Catalases and thioredoxin peroxidase protect *Saccharomyces cerevisiae* against Ca^{2+}-induced mitochondrial membrane permeabilization and cell death

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Abstract The involvement of reactive oxygen species in Ca^{2+}-induced mitochondrial membrane permeabilization and cell viability was studied using yeast cells in which the thioredoxin peroxidase (TPx) gene was disrupted and/or catalase was inhibited by 3-amino-1,2,4-triazole (ATZ) treatment. Wild-type *Saccharomyces cerevisiae* cells were very resistant to Ca^{2+} and inorganic phosphate or *t*-butyl hydroperoxide-induced mitochondrial membrane permeabilization, but suffered an immediate decrease in mitochondrial membrane potential when treated with Ca^{2+} and the dithiol binding reagent phenylarsine oxide. In contrast, *S. cerevisiae* spheroblasts lacking the TPx gene and/or treated with ATZ suffered a decrease in mitochondrial membrane potential, generated higher amounts of hydrogen peroxide and had decreased viability under these conditions. In all cases, the decrease in mitochondrial membrane potential could be inhibited by ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid, dithiothreitol or ADP, but not by cyclosporin A. We conclude that TPx and catalase act together, maintaining cell viability and protecting *S. cerevisiae* mitochondria against Ca^{2+}-promoted membrane permeabilization, which presents similar characteristics to mammalian permeability transition.

Key words: Mitochondrion; Yeast; Antioxidant; Mitochondrial permeability transition; Cell death

1. Introduction

The disruption of Ca^{2+} homeostasis plays a major role in the pathogenesis of cell injury in situations such as ischemia/reperfusion, xenobiotic poisoning, necrosis and apoptosis [1]. Under these conditions, cell death may be promoted by the Ca^{2+}-stimulated activity of cytosolic catalytic enzymes such as phospholipases [2]. However, in at least some cell death models, Ca^{2+} accumulation by mitochondria plays an essential role and inhibition of mitochondrial Ca^{2+} uptake prevents cell death, although it results in a more intense disruption of Ca^{2+} homeostasis [3].

In mammalian mitochondria, excessive Ca^{2+} accumulation promotes an inner mitochondrial membrane permeabilization, allowing solutes of molecular mass below 1.5 kDa to pass across the membrane (for reviews, see [4–6]). This membrane permeabilization is caused by the opening of a non-selective pore, which is promoted by Ca^{2+} and stimulated by an inducing agent such as inorganic phosphate (P_i) or an oxidant. Cyclosporin A is a potent inhibitor of this process, known as mitochondrial permeability transition (MPT). Based on detailed studies using different MPT inducers and inhibitors, we have determined that MPT is caused by the oxidation and cross-linkage of mitochondrial membrane protein thiol groups [5,7–10]. These alterations of mitochondrial membrane thiols may be caused directly by dithiol binding reagents, which are efficient MPT inducers [10], or by reactive oxygen species (ROS) generated at the mitochondrial respiratory chain [7,9]. Indeed, a considerable inhibition of mitochondrial membrane protein oxidation and MPT was observed when mitochondrial suspensions were incubated in the presence of substances which exhibit H_2O_2 removal activity, such as catalase, thioredoxin peroxidase (TPx) and ebselen [9].

Mitochondrial dysfunction related to oxidative stress and MPT has been shown to promote cellular necrosis under conditions such as ischemia/reperfusion or exposure to the tumor necrosis factor (for review, see [5,6,11]). Recently, the hypothesis that MPT may also be an initial event in apoptotic cell death has been presented. It has been determined that apoptotic cell death following MPT may occur through two mechanisms: (i) cytochrome *c* released from mitochondria during MPT can induce the activation of cytosolic caspases, resulting in nuclear DNA fragmentation [12–15], and/or (ii) the release of a mitochondrial intermembrane protein (the apoptosis inducing factor) causes nuclear DNA fragmentation [16]. Interestingly, in addition to preventing MPT [9], TPx is an efficient inhibitor of apoptosis in several experimental models [17], a finding that further supports the link between excessive mitochondrial ROS generation, MPT and cell death [5].

