Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically

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Saccharomyces cerevisiae cells grown either aerobically or anaerobically were tested for tolerance to a brief heat stress (52 °C, 5 min) or oxidative stress (20 mM H₂O₂, 15 min). Tolerance was related to growth phase, in that stationary phase cells were intrinsically more resistant to heat or oxidative stress than exponential phase cells. A mild heat shock (37 °C, 30 min) induced thermotolerance and oxidative tolerance in both aerobic and anaerobic cells. However, prior exposure to a low concentration of H₂O₂ (0.1 mM, 60 min) induced protection against the lethal concentration of H₂O₂ but not against the lethal temperature. Sensitivity to both heat and oxidative stress was dependent on membrane lipid composition. In the case of anaerobic cells, the most stress resistant had membranes enriched in saturated fatty acids, followed in order by cells enriched in oleic and linolenic acids. Aerobic cells with membranes enriched in palmitoleic and oleic acids showed the highest resistance to stress under all conditions. In both aerobic and anaerobic cells, a mild heat shock or oxidative shock induced markedly increased levels of thiobarbituric acid reactive substance (TBARS), indicative of malondialdehyde formation and lipid damage. Anaerobic cells with membranes enriched in linolenic acid had the highest TBARS, followed by cells enriched in oleic acid, with cells enriched in saturated fatty acids showing the lowest TBARS. The results suggest that heat and oxidative stress may share a common mechanism of damage through induction of oxygen-derived free radicals, resulting in membrane lipid damage. The extent of cellular damage was related to membrane lipid composition and correlated positively with increasing unsaturation of the phospholipid fatty acyl component.

Keywords: *Saccharomyces cerevisiae*, stress tolerance, membrane lipids, free radicals

INTRODUCTION

All living cells are subject to oxygen toxicity. The main oxygen-derived species causing damage are thought to be hydrogen peroxide, the superoxide anion (O₂⁻) and the highly reactive hydroxyl (OH·) radical. These species are formed during normal cell metabolism, changes in oxygen tension or by redox reactions (Halliwell & Gutteridge, 1989). Biological systems susceptible to attack by oxygen species include DNA, proteins and lipids (Sies, 1986). Membrane phospholipids are particularly susceptible to oxygen-derived free radical attack due to the abundance of polyunsaturated fatty acids and high solubility of

molecular oxygen in hydrophobic membranes relative to aqueous environments (Halliwell & Gutteridge, 1989).

In microbial systems, oxidative damage has been most intensively studied in the enteric bacteria *Escherichia coli* and *Salmonella typhimurium* (for a review, see Farr & Kogoma, 1991) and, in the case of eukaryotic microorganisms, *Saccharomyces cerevisiae* and *Neurospora crassa* (for a review, see Watson, 1990).

We have used yeast cells to study the interrelationships between oxidative and other stresses, particularly heat. The yeast cell provides an attractive model system for oxidative stress, not only because of rapid growth and relative genetic simplicity compared to higher organisms, but also because cellular composition and function may be readily manipulated by altering environmental conditions.

Abbreviation: TBARS, thiobarbituric acid reactive substance.

Our experimental system offers distinct advantages over previous systems which have been used for studies on oxidative stress. These include the controlled manipulation of membrane lipid unsaturation. The latter parameter is thought to be an important factor governing lipid peroxidation damage of biological membranes by oxygen-derived free radicals. Furthermore, yeasts can grow both aerobically and anaerobically, and thus a comparison of how cells cope with oxidative stress in aerobic and anaerobic environments may be directly tested in the same organism.

In this paper, we describe the response of aerobic and anaerobic *S. cerevisiae* cells with different membrane lipid compositions to oxidative stress, induced by H_2O_2 , and temperature shifts. A mild heat shock was found to induce tolerance against a lethal temperature and H_2O_2 challenge. However, pretreatment with low levels of H_2O_2 induced tolerance against a lethal H_2O_2 challenge but not a lethal temperature challenge.

