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Forum Review

Utilization of Yeast To Investigate the Role of Lipid Oxidation in Cell Death

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ABSTRACT

The yeast *Saccharomyces cerevisiae* is a powerful tool to investigate several aspects of the molecular mechanisms underlying programmed cell death, namely, the events involving mitochondria. Yeast has allowed new information to be gained about crucial aspects of the interaction between proapoptotic proteins Bax and Bid and mitochondria, namely, their addressing and insertion into the mitochondrial outer membrane and their ability to induce the relocalization of cytochrome *c*. Going one step further, the prooxidant effect of Bax can also be studied in yeast. Bax expression induces both the accumulation of reactive oxygen species and lipid oxidation. Lipid oxidation is involved in Bax-induced cell death and may be required for optimal insertion of Bax in mitochondria. The easy modulation of lipid composition in yeast is a powerful tool to investigate this process further, and studies can be extended to other regulators of apoptosis, such as proapoptotic Bid or antiapoptotic Bcl-x_L. Also, yeast is a model for the study of other types of cell death, such as autophagy-related forms of death, for which a role of lipid oxidation has also been evidenced. *Antioxid. Redox Signal.* 6, 259–267.

INTRODUCTION

PROGRAMMED CELL DEATH is an integral part of life. It participates in development and tissue homeostasis by eliminating useless or potentially hazardous cells. The major form of programmed cell death in mammalian cells is apoptosis, but other forms of programmed cell death may exist in lower eukaryotes (3). Apoptosis is an intense research field because defects of this process are involved in both major degenerative and proliferative diseases.

The central role of mitochondria in the regulation of apoptosis is now widely accepted. Typically, mitochondria are the targets of a family of apoptosis regulators termed "Bcl-2 family," which modulate their permeability by a still debated mechanism (13). Following the action of proapoptotic members of this family, mitochondria release a number of proteins, sometimes referred together as "apoptogenic factors," which activate the final steps of apoptosis leading to the degradation of the cell from the inside.

Besides these central events, a number of side phenomena occur, which participate in the regulation of apoptosis. One major (and mostly mitochondrial) event is the production of reactive oxygen species (ROS). Bioenergetic dysfunctions following early mitochondrial events result in the accumulation of superoxide ion and hydrogen peroxide (H_2O_2) and eventually the generation of highly hazardous hydroxyl radical. This oxidative shock may directly target biological molecules, inducing their acute and irreversible degradation but, more moderately, may also play a role by affecting the redox balance of the cell (for instance, the redox potential of glutathione) or oxidize selective targets, among which stand the acyl chains of lipids.

The budding yeast *Saccharomyces cerevisiae* has proved to be a useful tool to study partial steps of apoptosis (46). Although it does not support a typical apoptotic cell death, part of the process can be reproduced in yeast, following the heterologous expression of apoptosis regulators from other organisms, including mammals (18, 32, 46), plants (22, 50), and *C.*

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elegans (11, 55). It is noteworthy that the events accompanying the heterologous expression of nonyeast proteins, including death, are closely related to "physiological" phenomena of cell death occurring in yeast following mutations of proteins involved in the secretory pathway (2), moderate oxidative stress (30, 31), acetic acid stress (28), senescence (25), and autophagy (8).

The aim of this overview is to show how the easy manipulation of lipid in yeast can be a powerful tool for the understanding of the role of lipid oxidation in programmed cell death, in both yeast and higher eukaryotes.

BAX TRANSLOCATION TO MITOCHONDRIA

To understand what might be the crucial role of alterations of lipid during programmed cell death, one must keep in mind how proapoptotic members of the Bcl-2 family interact with mitochondria. Bax is by far the most studied protein, given its crucial role in the apoptotic process. In healthy mammalian cells, Bax is a cytosolic protein. Following apoptotic induction, Bax is relocalized to the outer mitochondrial membrane. This relocalization is under the control of conformational changes affecting both the N-terminal sequence (called ART for apoptotic regulation of targeting) and the C-terminal α-helix, of which the actual function is still debated (9, 10, 17, 47, 56, 60). Following this translocation, Bax is able to induce the release of apoptogenic factors from the mitochondria intermembrane space to the cytosol. The molecular mechanism underlying this process is still debated, but all the hypotheses are based on the activation of endogenous channels, such as the permeability transition pore (36), or the formation of *de novo* channels (24, 42). Apoptogenic factors released from mitochondria play different functions in the postmitochondrial steps of apoptosis: cytochrome *c* associates with APAF-1 (apoptotic protease activating factor) to activate caspases, a family of apoptosis-specific proteases; smac/DIABLO inhibits IAPs (inhibitor of apoptotic proteases), thus allowing further caspase activation; and AIF (apopotosis-inducing factor) and endonuclease G are probably directly translocated to the nucleus where they participate in chromatin degradation.