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Abbreviations: ATZ, 3-amino-1,2,4-triazole; EGTA, ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; DTT, dithiothreitol; FCCP, carbonyl cyanide *m*-clorophenyl-hydrazone; HEPES, *N*-(hydroxyethyl)piperazine-*N′*-(4-butanesulfonic acid); MPT, mitochondrial permeability transition; ROS, reactive oxygen species; WTSC, wild-type *Saccharomyces cerevisiae* spheroblasts; ΔTSASC, *Saccharomyces cerevisiae* spheroblasts lacking thioredoxin peroxidase; ATZ–WTSC, 3-amino-1,2,4-triazole-treated wild-type *Saccharomyces cerevisiae* spheroblasts; ATZ–ΔTSASC, 3-amino-1,2,4-triazole-treated *Saccharomyces cerevisiae* spheroblasts lacking thioredoxin peroxidase; P_i, inorganic phosphate; *t*-bOOH, *t*-butyl hydroperoxide; PhAsO, phenylarsine oxide; TPx, thioredoxin peroxidase

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In this work, we wished to verify the effect that naturally occurring antioxidants such as TPx and catalase present on the maintenance of mitochondrial integrity and cell viability. To do so, we chose to study Ca^{2+}-induced injury to the mitochondria of *Saccharomyces cerevisiae* cells, due to the ready availability of a strain lacking the TPx gene (*TSA1*). We characterized a Ca^{2+}-dependent permeabilization of the mitochondrial inner membrane in *S. cerevisiae* and studied its inhibition by TPx and catalase. Our results indicate that ROS mediate this permeabilization and that these reactive species may also be involved in Ca^{2+}-induced loss of cell viability.

2. Materials and methods

2.1. Yeast strain and culture conditions

The *TSA1* gene which encodes TPx was disrupted in the JD7-7C *S. cerevisiae* strain by Chae et al. [18]. The JD7-7C strain with the *TSA1* gene disrupted is referred to herein as ΔTSASC, while the wild-type strain is referred to as WTSC. Inoculates from WTSC and ΔTSASC were prepared by dilution of fresh yeast colonies from plates into liquid YPD medium (yeast extract, peptone and 2% glucose). Yeast catalases were specifically inhibited by addition of 3-amino-1,2,4-triazole (ATZ) (1 mM) into the inoculates [19]. Treatment of yeast with higher ATZ concentrations (up to 5 mM) did not significantly change the experimental results, indicating that ATZ (1 mM) is at saturating concentrations. The wild-type and mutant cells in which catalase was inhibited by ATZ are referred to herein as ATZ–WTSC and ATZ–ΔTSASC, respectively. Cells were incubated overnight at 30°C, with shaking at 250 rpm. The yeast cells obtained were further processed as described below.

2.2. Spheroblast preparation

Cells were harvested by centrifugation (10 min at 3000 rpm), weighed and suspended in 3 ml/g of 1 M sorbitol, 50 mM Tris buffer pH 7.5, 10 mM Mg^{2+} and 30 mM dithiothreitol (DTT). After 15 min incubation at 30°C, the suspension was centrifuged, and the resulting pellets were resuspended in 5 ml/g of 1 M sorbitol, 50 mM Tris buffer pH 7.5, 10 mM Mg^{2+} and 1 mM DTT, containing 1–2 mg/g of lyticase. The suspensions were incubated at 30°C for 40–60 min, until conversion to spheroblasts was observed, as assessed by osmotic swelling after suspension in deionized water. The spheroblast suspension was then centrifuged and washed twice with 1 M sorbitol, 50 mM Tris buffer pH 7.5 and 10 mM Mg^{2+}. The final pellet was suspended to a concentration of approximately 50 mg/ml in the same buffer, and kept on ice.

2.3. Standard incubation procedure

Unless specified in the figure legend, incubations were conducted at 28°C in a standard reaction medium containing 125 mM sucrose, 65 mM KCl, 10 mM *N*-(hydroxyethyl)piperazine-*N*′-(4-butanesulfonic acid) (HEPES) buffer pH 7.2 and 500 μM ethanol. The results shown in Figs. 1–3 are representative of a series of at least three experiments conducted using different preparations. The results presented in Fig. 4 are the averages ± S.D. of a series of three experiments.

2.4. Determination of mitochondrial membrane potential ($\Delta\Psi$)

Mitochondrial $\Delta\Psi$ was estimated through fluorescence changes of safranin O (5 μM), recorded on a Hitachi F-4010 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan) operating at excitation and emission wavelengths of 495 and 586 nm, with a slit width of 5 nm.

2.5. Determination of H_2O_2 generation

H_2O_2 production was assessed by the oxidation of scopoletin by horseradish peroxidase (HRP) in the presence of H_2O_2 [20] as described in [9].