METHODS

Growth conditions. *Saccharomyces cerevisiae* strain K7 (ATCC 26422) was used. Cells were grown aerobically at 25 °C in an orbital shaker (150 r.p.m.) in medium containing (per litre): 10 g yeast extract (Oxoid), 5 g bacteriological peptone (Oxoid), 3 g KH_2PO_4 , 3 g $(NH_4)_2SO_4$, 25 mg $CaCl_2 \cdot 2H_2O$, 25 mg $MgSO_4 \cdot 7H_2O$ and 20 g glucose, pH 7.5 (YEP medium). Anaerobic growth was in YEP medium as previously described (Watson & Rose, 1980) with a constant stream of high-purity nitrogen gas passing through the medium. Lipid supplements, when provided, were as ergosterol (5 mg l⁻¹) and either oleic (30 mg l⁻¹), linoleic (30 mg l⁻¹) or linolenic (30 mg l⁻¹) acids. Lipid analysis, as presented in Results, confirmed the aerobic and anaerobic environments of these growth conditions (for a review, see Watson, 1984). Cells grown anaerobically without lipid supplements showed a low content, generally less than 10%, of monounsaturated fatty acids. On the other hand, cells grown anaerobically with lipid supplements were typically enriched in the fatty acid supplement (Watson & Rose, 1980). Cells grown aerobically under our conditions were enriched in monounsaturated fatty acids with a composition characteristic of aerobic *S. cerevisiae* (Watson, 1984).

Lipid analysis. Lipids were extracted as previously described (Watson & Rose, 1980). Total phospholipids were separated from neutral lipids by TLC using silica gel G plates (Merck; 0.25 mm thick) and a solvent system of light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (68:29:2, by vol.). The phospholipids, which remained at the origin, were scraped off the plates and methylated with BF_3 /methanol at 80 °C for 30 min.

The fatty acid methyl esters were extracted into hexane and concentrated to a small volume prior to analysis. Samples were analysed using a Hewlett-Packard model 5890A gas chromatograph equipped with a flame ionization detector. Conditions were as follows: 2 m glass 10% Silar 10C column (Applied Sciences), carrier gas (nitrogen) flow rate 30 ml min⁻¹, detector and injection temperature 200 °C and oven temperature 180 °C. Fatty acid methyl esters were identified from their retention times relative to appropriate standards (Sigma), and percentage fatty acid composition was determined using the Delta chromatography data system (Digital Systems).

Thiobarbituric acid reactive substance (TBARS). TBARS was estimated as outlined by Jain (1988) and modified for yeast cells as follows. Cells were pelleted at 2000 g for 2 min and the pellet was washed twice with distilled Millipore purified H_2O . Washed cells were disrupted by vortexing for six periods of 20 s each with an equal volume of glass beads (0.5 mm diameter) in phosphate-buffered saline and 10% (w/v) trichloroacetic acid. Cells were kept on ice, except during vortex mixing. Extracts were centrifuged at 2000 g for 3 min and the supernatant mixed with 0.1 ml 0.1 M EDTA and 0.6 ml 1% (w/v) thiobarbituric acid in 0.05 M NaOH. The reaction mixture was incubated in a boiling waterbath for 15 min and, after cooling, the A_{532} was measured. Results were expressed as μ mol malondialdehyde per mg protein.

Studies on the heat-shock response. Cells were grown at 25 °C in batch culture and, at various times of the growth phase, were heat shocked at 37 °C for 30 min. For heat-stress experiments, cells were rapidly heated to 52 °C for 5 min following heat shock (37 °C for 30 min, heated directly to 52 °C – induced thermotolerance) or, alternatively, cells at 25 °C were rapidly heated directly to 52 °C for 5 min (intrinsic thermotolerance). Following heat stress, cells were cooled to 25 °C in an ice bath before plating onto YEP media for viability measurements. Viable cells were determined after incubation at 28 °C for 2–3 d.

Studies on H_2O_2 tolerance. Preliminary experiments were performed to determine appropriate concentrations of H_2O_2 for lethal and sublethal stresses, as detailed in Results. In later experiments for H_2O_2 shock, cells were incubated with 0.1 mM H_2O_2 for 30 min and then washed in fresh medium or subjected to H_2O_2 stress. For H_2O_2 stress, cells were incubated with 20 mM H_2O_2 for 15 min and then washed in fresh medium. Viable cells were determined as above.

Cell protein. Protein content was assayed by a modification of the Bradford procedure (Bradford, 1976) using the Coomassie blue microassay method (Pierce). BSA was used as a standard.

RESULTS

Membrane fatty acid composition

The percentage fatty acid composition of phospholipids from cells grown aerobically or anaerobically with lipid supplements is presented in Fig. 1. Aerobic cells in the exponential growth phase contained about 80% mono-unsaturated fatty acids, consisting of 40% palmitoleic ($C_{16:1}$) and 38% oleic ($C_{18:1}$) acids. There was little change in percentage unsaturated fatty acid as cells grew into the stationary growth phase.