In yeast, postmitochondrial events are less clear: Bax induces cell death in a way that is largely independent of cytochrome *c* (44) and is related to autophagy (8; I. Kissova, S. Manon, and N. Camougrand, unpublished observations).

BAX-INDUCED ROS ACCUMULATION IS NOT COMPLETELY RESPONSIBLE FOR CELL DEATH

The lethal effect of Bax expression in yeast is strictly dependent on the presence of oxygen, because Bax loses its killing effect under anaerobic conditions (30). This requirement for oxygen does not reflect a requirement for the mitochondrial respiratory chain, because Bax is able to kill rho_0 mutants (18), which do not have a functional chain. The killing effect of Bax is therefore dependent on oxidation event(s), but not on mitochondrial respiration.

On the basis of phenotypic similarities between Bax-induced cell death and H_2O_2-induced cell death, Madeo *et al.* proposed that both phenomena could be related (30). This group reported that typical "pseudo-apoptotic" characteristics identified in Bax-expressing yeast could be reproduced following treatment with a moderate concentration of H_2O_2 (27, 30). These characteristics were the nuclear DNA degradation, evidenced by TUNEL experiments, and the exposition of phosphatidylserine on the outer leaflet of the plasma membrane, evidenced by the binding of annexin V. A "caspase-like" protease, Yca1p, was found to be involved in the effect of moderate H_2O_2 treatment, supporting the hypothesis that this treatment induced a "pseudo-apoptotic" cell death in yeast (31). It should be noted, however, that the involvement of Yca1p in Bax effects has not been demonstrated to date.

The stimulating effect of Bax expression on ROS accumulation is not questioned. This accumulation has been shown with different ROS-sensitive fluorescent probes (30, 45). It could be prevented by treatment of the cells with chemical reducers such as *N*-acetylcysteine or Tiron. The use of a moderate expression system has allowed the observation of a stronger response of probes having some selectivity for H_2O_2 (dichlorodihydrofluorescein diacetate) than probes having selectivity for superoxide ion (dihydroethidium) (45). This could be correlated to a stronger collapse of catalase activity than of superoxide dismutase activity (45). This leads to the question of the origin of this ROS accumulation.

Like apoptotic mammalian cells, Bax-expressing yeast cells support a release of cytochrome *c* from the mitochondrial intermembrane space to the cytosol (34). The molecular mechanism underlying this release is not the focus of this review, but might be caused by the formation of a giant channel in the mitochondrial outer membrane (24, 42). The partial collapse of the electron transport chain caused by the release of part of cytochrome *c* induces an increase of the reduction status of upward electron carriers, namely, flavine and coenzyme Q. These carriers are then able to reduce molecular oxygen to superoxide ion. Also, when present in the cytosol, cytochrome *c* is likely to unbalance the redox potential, thus favoring the formation of ROS. Both phenomena (depletion of mitochondrial cytochrome *c*, accumulation of cytosolic cytochrome *c*) may contribute together to an increase of ROS production, but it is difficult to estimate the degree of their respective contributions. The increase of ROS production added to the decrease of ROS scavenging caused by the significant collapse of catalase activity would be thus responsible for the massive ROS accumulation following Bax expression.

Is this ROS accumulation a major determinant in Bax-induced yeast cell death? This question was investigated in two ways (45). First, chemical scavenging of ROS by *N*-acetylcysteine or Tiron does not have a significant effect on the rate of Bax-induced cell death. Second, overexpression of cytosolic superoxide dismutase, cytosolic catalase, or peroxysomal catalase in active forms did nor result in a decrease of the rate of Bax-induced cell death (overexpression of mitochondrial superoxide dismutase had, by itself, deleterious ef-

fects on cell survival). From these data, there is no evidence for a crucial involvement of ROS accumulation in Bax-induced cell death.

BAX-INDUCED LIPID OXIDATION

Lipid oxidation has often been observed as an accompanying event of apoptosis, but also of the inhibition of apoptosis, leading to contradictory proposals about its exact role (53, 54). It should be noted that different lipoxygenases that target fatty acyl chains at different positions might well have opposite functions.