2.6. Determination of the intracellular Ca^{2+} content

Freshly prepared spheroblasts (25 mg/ml) were incubated for 60 min at 40°C in standard reaction medium in the presence of 0.1 mg/ml of bovine serum albumin and 10 μM Fura-2AM. The suspension was then centrifuged and washed twice in standard reaction medium. The excitation fluorescence spectrum of the suspension was determined on a Hitachi F-4010 fluorescence spectrophotometer with an emission wavelength fixed at 510 nm. Intracellular Ca^{2+} content was calculated relative to the fluorescence of free Fura and Fura saturated with Ca^{2+}, as described in [21].

2.7. Determination of spheroblast viability

The fluorescence of the spheroblast suspension was determined at excitation and emission wavelengths of, respectively, 365 and 450 nm, on a Hitachi F-4010 fluorescence spectrophotometer, previous to the addition of 50 μM ethidium bromide and followed by the addition of 0.5% Triton X. Viability was calculated as the difference between the fluorescence after ethidium bromide addition and the fluorescence after the addition of Triton X, as a percentage of the total increase in fluorescence promoted by the addition of both ethidium bromide and Triton X [22].

2.8. Materials

Catalase (C-10), HRP (type IV-A), ADP, ATZ, P_i, *t*-butyl hydroperoxide (*t*-bOOH), phenylarsine oxide (PhAsO), cyclosporin A, ethylene glycol-bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), HEPES, rotenone, ethidium bromide, safranin, scopoletin, lyticase and ethanol were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other reagents were commercial products of the highest purity grade available.

3. Results

Many studies have shown that it is possible to study the functional state of mitochondria in intact cells by permeabilizing the plasma membrane with digitonin [23–25]. We observed that it was possible to study *S. cerevisiae* spheroblasts (yeast depleted enzymatically of the cell wall; see Section 2) through digitonin permeabilization [26], and to determine their mitochondrial membrane potential using the fluorescent probe safranin. Studying in situ mitochondria in this manner has the advantage of better preserving mitochondrial function [27]. Moreover, it is possible to study the effects of non-mitochondrial proteins, such as TPx and catalases, on the physiology of this organelle. In situ *S. cerevisiae* mitochondria presented active oxidative phosphorylation and did not show evidence of a high activity Ca^{2+} uptake, an observation in agreement with previous results using isolated yeast mitochondria (results not shown, see [28,29]). In addition, these mitochondria were capable of maintaining their membrane potential supported by ethanol for up to 40 min even in the absence of added P_i, in contrast to what is observed with isolated *S. cerevisiae* mitochondria [29,30].

Initially, the sensitivity of *S. cerevisiae* mitochondrial membrane potential to three classical mammalian MPT inducers was studied (Fig. 1): P_i (lines a), *t*-bOOH (lines b) and PhAsO (lines c). Because yeast mitochondria do not present a high activity Ca^{2+} uptake, the MPT inducers P_i and *t*-bOOH, which cause MPT in a manner dependent on the presence of intramitochondrial Ca^{2+} [4–9], were added together with a high Ca^{2+} concentration (500 μM), to drive a mitochondrial uptake of the ion through a concentration gradient. PhAsO, which promotes MPT in a manner stimulated by extramitochondrial Ca^{2+} [10], was added together with 50 μM Ca^{2+}. Using wild-type *S. cerevisiae* spheroblasts (WTSC), we observed that the mitochondrial membrane potential was dissipated only by the addition of PhAsO (line c), while the spheroblasts treated with P_i (line a) or *t*-bOOH and Ca^{2+} (line b), in concentrations sufficient to promote a complete and fast dissipation of the membrane potential in rat liver [7–10], were capable of maintaining their membrane potential for

long periods. These results are in agreement with those obtained by Jung and co-workers [29], which show that isolated *S. cerevisiae* mitochondria do not suffer mitochondrial permeabilization when treated with P_i, Ca^{2+} and an electrophoretic Ca^{2+} ionophore. Interestingly, when *S. cerevisiae* spheroblasts lacking the TPx gene (ΔTSASC) were used, a small but continuous loss of mitochondrial membrane potential could be observed after treatment of the mitochondrial suspension with P_i or *t*-bOOH and Ca^{2+} (lines a and b). ΔTSASC mitochondria could also be completely permeabilized by PhAsO (line c).