In cells grown anaerobically without lipid supplements, the phospholipids were typically enriched in saturated fatty acids (90%) and low in monounsaturated fatty acids (10%). The phospholipids of lipid-supplemented anaerobically grown cells were typically enriched in the fatty acid growth supplement. In cells in the exponential growth phase, membranes were enriched to more than 60% in $C_{18:1}$ supplement or linoleic acid ($C_{18:2}$) supplement and about 70% in linolenic acid ($C_{18:3}$) supplement growth media. Saturated fatty acids, mainly $C_{16:0}$ palmitic acid, constituted 30–35% of the total. In contrast, phospholipids from cells in stationary growth phase were considerably lower in the lipid supplements, typically a 40–50% enrichment (results not shown). A corresponding increase in saturated fatty acid, particularly $C_{16:0}$ and

Fig. 1. Fatty acid composition of phospholipids from exponential phase cells. □, Aerobic; ▨, anaerobic unsupplemented; ▩, anaerobic with C_{18:1} supplement; ▤, anaerobic with C_{18:3} supplement. The data shown are from a representative experiment.

Fig. 2. Intrinsic and induced thermotolerance of cells in relation to culture time. The growth phase of cells treated at each time is shown on the figure. (a) Intrinsic thermotolerance was measured by heating from 25 °C to 52 °C for 5 min. (b) Induced thermotolerance was measured by heating from 25 °C to 37 °C for 30 min and then 52 °C for 5 min. ○, Aerobic; ●, anaerobic unsupplemented; ▽, anaerobic with C_{18:1} supplement; ▼, anaerobic with C_{18:3} supplement. The data shown are from a representative experiment.

short-chain (C₁₀–C₁₄) fatty acids, was noted. Anaerobically grown cells were typically low in C_{16:1}, the major monounsaturated fatty acid of aerobic *S. cerevisiae*.

Thermotolerance

Intrinsic and heat-shock-induced thermotolerance was tested at selected points throughout the growth cycle of the yeast. In aerobic cells, intrinsic thermotolerance, measured as percentage cell survival after a challenge at 52 °C for 5 min, was extremely low in exponential phase cells (Fig. 2a). As the culture approached mid to late exponential growth, there was a progressive increase in intrinsic thermotolerance, which peaked at about 20 % survival at early stationary phase.

Heat-shock-induced thermotolerance, defined as a 37 °C heat shock for 30 min followed by a challenge at 52 °C for 5 min, showed a similar pattern with respect to the growth curve (Fig. 2b). However, heat-shock-induced thermotolerance was always higher than intrinsic thermotolerance of cells from the same culture, with a maximum 40–50 % cell survival at late exponential growth phase.

Anaerobically grown cells showed the same pattern of increased intrinsic and acquired thermotolerance as cultures progressed from early exponential to late stationary growth phase. A trend may be noted that for both intrinsic and induced thermotolerance, aerobic cells were the most resistant, followed in decreasing order by anaerobic unsupplemented, anaerobic C_{18:1} enriched and anaerobic C_{18:3} enriched cells. Maximum induced thermotolerance was roughly 40 % in anaerobic unsupplemented cells, 30 % in C_{18:1} cells and 20 % in C_{18:3} cells, as compared to about 45 % in aerobic cells. Cells enriched in C_{18:2} showed between 20–30 % survival (data not shown).

H₂O₂ tolerance

The effect of different concentrations (1–50 mM) of H₂O₂ on cell survival was tested in exponential (Fig. 3a) and stationary phase cells (data not shown). As shown in Fig. 3(a) for cells in the exponential growth phase, there were marked differences in cell survival at H₂O₂ concentrations from 1–10 mM.

As found for intrinsic and induced thermotolerance, aerobic cells were the most resistant, followed in order by C_{18:1} and C_{18:3} anaerobic cells. At and beyond 20 mM H₂O₂, all three cell types showed low percentage survival. The time-course of incubation of cells in the exponential phase with 10 mM H₂O₂ clearly demonstrated differences in sensitivity to H₂O₂ (Fig. 3b). Differences were most noticeable after 15 min and 30 min incubation with H₂O₂, with aerobic cells showing 60 % and 35 % viability, respectively, as compared to 35 % and < 5 % viability with C_{18:3} cells. Differences in sensitivity to H₂O₂ were not as marked in cells in stationary growth phase. For example, at 10 mM H₂O₂, the percentage survival rates were approximately 60 %, 70 % and 75 % for C_{18:3}, C_{18:1} and aerobic cells, respectively.