It was first investigated whether the early events involved in the mitochondrial steps of apoptosis, which are reconstituted in yeast following heterologous expression of an active Bax variant (*c-myc*-tagged human Bax; see 46 and 47 for an explanation of the reason why a tagged protein was used), would result in lipid oxidation, in addition to the ROS accumulation described above.

When grown under standard conditions, yeast contains only saturated and monounsaturated fatty acids and no polyunsaturated fatty acids. The oxidation of monounsaturated fatty acids is expected to result in their disappearance, the formation of medium-chain alkanes, alkenes, and aldehydes, and the subsequent decrease in the ratio between unsaturated and saturated fatty acids (48).

Bax expression in lactate-grown yeast (having highly differentiated mitochondria) results in a strong decrease of the unsaturation of fatty acids, namely, in mitochondria (45). This decrease targets all classes of phospholipids. The demonstration that this decrease of unsaturated fatty acid content actually reflects oxidation was addressed in two ways.

In whole cells, peroxidation can be evaluated as the amount of thiobarbituric acid-reactive species (TBARS). As the oxidation of $C_{16:1}$ and $C_{18:1}$ normally found in yeast does not result in the appearance of such molecules, cells were grown in the presence of $C_{18:2}$. The exogenous fatty acid inhibits Δ_9-acyl-coenzyme A desaturase, thus preventing the endogenous formation of $C_{16:1}$ and $C_{18:1}$ and forcing cells to use exogenous $C_{18:2}$ to build their phospholipids. Under these conditions, lipid peroxidation results in the formation of TBARS. Bax expression induced a twofold increase in TBARS content of yeast cells grown in the presence of $C_{18:2}$, showing that lipid peroxidation occurs (45).

Lipid peroxidation could also be evidenced on isolated mitochondria. The polyunsaturated fatty acid *cis*-parinaric acid can be inserted *in vitro* into biological membranes, and it then supports the same alterations as endogenous fatty acids. Its oxidation results in a strong decrease of its natural fluorescence. Mitochondria were isolated from control and bax-expressing cells and incubated with *cis*-parinaric acid, under conditions allowing its insertion in mitochondrial membranes. Functioning of the mitochondrial respiratory chain was triggered, and the fluorescence of *cis*-parinaric acid was followed. In control mitochondria, a slow decrease of *cis*-parinaric acid fluorescence could be monitored versus time, which was three- to fourfold stimulated in Bax-expressing mitochondria (45). From these data, the presence of Bax strongly increased mitochondrial lipid oxidation induced by the normal function of the respiratory chain.

Is there a link between Bax-induced lipid oxidation and Bax-induced ROS production? Addition of chemical ROS scavengers Tiron or *N*-acetylcysteine did prevent the massive Bax-induced ROS accumulation, but did not have any detectable effect on Bax-induced lipid oxidation, thus supporting the view that the two phenomena are not related (45). However, one should consider that subdetectable amounts of ROS, produced closed to or within the membrane (namely, by the mitochondrial respiratory chain), might not be scavenged by hydrophilic Tiron or *N*-acetylcysteine, and that this "membrane-associated" production might be responsible for lipid oxidation.

BAX-INDUCED LIPID OXIDATION IS INVOLVED IN BAX-INDUCED CELL DEATH

Lipid oxidation can be prevented by hydrophobic antioxidants, such as α-tocopherol or resveratrol. Both molecules were assayed for their effect on Bax-induced cell death, under the same conditions as above (lactate-grown yeast cells with fully differentiated mitochondria). In contrast with hydrophilic ROS scavengers, both α-tocopherol and resveratrol strongly delayed Bax-induced cell death (45). This supports the hypothesis that lipid oxidation is a more crucial determinant for cell death than ROS accumulation.

The sensitivity of lipid to oxidation can be modulated by the unsaturation ratio of their fatty acids. This had been done in two ways. As discussed above, the addition of exogenous unsaturated fatty acids results in a preferential incorporation of these fatty acids into phospholipids. The effect of Bax expression on cell death was then measured on control, $C_{18:1}$-supplemented, and $C_{18:2}$-supplemented cells. The rate of Bax-induced cell death was much higher in $C_{18:2}$-supplemented cells than in $C_{18:1}$-supplemented cells, which was itself slightly higher than in control cells (45). This suggests a strong correlation between the sensitivity of lipid to oxidation and the rate of Bax-induced cell death. Also, some chemicals are able to modulate the unsaturation degree of fatty acids. Among them, dioctyl phthalate has been shown to be particularly efficient in increasing this ratio. Dioctyl phthalate has been shown to strongly increase the rate of Bax-induced cell death (45).