Since TPx is able to decompose peroxides at the expense of sulfhydryl groups [9,31], and it has been demonstrated that both P_i- and *t*-bOOH- but not PhAsO-induced MPT in mammalian mitochondria is dependent on ROS generated by the respiratory chain [5,7,9], we imagined that the low sensitivity of WTSC mitochondria to Ca^{2+}-induced permeabilization might be related to the presence of multiple pathways to decompose peroxides in these cells. Indeed, *S. cerevisiae* possesses two naturally occurring catalases, one peroxisomal and the other cytosolic, in addition to TPx (for review, see [32]). When the catalases of WTSC were specifically inhibited through the incubation of these cells with ATZ (Fig. 1, ATZ–WTSC), we observed a more extensive decrease in mitochondrial membrane potential induced by P_i or *t*-bOOH and Ca^{2+} (lines a and b). This dissipation of mitochondrial membrane potential was even more pronounced when ΔTSASC were treated with ATZ (ATZ–ΔTSASC). Since the depletion of TPx and catalase stimulates mitochondrial permeabilization in an additive fashion, we believe that these proteins are acting together, degrading H_2O_2 generated in these cells and protecting mitochondria from permeabilization induced by Ca^{2+} and P_i or by Ca^{2+} and *t*-bOOH.

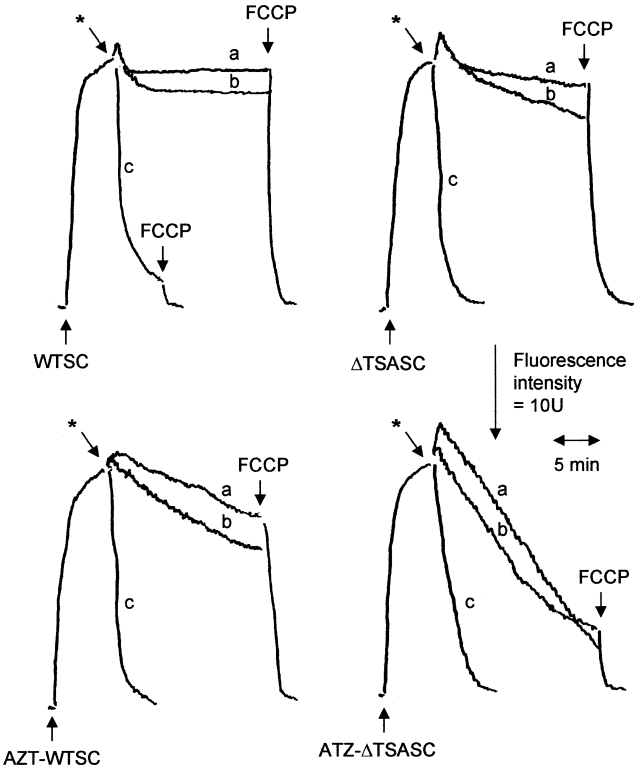

Fig. 1. Effect of MPT inducers on the membrane potential of in situ *S. cerevisiae* mitochondria. Different *S. cerevisiae* spheroblast preparations (see Section 2) were incubated at 1 mg/ml in standard reaction medium containing 0.002% digitonin and 5 μM safranin. Where indicated (*), 500 μM Ca^{2+} and 2 mM P_i (lines a), 500 μM Ca^{2+} and 500 μM *t*-bOOH (lines b) or 50 μM Ca^{2+} and 30 μM PhAsO (lines c) were added. Carbonyl cyanide *m*-clorophenyl-hydrazone (FCCP) (1 μM) was added at the end of each trace, as shown.

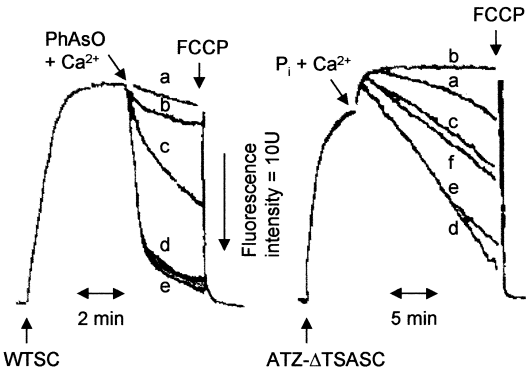

Fig. 2. Effect of MPT inhibitors on *S. cerevisiae* mitochondrial membrane potential dissipation promoted by Ca^{2+} and P_i or PhAsO. Different *S. cerevisiae* spheroblast preparations (see Section 2) were incubated at 1 mg/ml in standard reaction medium containing 0.002% digitonin and 5 μM safranin, in the presence of 5 mM DTT (lines a), 1 mM EGTA (lines b), 300 μM ADP (lines c), 1 μM cyclosporin A (lines d), no further additions (lines e) or 2 μM catalase (line f). 50 μM Ca^{2+} and 30 μM PhAsO or 500 μM Ca^{2+} and 2 mM P_i were added where indicated. FCCP (1 μM) was added at the end of each trace, as shown.