Tolerance to 20 mM H₂O₂ could be induced in exponential phase cells by a prior incubation with low levels of H₂O₂ (Fig. 4). Maximum induced tolerance (approaching 60 % survival) to 20 mM H₂O₂ was observed by pre-treatment of cells with 0.1 mM H₂O₂. Exposure of cells to

Fig. 3. Effect of H_2O_2 on survival of exponential phase cells. (a) Cells incubated with H_2O_2 for 15 min, washed with fresh medium and cell survival determined by plate counts. (b) Timecourse of survival in 10 mM H_2O_2 . ○, Aerobic; ▽, anaerobic with $C_{18:1}$ supplement; ▼, anaerobic with $C_{18:3}$ supplement. The data shown are from a representative experiment.

Fig. 4. Induction of H_2O_2 tolerance in aerobically grown cells in exponential growth phase. Cells were pretreated with various concentrations of H_2O_2 (as indicated on the abscissa) for 30 min, prior to exposure to 20 mM H_2O_2 for 15 min. The data shown are from a representative experiment.

lower or higher levels of H_2O_2 was less effective in inducing tolerance to 20 mM H_2O_2 .

The results for intrinsic (Fig. 5a) and induced (Fig. 5b)

Fig. 5. Intrinsic and induced tolerance to H_2O_2 in aerobic and anaerobic cells. The growth phase of cells treated at each time is shown on the figure. (a) Intrinsic tolerance was measured by incubating cells with 20 mM H_2O_2 for 15 min. (b) Induced tolerance was measured by prior incubation with 0.1 mM H_2O_2 for 30 min prior to treatment with 20 mM H_2O_2 for 15 min. ○, Aerobic; ●, anaerobic unsupplemented; ▽, anaerobic with $C_{18:1}$ supplement; ▼, anaerobic with $C_{18:3}$ supplement.

tolerance to H_2O_2 in aerobic and anaerobic cells indicated that maximum tolerance occurred in cells towards the end of exponential and early stationary growth phase, with aerobic cells the most resistant, followed in order by anaerobic unsupplemented, $C_{18:1}$ -enriched and $C_{18:3}$ -enriched cells.

Cross-tolerance

The response of cells to heat and H_2O_2 stress appeared to have similar kinetics, and experiments were therefore conducted to determine the degree of cross-tolerance to the two stresses. Resistance to 20 mM H_2O_2 could be induced by a prior heat shock at 37 °C for 30 min in aerobic (Fig. 6) and anaerobic cells (data not shown). Induced tolerance in aerobic cells was generally about fourfold higher than intrinsic tolerance and about threefold higher in anaerobic cells. It was noteworthy that prior exposure of cells to low levels of H_2O_2 for 30 min did not induce tolerance to heat stress (52 °C for 5 min; data not shown).

TBARS

Cells in the exponential growth phase were subjected to H_2O_2 stress (20 mM for 15 min), and TBARS (expressed as μmol malondialdehyde per mg protein) was measured

Table 1. TBARS induced by H₂O₂ in exponential phase cells

Data are the means of duplicates from a representative experiment.

Mode of growth	Supplement*	H ₂ O ₂ †	Malondialdehyde [μmol (mg protein) ⁻¹]	Survival (%)
Aerobic	None	—	5.2	100
		+	9.7	20
Anaerobic	None	—	3.9	100
		+	13.4	16
Anaerobic	C _{18:1}	—	3.2	100
		+	17.7	9.6
Anaerobic	C _{18:3}	—	3.9	100
		+	26.3	6.8

* See Methods for details.

† Cells incubated with 20 mM H₂O₂ for 15 min.

Fig. 6. Induction of tolerance to 20 mM H₂O₂ (15 min) by H₂O₂ and heat shock in aerobic cells. The growth phase of cells treated at each time is shown on the figure. Heat shock was produced by heating cells from 25 °C to 37 °C (30 min) and H₂O₂ shock was produced by treating cells with 0.1 mM H₂O₂ (30 min). ○, Intrinsic tolerance; □, induced tolerance after H₂O₂ shock; ■, induced tolerance after heat shock.

in control and oxidatively stressed cells (Table 1). In aerobic cells, there was a twofold elevation of TBARS following a H₂O₂ stress. Anaerobic cells grown in medium without lipid supplements showed a threefold increase in TBARS. In the case of anaerobic cells supplemented with C_{18:1} fatty acid there was a 5.5-fold increase and with C_{18:3} fatty acid a 6.5-fold increase (Table 1). There was a positive correlation between the amount of TBARS produced and the percentage cell survival following H₂O₂ stress. Similarly, cells subjected to a heat stress (52 °C for 5 min) showed a marked increase in TBARS and a decrease in percentage cell survival (Table

2). Furthermore, prior exposure of cells to a mild oxidative (0.1 mM H₂O₂ for 30 min) or heat (37 °C for 30 min) shock followed by a higher stress also resulted in a marked increase in levels of TBARS. These pretreatment experiments characteristically induced an increase in cell survival compared to untreated controls (Table 2).