These data suggest that, in contrast with ROS accumulation, which has no major incidence on the rate of Bax-induced cell death, the oxidation of lipid might be a major factor in the killing effect of Bax.

LIPID OXIDATION AND CARDIOLIPIN

The mitochondrial inner membrane is characterized by the presence of cardiolipin. This phospholipid has often been suggested to play a key role in the function of crucial mitochondrial proteins, such as cytochrome *c* oxidase (respiratory complex IV) or the adenine nucleotide translocator. It should be noted, however, that a cardiolipin-less yeast mutant is still able to grow vigorously on a nonfermentable carbon source (lactate), although at a significantly lower rate, suggesting that the role

of cardiolipin in maintaining bioenergetics function might not be that crucial, at least under standard growth conditions (57, 58).

The role of cardiolipin might be sought elsewhere. Its unique structure, with the large hydrophobic domain formed of four acyl chains and a relatively small polar head, allows it to acquire a unique organization known as hexagonal phase II (H_{II}), looking like "reverse" micelles. This structure, which is favored by the neutralization of the negative charges by divalent cations, namely, Ca^{2+}, has been proposed to play a role in the dynamics of contact sites between the inner and outer mitochondrial membranes (19).

Evidence has shown that cardiolipin could play a crucial role in the regulation of mitochondrial steps of apoptosis, as the target of BH3-only Bcl-2 family member Bid (37). Although generally not considered a proapoptotic protein by itself, Bid might be a positive regulator of apoptosis by at least two distinct mechanisms: inhibition of the antiapoptotic function of Bcl-2, and activation of the proapoptotic function of Bax. The first mechanism would involve a simple interaction between BH3 domains of Bcl-2 and Bid, leading to the formation of a stable heterodimer and further inactivation of Bcl-2 function. The second mechanism would involve the participation of Bid in the process of targeting/insertion of Bax into mitochondria. This second mechanism would involve cardiolipin.

Following the induction of apoptosis by death receptors, Bid has been shown to support a cleavage by activated caspase-8, leading to the accumulation of a truncated form, tc-Bid, corresponding to the 15-kDa C-terminal end of Bid. This truncated form was shown to stimulate Bax insertion (4, 26). On the basis of *in vitro* experiments on artificial membranes, it has been shown that cardiolipin is required for optimal tc-Bid insertion in a lipid bilayer (29). Therefore, the three partners (Bax, tc-Bid, and cardiolipin) would be required for the optimal formation of a proapoptotic complex in mitochondria (24).

Another aspect of the potential role of cardiolipin in the effects of Bcl-2 family members is the release of cytochrome *c*. At intracellular pH, cytochrome *c* is a positively charged protein. Although it is a soluble protein, it is thought to interact tightly with the mitochondrial inner membrane. It has long been known that treatment of mitochondria with high salt concentration is required to completely remove cytochrome *c*, showing that the interaction is mostly of an ionic nature. Cardiolipin is the most negatively charged phospholipid and has been suggested to play a crucial role in this interaction. Experiments on artificial systems show that cytochrome *c* interacts more efficiently with liposomes containing cardiolipin (diphosphatidylglycerol) than with liposomes containing a twofold higher concentration of (mono)phosphatidylglycerol, suggesting that not only the charge, but also the unique structure of cardiolipin may play a role in the interaction between cytochrome *c* and membrane (12, 49). Interestingly, it has been shown that oxidation of acyl chains of cardiolipin impaired the interaction with cytochrome *c* (52). As the polar heads of cardiolipin are not altered by oxidation, this result might be interpreted as a role of the structure of cardiolipin in the interaction of cytochrome *c* with the lipid bilayer, which is lost in oxidized cardiolipin following the rupture of one (or more) acyl chains. It should be noted that, in mammalian mitochondria, acyl chains of cardiolipin are significantly more unsaturated than those that of other mitochondrial phospholipids (19), potentially making cardiolipin particularly sensitive to oxidation.