To characterize this Ca^{2+}-induced permeabilization of yeast mitochondria, the effect of different mammalian MPT inhibitors [4] on the dissipation of WTSC membrane potential induced by Ca^{2+} and PhAsO or the dissipation of ATZ–ΔTSASC membrane potential induced by Ca^{2+} and P_i were analyzed (Fig. 2). We observed that, in both situations, the membrane potential decrease could be prevented by the presence of the Ca^{2+} chelator EGTA (lines b), DTT (lines a) or ADP (lines c). The addition of catalase (line f) or isolated TPx (not shown) prevented Ca^{2+}- and P_i-induced permeabilization in ATZ–ΔTSASC. Among all classical MPT inhibitors studied, only cyclosporin A did not prevent mitochondrial membrane potential dissipation in *S. cerevisiae* (lines d).

Mitochondrial permeabilization induced by Ca^{2+} and P_i in rat liver mitochondria is preceded by a burst in mitochondrial H_2O_2 generation [9]. We observed that the addition of Ca^{2+} and P_i also induced a burst in H_2O_2 generation in *S. cerevisiae*, measured as the degradation of scopoletin in the presence of HRP (Fig. 3). This burst clearly preceded the decrease in mitochondrial membrane potential observed in Fig. 1, and was more evident in ATZ–ΔTSASC, AZT–WTSC and ΔTSASC than in WTSC. This is most probably related to the competition between catalase, TPx and HRP for the reaction with H_2O_2 generated. Indeed, similar results were obtained when these purified enzymes were added to isolated mammalian mitochondria [9]. In addition, as observed in Ca^{2+}- and P_i-induced permeabilization of rat liver mitochondria [9], the membrane protein thiol content of *S. cerevisiae* mitochondria treated with Ca^{2+} and P_i decreased proportionally to the mitochondrial permeabilization observed, i.e. more intensely in the mitochondria of yeast cells which do not contain the TPx gene and/or active catalase (results not shown).

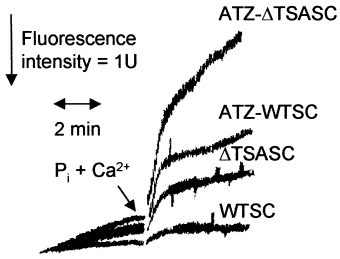

Fig. 3. Effect of Ca^{2+} and P_i on *S. cerevisiae* spheroblast generation of H_2O_2. *S. cerevisiae* spheroblasts were incubated at 1 mg/ml in standard reaction medium containing 0.002% digitonin, 1 μM HRP and 1 μM scopoletin. 500 μM Ca^{2+} and 2 mM P_i were added where indicated.

The results presented until now demonstrate that the naturally occurring yeast antioxidant enzymes catalase and TPx protect the mitochondria of WTSC against mitochondrial oxidative stress induced by Ca^{2+} in the presence of P_i or *t*-bOOH. We were then interested to know if the mitochondrial permeabilization observed could occur in non-permeabilized spheroblasts, and affect cell function. To do so, we incubated non-permeabilized *S. cerevisiae* spheroblasts in reaction medium in the absence (Fig. 4A) or presence (Fig. 4B) of 1 mM Ca^{2+}. Intracellular Ca^{2+} determinations, conducted using Fura-2, showed that the Ca^{2+} content of the spheroblasts incubated in the absence of Ca^{2+} (146 ± 53 nM) was significantly increased ($P < 0.01$) after 10 min incubation in the presence of Ca^{2+} (367 ± 41 nM). These results are in line with intracellular Ca^{2+} determinations in intact *S. cerevisiae*, showing that these cells present an intracellular Ca^{2+} concentration in the 100–150 nM range, which increases up to 800 nM in cells incubated in the presence of Ca^{2+} and in the absence of a respiratory substrate [33]. Under these conditions, after different incubation periods, spheroblast viability was determined by calculating the relative permeability of the suspension to ethidium bromide (see Section 2). We observed that all *S. cerevisiae* spheroblast preparations suffer a continuous decrease in cell viability with time (Fig. 4A), in a very similar proportion. However, when the spheroblasts were incubated in the presence of Ca^{2+} (Fig. 4B), only WTSC (●)

