not related to an absence of this target protein or to a failure of CsA to inhibit its peptide bond isomerase activity. It may indicate that the PTP in yMt and mMt are unrelated, or that the involvement of cyclophilin in forming/regulating the mPTP evolved relatively recently.

Possible Relationships between the yPTP, ATP-induced Ion Transport Pathways, and Ion-conducting Channels in yMt—

ATP-induced ion transport pathways for cations and anions in yMt have been described by three different laboratories (54–58). Prieto et al. (56, 58) reported that ATP activates an anion-conducting pathway by binding to a site on the outer surface of the inner membrane. Guerin et al. (54) described an ATP-induced nonspecific pathway that gives rise to swelling in K⁺ or Na⁺ salts of gluconate, glutamate, chloride, or acetate when the medium pH is neutral. The ATP-induced swelling was inhibited by ADP, apparently by acting at the ATP-binding site on the external side of the inner membrane (54). Roucou et al. (55, 57) described an ATP-induced electrophoretic K⁺ transport pathway in respiring yMt. It differs from the one described by Prieto et al. (56, 58) in that it requires KCl. Unlike the nonspecific activity (54), the latter activity is specific for K⁺ over Na⁺ while the ATP regulatory site is located in the matrix (57). In similarity to the yPTP described here, each of these ATP-induced transport pathways is inhibited by Pi. Thus, it seems possible that one or more of these is related to the yPTP.

The same is true as regards the ion conducting channels in yMt that are observed by patch clamp techniques since the larger examples display conductance values similar to those of the mPTP (15, 21–23). In addition, ATP favors the open forms although the site is on the matrix side (21, 23), and additional regulatory features do not parallel those of the yPTP reported here. The substantial differences in techniques, preparations, and conditions that were used in these studies, and those involving ATP-regulated ion transport, discourage more specific cross-comparisons at present. However, it is worthwhile to note the mPTP displays substrates that are solute selective under some circumstances (4, 59). Thus, further investigation of the yPTP and its relationship to other ion transporters and channels in yMt is warranted.

Potential Physiological Functions—As with the mPTP, the physiological function of the yPTP is not clear, but it is useful nevertheless to consider possibilities. The differences in regulatory features of the yPTP and mPTP may indicate that the functions are not equivalent and may also reflect the differing environmental challenges faced by yeast and mammalian cells. If only the regulators identified in this study are considered, the yPTP would be expected to open if the cell attained a high phosphorylation potential. During growth on a nonfermentable carbon source, the resultant uncoupling could provide a mechanism to dispose of excess reducing equivalents generated during carbon flux into the biosynthetic pathways. Rial and co-workers (56, 58) propose this function for their ATP-regulated proton conducting pathway, which may be related to the yPTP (or a substate) as noted above. Lohret and Kinally (15) propose that the yeast megachannel is involved in protein import since leader peptides decrease the open probability. This pos-
sibility also applies to the yPTP since these structures may again be related. Additional regulators of all these structures will probably be identified and such studies should help to test these potential functions and interrelationships.

It is also possible the yPTP remains closed during rapid growth but is present to facilitate a response to changing conditions, such as a change of carbon source or the onset of a nutrient deficiency. Such environmental factors initiate complex signaling cascades (60) that cause the dynamic adjustment of mitochondrial properties, including structure, metabolic capacity, location, and numbers per cell (61, 62). In cells grown on a nonfermentable carbon source, for example, yMt represent 10–13% of cell volume but only ~3% in cells grown on glucose (62). During adaptation, only 1 h of glucose repression produces a fraction of yMt that are swollen and contain few cristae (63). Although the underlying mechanisms are not known, opening the yPTP may well be involved since this produces a collapse of ion gradients and swelling. In this way, the yPTP might function as the target of signals that terminate yMt function and initiate degradation/nutrient recycling.

A role for the yPTP in yeast cell death can also be envisioned since mPTP is implicated in mammalian cell apoptosis (e.g. Refs. 17–19), where members of the Bcl-2 protein family influence opening of the mPTP (64) and the progression to death (18, 65–67). As shown here, these components rescue them, suggesting that components of mammalian systems may be related. Additional regulators of all these structures will probably be identified and such studies should help to test these potential functions and interrelationships.

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