Yeast can be used as a tool to address both aspects of the role of cardiolipin in the action of Bcl-2 family members on mitochondria. A cardiolipin synthase-less strain, although exhibiting a slight reduction of growth rate, is still fully able to grow on a strict respiratory carbon source (57). Mitochondria isolated from this strain do not contain cardiolipin, which is partially replaced by phosphatidylglycerol (58) and can be used as a tool to test if cardiolipin plays a role in Bax, Bid, and tc-Bid effects on mitochondria. The heterologous expression of Bid or tc-Bid alone in yeast does not result in any loss of viability (47). Both proteins are only marginally present in yeast mitochondria. The next step was to test whether Bid or tc-Bid had a stimulating effect on Bax-induced cell death. Bax was introduced in yeast under the control of the moderate-strength promoter *tet*-off, in the absence or in the presence of a Bid- or tc-Bid-encoding plasmid. Whereas Bid did not have any effect on the rate of Bax-induced cell death, tc-Bid slightly, but significantly increased this rate. The stimulating effect of tc-Bid on Bax effects was more clearly evidenced on Bax-induced release of cytochrome *c,* which was strongly stimulated by tc-Bid (Fig. 1). These data confirm the hypothesis that, although not having a direct effect by itself, tc-Bid stimulates the proapoptotic function of Bax by helping the release of cytochrome *c*.

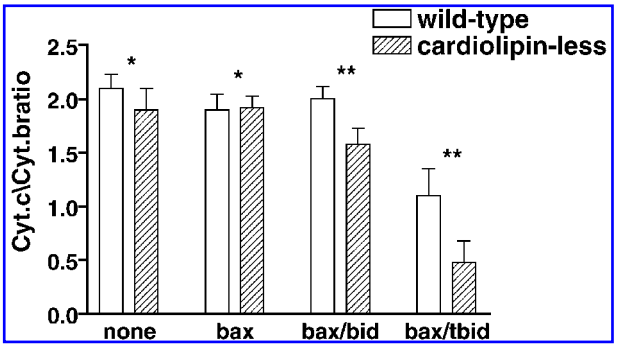

FIG 1. Effect of Bid and tc-Bid on Bax-induced release of cytochrome *c*. Wild-type strain W303-1B and a derived Δcls1 null mutant (cardiolipin synthase-less) were transformed with expression plasmids pCM189/Bax-*c-myc*, pYES2/Bid, or pYES2/tc-Bid. Bax-*c-myc* expression is controlled by a *tet*-off promoter, and expression of Bid and tc-Bid is controlled by a GAL1/10 promoter. Cells were grown aerobically in a medium supplemented with 2% lactate as a carbon source until early exponential growth phase (OD_{550nm} = 1). Galactose at 0.5% was added to induce Bid or tc-Bid expression and, after 8 h, doxycyclin was removed by washing the cells to induce the expression of Bax-*c-myc*. After an additional 8 h, cells were harvested, mitochondria were isolated, and cytochrome content was measured for redox spectra, as described (24). Data are means ± SD of at least four independent experiments. *Nonsignificant differences between wild-type and cardiolipin-less strains ($p > 0.2$). **Significant differences between wild-type and cardiolipin-less strains ($p < 0.05$).

The same experiment was done in the cardiolipin synthase-less mutant. Quite unexpectedly, an increase of the stimulating effect of tc-Bid was observed on both Bax-induced cell death and the release of cytochrome *c* (Fig. 1). Moreover, in this mutant, Bid also gained a moderate stimulating effect. These data suggest that tc-Bid stimulates Bax-induced cytochrome *c* release more easily in the absence of cardiolipin. This suggests that cardiolipin is not involved in the binding of tc-Bid to mitochondria. On the contrary, the absence of cardiolipin would favor the binding of tc-Bid (and Bid) and its further stimulating effect on the release of cytochrome *c*. A possible hypothesis is that Bax-induced lipid oxidation, including cardiolipin, may have a similar effect as the absence of cardiolipin in the stimulation of tc-Bid interaction with mitochondria. In support of this hypothesis, it has been observed that if tc-Bid did not increase the binding of Bax to mitochondria, the presence of Bax did increase the binding of tc-Bid to mitochondria (47). It is therefore tempting to speculate that Bax-induced lipid oxidation, and namely cardiolipin oxidation, would help tc-Bid to gain a stimulating effect over Bax-induced release of cytochrome *c*. This parallels the suggestion that neither Bax-induced permeabilization of the mitochondrial outer membrane nor the oxidation of cardiolipin is independently able to induce the release of cytochrome *c* in apoptotic mammalian cells, but that both are required (40).

This hypothetical role of Bax-induced lipid oxidation remains to be confirmed in yeast and to be further extended to the physiological situation in apoptotic mammalian cells. The use of a combination of inhibitors of lipid peroxidation and of strains carrying an inducible expression system for cardiolipin synthase is under way, and yeast is an adequate tool to verify the hypothesis proposed by Ott *et al.* (40).