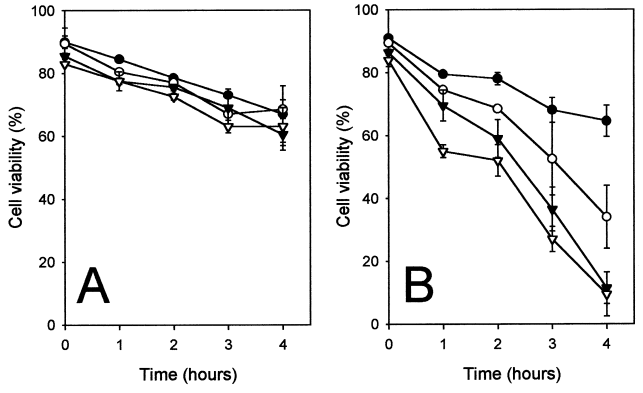

Fig. 4. Effect of Ca^{2+} on spheroblast viability. *S. cerevisiae* spheroblasts were incubated at 0.5 mg/ml in reaction medium containing 250 mM sucrose and 10 mM HEPES pH 7.2, at 28°C, in the absence (A) or presence (B) of 1 mM Ca^{2+}. After the incubation times indicated, cell viability was determined as described in Section 2. WTSC (●), ΔTSASC (○), ATZ–WTSC (▼) or ATZ–ΔTSASC (▽) were used.

were capable of maintaining a cell viability similar to spheroblasts incubated in the absence of Ca^{2+}. ΔTSASC (○) and ATZ-treated preparations (▼ and ▽) suffered a large decrease in cell viability with time. These results show that, under situations of Ca^{2+} overload, naturally occurring H_2O_2 removing enzymes are important to ensure cell integrity.

4. Discussion

Yeast has proved to be an important tool for apoptosis research, since this organism is fast growing and can be readily manipulated genetically [34]. Although *S. cerevisiae* does not possess all the components of the pathway that leads to apoptosis, diagnostic markers of apoptosis, such as chromatin condensation and fragmentation, DNA breakage, exposure of phosphatidyl serine and plasma membrane blebbing are present in yeast cells expressing Bax [35]. Also, the pro-apoptotic protein Bax induces growth arrest in this organism, which is rescued by anti-apoptotic Bcl-2, suggesting that important steps of the apoptotic process may be conserved [36–38]. One of the apoptotic steps, namely the cytochrome *c* release from mitochondria, was observed in Bax-expressing *S. cerevisiae*, in a manner reversed by coexpression of Bcl-$_{XL}$ [39–41]. In addition, evidences for the participation of ROS on the apoptotic cell death in yeast have been reported [42,43].

In this paper, we employed the yeast system to study the effects of classical MPT inducers on in situ mitochondria of *S. cerevisiae*, with the intention of determining the effects of naturally occurring antioxidants on Ca^{2+}-induced mitochondrial permeabilization. Our results show that yeast is very resistant to mitochondrial membrane permeabilization induced by Ca^{2+} and P_i or *t*-bOOH (Fig. 1), potent inducers of MPT in mammalian mitochondria [4,5,7,9]. This is in agreement with previous reports [29] showing that isolated yeast mitochondria are resistant to inner membrane permeabilization induced by Ca^{2+} ions. However, we found that yeast mitochondria do suffer a Ca^{2+}-stimulated permeabilization, with characteristics similar to MPT, when treated with the thiol cross-linking reagent PhAsO (see Figs. 1 and 2). In addition, when depleted of catalase and TPx, *S. cerevisiae* spheroblasts present a drop in mitochondrial membrane potential when treated with an oxidant or P_i, in the presence of high Ca^{2+} concentrations (Figs. 1 and 2). This suggests that wild-type yeasts are resistant to mitochondrial permeabilization for two reasons: the lack of a high affinity mitochondrial Ca^{2+} uptake and the abundant presence of antioxidants such as catalase and TPx. Thus, if treated with a thiol cross-linking reagent, which directly oxidizes thiol groups to promote MPT, or depleted of naturally occurring antioxidants, yeast mitochondria are capable of undergoing an inner membrane permeabilization very similar to that observed in mammalian mitochondria (see Fig. 2).