One must keep in mind, however, that yeast allows the study of the initial mitochondrial steps of apoptosis, up to the release of cytochrome *c,* and that subsequent events leading to the full permeabilization of mitochondria via a permeability transition never occur in yeast; lipid oxidation is likely also to play a role in these final mitochondrial steps of apoptosis, for which yeast is not an adequate tool.

REMODELING MITOCHONDRIA

In recent years, progress in electron microscopy allied to the development of tomography and computerized imaging led investigators to reconsider the classical view of the structure of mitochondria. This demonstrated the complexity of mitochondrial cristae (16, 33) and pointed out the fact that the release of cytochrome *c* might require a profound remodeling of mitochondrial inner membrane structure (51). Recent data suggest that this remodeling might involve the same system responsible for the process of fission of mitochondria (15, 21). It is noteworthy that the formation of a cytochrome *c*-permeable channel through the mitochondrial outer membrane might not be enough to allow the release of cytochrome *c*. When the MAC (mitochondrial apoptosis-induced channel) is formed in yeast cells expressing Bax under the control of a moderate-strength promoter, only a marginal proportion of cytochrome *c* is actually released even though MAC is detected with high probability (42). In addition to the problem of the tight association of cytochrome *c* with the mitochondrial inner membrane that was discussed above, cytochrome *c* should reach the outer membrane from tubular cristae that are sometimes as long as 1 μm (33). Here again, lipid oxidation, and especially cardiolipin oxidation, might provide a basis for the structural changes required for the reorganization of cristae. In addition, the availability of yeast mutants having an altered morphology of cristae (41) will be useful for such a study.

NONMITOCHONDRIAL LIPID OXIDATION

Until now, we have focused on mitochondrial events, because they are a major step in the implementation of apoptosis. However, Bax is able to kill yeast grown on a strictly fermentable carbon source, containing a high concentration of glucose, although at a much slower rate than on a nonfermentable carbon source (35, 44). An interesting observation is a paradoxical effect on plasma membrane permeability. When grown under these conditions, wild-type cells support a stress caused by the accumulation of ethanol, which cannot be further oxidized because of the glucose-induced catabolic repression of respiratory enzymes. This ethanolic stress causes an increase in the plasma membrane permeability and further cell death. Bax expression increases the rate of cell death but, on the other hand, limits the extent of ethanol-induced permeabilization (35).

The protective effect of Bax over plasma membrane permeability may depend on lipid oxidation, because it is prevented by the addition of both resveratrol and α-tocopherol. This appears to indicate that the oxidation of lipid that occurs following Bax expression has a protective effect over plasma membrane permeability.

It has been reported recently that the deletion of cytosolic superoxide dismutase Sod1p, although expectedly increasing the sensitivity to oxidative stress, also improves the adaptative response of yeast. The adaptative response of yeast is the resistance to an acute stress that appears following exposure to a moderate stress. Data reported by Pereira *et al.* (43) suggest that the deletion of Sod1p, by inducing an unbalancing of redox potential, alters the levels of lipid peroxidation and/or the metabolism of glutathione, thus allowing yeast to gain tolerance against further oxidative stress, just like wild-type cells preexposed to a moderate oxidative stress.

As Bax expression, like Sod1p inactivation, induces an increase of lipid oxidation, we investigated whether a similar tolerance against other stress, in addition to ethanol, could be evidenced. Plasma membrane permeability was used as a marker of cell response. Bax expression induced an increased sensitivity of cells to both oxidative stress (20 m*M*/100 m*M* H_2O_2 treatment) and heat stress (exposure at 55°C/60°C). However, adaptative conditions (1 m*M* H_2O_2 treatment and growth at 37°C, respectively) induced a further increase in tolerance against acute stress in Bax-expressing cells as compared with control cells (Fig. 2): Bax-expressing cells are more resistant than wild-type to higher heat stress (60°C) and higher H_2O_2 concentration (100 m*M*). The response of the cells to Bax ex-

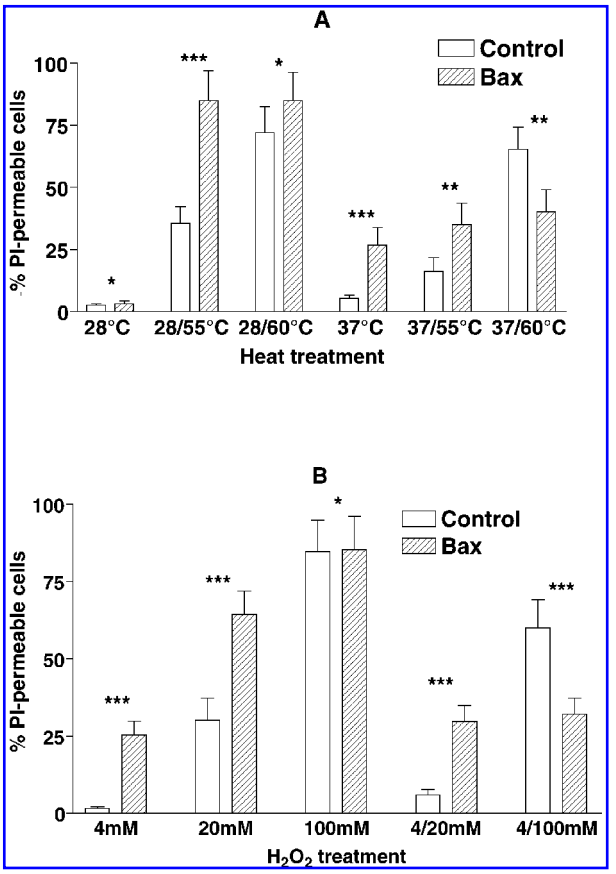

FIG. 2. Effect of Bax expression on plasma membrane permeability induced by heat stress or oxidant stress. (**A**) Wild-type cells or Bax-*c-myc*-expressing cells under the control of the *tet*-off promoter were grown aerobically in a medium supplemented with 2% lactate as a carbon source at 28°C or 37°C. Cells were then submitted to a shift at 55°C for 30 min or 60°C for 10 min. After the cells were incubated in the presence of 100 μ*M* propidium iodide (PI), the % of permeable cells was counted by flow cytometry as described (35). (**B**) Wild-type or Bax-*c-myc*-expressing cells were grown aerobically in a medium supplemented with 2% lactate as a carbon source at 28°C in the absence or in the presence of 4 m*M* H_2O_2. Cells were then submitted to 20 m*M* or 100 m*M* H_2O_2 for 30 min, before permeability to propidium iodide was measured as in A. Data are means ± SD of at least four independent experiments. *Non-significant differences between wild-type and Bax-expressing cells ($p > 0.2$). **Significant differences between wild-type and Bax-expressing cells ($p < 0.05$). ***Significant differences between wild-type and Bax-expressing cells ($p < 0.01$).

pression is thus more complex than the response to Sod1p inactivation, because the sensitivity of Bax-expressing cells to a primary stress is actually increased, and not decreased. But the acquisition of stress tolerance is markedly increased by Bax expression, which might indicate, like in the Sod1p-inactivated strain, a crucial role of lipid oxidation and glutathione metabolism (which has not been investigated so far in Bax-expressing cells) in the process of the acquisition of yeast resistance to oxidant stress, heat stress, and ethanolic stress. Here again, the manipulation of lipid metabolism will now be used to investigate further this phenomenon.

BAX-INDUCED CELL DEATH AND OTHER CELL DEATH TRIGGERS: A COMMON, LIPID-DEPENDENT, PATHWAY?

A genetic approach allowed the identification of the yeast gene *UTH1* as a mitochondrial partner involved in Bax-induced cell death (8, 59). Also, the absence of *UTH1* strongly delays the effects of rapamycin, a drug known to induce autophagic degradation. Recent data showed that, in the absence of *UTH1*, mitochondria appeared to be "protected" against autophagic degradation (I. Kissova, S. Manon, and N. Camougrand, unpublished observations). This strongly supports the hypothesis that (a) Uth1p is involved in the recognition of mitochondria by the components of the autophagic pathway and (b) Bax interferes with this process. The absence of Uth1p not only delays Bax-induced cell death under respiratory conditions, but also prevents all the other characteristics that the cells acquire following Bax expression, namely, the increase of ROS production and the protective effect against ethanolic stress, under fermentative conditions (8).