In a previous publication, Jung and co-authors [29] reported that isolated yeast mitochondria did not present a Ca^{2+}-induced inner membrane permeabilization. These authors characterized a P_i-inhibited, ATP- and respiration-stimulated inner membrane permeabilization, which was interpreted as a yeast form of the MPT. Despite the fact that this permeabilization occurred through the opening of an inner membrane pore similar in size to the mammalian MPT pore, the induction and inhibition patterns of this pore

studied by Jung et al. are quite distinct from classical MPT. For this reason, it is believed that the pore described is a different unselective inner mitochondrial membrane pore (see [44]). Indeed, the channel described by Jung et al. bears a much closer resemblance to the yeast proton-conducting pathway originally described by Prieto et al. [30]. Here, we demonstrate that under quite distinct conditions, yeast mitochondria may present a permeabilization with characteristics very similar to MPT. As observed in mammalian mitochondria, this permeabilization is induced by Ca^{2+} in the presence of P_i, prooxidants or dithiol reagents, and inhibited by naturally occurring H_2O_2 removing enzymes (Fig. 1), ATP and DTT (Fig. 2). As also described in mammalian mitochondria, Ca^{2+}-dependent mitochondrial permeabilization in yeast was preceded by a burst in H_2O_2 generation (Fig. 3) followed by a decrease in membrane protein thiol content (results not shown). Despite these similarities, the *S. cerevisiae* mitochondrial permeabilization observed by us was not prevented by cyclosporin A, which strongly inhibits mammalian MPT. The reason for this lack of sensitivity to cyclosporin A remains to be determined.

It has become clear through our study that yeast mitochondria present a strong resistance to Ca^{2+}-stimulated mitochondrial permeabilization due to the abundant availability of naturally occurring antioxidants. In fact, other naturally occurring antioxidants may defend *S. cerevisiae* mitochondria against oxidative damage, since the mitochondrial permeabilization observed in spheroblasts lacking TPx and depleted of catalase is still much slower and less intense than in rat liver mitochondria [7–9]. *S. cerevisiae* possesses multiple mechanisms to defend itself against oxidative stress. Besides TPx and the two catalases, yeast has two superoxide dismutases (one cytosolic and the other mitochondrial), cytochrome *c* peroxidase, thioredoxin, glutathione, metallothionein (for review, see [32]) and a new alkyl hydroperoxidase recently found in peroxisomes [45,46]. Moreover, recently, it has been reported that yeast possesses three genes similar to the mammalian selenium-dependent glutathione peroxidase, which seem to be related to the antioxidant defense of yeast [47]. *S. cerevisiae* also possesses enzymes that repair oxidative damage such as methionine sulfoxide reductase [48] and 8-oxoguanine glycosylase [49]. Therefore, even when yeasts are unable to utilize TPx and the two catalases (ATZ–ΔTSASC), other means to protect these cells from oxidative stress still remain. In any case, we show here that cells depleted of catalase and/or lacking TPx are more susceptible to accumulate H_2O_2 (Fig. 3), suffer mitochondrial membrane protein thiol oxidation (results not shown), non-specific permeabilization (Fig. 1) and loss of cell viability (Fig. 4) than wild-type cells. Our results are in agreement with the work of several groups showing that *S. cerevisiae* survival is dependent on the availability of naturally occurring antioxidants [18,42,43,47,48]. These studies suggest an important role for intracellular antioxidant enzymes in the defense of the cell against potentially damaging ROS generated mainly by the mitochondrial respiratory chain.

In conclusion, we demonstrate here that *S. cerevisiae* mitochondria studied in situ present an inner mitochondrial membrane permeabilization which is in every way similar to mammalian MPT, with the exception of its sensitivity to cyclosporin A. We also show that naturally occurring antioxidants such as TPx and catalases strongly inhibit this permeabilization of yeast mitochondria, which explains why it was not observed in previous studies. Furthermore, we observed that a Ca^{2+}-stimulated decrease in *S. cerevisiae* spheroblast viability is prevented by naturally occurring antioxidants in yeast, further supporting the notion that, similarly to mammalian cells, yeast viability is hampered by Ca^{2+}-stimulated mitochondrial oxidative stress and dysfunction.

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