Interestingly, the absence of Uth1p has been previously associated with altered responses to other different stress; as compared with its parental stain, the null-mutant strain is more resistant to heat stress, menadione, and Cd^{2+}, but more sensitive to Cu^{2+}, H_2O_2, and paraquat (5, 7, 53). Recent data showed that the lipid composition of the null mutant is altered, containing significantly more saturated fatty acids. This seems to contradict the observation of a direct correlation between the unsaturation degree of fatty acids and the yeast sensitivity to Cu^{2+} and Cd^{2+} (4, 20). The altered responses of *UTH1*-null mutant suggest that the role of lipid oxidation may not be such a simple, direct link between the sensitivity of lipid to oxidation and the efficiency of stress-induced cell death.

There is evidence that in neuronal cells, lipid oxidation, and especially cardiolipin oxidation, might be part of the signal that points at damaged mitochondria that should be degradated by an autophagic process (23). The activation of autophagy in otherwise healthy cells will lead to the degradation of damaged mitochondria and the recycling of their components. But this system might lose efficiency in unhealthy cells, due to an increase in the number of damaged mitochondria and/or a collapse of the components of the autophagosomal/lysosomal degradation pathway (6), then leading to apoptotic cell death.

The proteins involved in yeast autophagy are identified and the whole process is very conserved from yeast to mammals, with the difference that well differentiated autophagosomes are ultimately degraded in the vacuole instead of involving a lysosomal degradation (1). Thus, yeast can be a useful tool to understand the potential role of lipid oxidation in the process of recognition of mitochondria (as well as other intracellular structures) as targets of the autophagic system.

PERSPECTIVES

The utilization of yeast both as a tool and as a model to understand the role of lipid oxidation in cell death is at its very begining. The role of the mitochondrion, both as the major location of chemical reactions involving molecular oxygen and

as a central regulator of cell death, is crucial. Cardiolipin, which is exclusively located in mitochondria, is an obvious candidate for being a target of the process of lipid oxidation because its capacity to form the hexagonal phase H_{II} is challenged by oxidation of its acyl chains. In addition, at least in mammalian cells, the fatty acyls of cardiolipin are more unsaturated than those of other phospholipids, and therefore potentially more sensitive to oxidation.

Despite the limits of the tool, namely, the fact that yeast does not support an orthodox form of apoptotic cell death, but rather an autophagy-related cell death, yeast has several advantages.

- It is easy to modulate fatty acyl composition of yeast phospholipids, because yeast preferably uses exogenous fatty acids rather than synthesizes them *de novo*. Data from Howlett and Avery (20) show that up to 65% of the plasma membrane acyl chain originates from exogenous fatty acids. It is reasonable to hypothesize that similar high levels of incorporation occur in other cellular membranes. These authors easily introduced $C_{18:2}$ and $C_{18:3}$ in phospholipids in yeast grown with only *de novo* synthesized acyl chains containing negligible amounts of these fatty acids. Also, several drugs are able to modulate yeast lipid composition.
- The second advantage of yeast is genetics. Yeast is a long-time model for molecular genetic studies. This adds to its nature of facultative aerobe, making it an adequate tool for studies of mitochondrial functions. As examples, mitochondrial protein importation, the regulation of mitochondrial biogenesis, mitochondrial fission and fusion, autophagy, and nuclear/mitochondrial genome interactions are major research fields where yeast molecular genetics has proved to be not only useful, but even absolutely required for the advancement of knowledge. Null mutant strains in several enzymes, such as cardiolipin synthase, superoxide dismutase(s), or catalase(s), have already proven to be useful. Future research will be directed toward the identification of the enzymes involved in the metabolism of the products of lipid oxidation. In yeast, no lipoxygenase has been identified so far on the basis of sequence homology analyses. Reliable enzymatic assays will therefore be required to identify putative lipoxygenases involved in yeast cell death by comparing, for instance, these activities in control cells and Bax-expressing cells.

Also, the recent evidence that Bax-induced cell death in yeast is related to an autophagic process (2, 8; I. Kissova, S. Manon, and N. Camougrand, unpublished observations) must be considered in the light of growing evidence for the role of autophagy in an "alternative pathway" to cell death (39). As the basis of the autophagic process is the recognition of altered structures by a vesicular system, the potential role of products of lipid oxidation (and the consequence of their presence on the organization of membranes and membrane proteins) is an exciting investigation pathway, for which yeast is likely to be the most powerful tool.

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ABBREVIATIONS

H_{II}, hexagonal phase II; H_2O_2, hydrogen peroxide; MAC, mitochondrial apoptosis-induced channel; ROS, reactive oxygen species; Sod1p, cytosolic superoxide dismutase; TBARS, thiobarbituric acid-reactive species; tc-Bid, truncated Bid.

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