DISCUSSION

Oxygen is obligatory for all forms of aerobic life. Paradoxically, all living cells are subject to oxygen toxicity. Oxygen-derived species have been implicated as

Table 2. TBARS induced by H₂O₂ and heat challenge in exponential phase cells

Data are the means of duplicates from a representative experiment.

	H ₂ O ₂		Heat	
	Malondialdehyde [μmol (mg protein) ⁻¹]	Survival (%)	Malondialdehyde [μmol (mg protein) ⁻¹]	Survival (%)
Cells grown aerobically				
Control	3.8	100	6.1	100
Shock*	7.9	89	8.3	95
Stress†	15.2	11	16.2	9
Shock, stress	16.1	53	16.8	47
Cells grown anaerobically‡				
Control	5.1	100	5.0	100
Shock	10.5	76	8.5	93
Stress	26.9	5	20.0	2
Shock, stress	28.4	30	22.6	28

* 0.1 mM H₂O₂ for 30 min or 37 °C for 30 min (heat).

† 20 mM H₂O₂ for 15 min or 52 °C for 5 min (heat).

‡ Anaerobically grown cells were enriched in C_{18:3} fatty acid.

key causative agents of ageing, tumour promotion, cancer, inflammatory-immune injury, radiation damage and other clinical disorders (Halliwell, 1987; Halliwell & Grootveld, 1987).

In prokaryotic micro-organisms, cellular responses to oxidative stress have been primarily focused on *Escherichia coli* and *Salmonella typhimurium* (Farr & Kogoma, 1991). Ames and co-workers (Bochner *et al.*, 1984; Lee *et al.*, 1983) made the early suggestion that adenylated nucleotides or alarmones may be the common signal for the onset of oxidative and heat stress. Recent evidence has accumulated against this concept (Van Bogelen *et al.*, 1987), although a significant overlap exists between peroxide and heat-shock-inducible proteins in *Sal. typhimurium* (Morgan *et al.*, 1986) but less so in *E. coli* (Van Bogelen *et al.*, 1987).

In this report, we describe the response of aerobic and anaerobic *S. cerevisiae* cells to oxidative stress, induced by H_2O_2 , and temperature shifts. Intrinsic resistance to heat stress (52 °C, 5 min; Fig. 2) and oxidative stress (20 mM H_2O_2 , 15 min; Fig. 5) varied during the growth phase in batch culture, with cells in the exponential growth phase much more sensitive than cells in the stationary phase. It is well documented that yeast cells in the stationary phase are intrinsically more resistant to temperature stress as compared to cells in the exponential phase (Schenberg-Frascino & Moustacchi, 1972; Parry *et al.*, 1976; Walton *et al.*, 1979). Furthermore, in the case of *Bacillus subtilis*, it was reported that cells in the stationary phase were remarkably resistant to high levels (10 mM) of H_2O_2 (Murphy *et al.*, 1987). The present study extends these observations to include cellular physiology and membrane composition.

Early studies on *E. coli* by Overath *et al.* (1970) showed that $C_{18:1}$ -supplemented cells could grow at temperatures up to 45 °C, whereas $C_{18:3}$ -supplemented cells were unable to grow at temperatures about 40 °C. In later studies, Yatvin (1977) reported that $C_{18:1}$ -supplemented *E. coli* cells were more thermoresistant than $C_{18:3}$ -supplemented cells. It is clear from the present study that the membrane lipid composition, while important, is not the only parameter affecting thermotolerance and oxytolerance. The trend in anaerobically grown yeast cells was for a decrease in stress tolerance with increasing unsaturation of membrane fatty acids, which is in keeping with the previous observations on *E. coli*. On the other hand, aerobically grown cells enriched in mono-unsaturated fatty acids (for *S. cerevisiae*, characteristically 40% $C_{16:1}$ and 38% $C_{18:1}$) were the most stress tolerant under all experimental conditions.

We are led to suggest, therefore, that the metabolic state of the cells may also be an important factor in stress tolerance. This conclusion is in keeping with recent studies on stress tolerance of yeast cells grown on fermentable and non-fermentable carbon sources (Hou *et al.*, 1991; Sanchez *et al.*, 1992; Lewis *et al.*, 1993a; Elliot & Futcher, 1993). These studies have shown that yeast cells grown on a non-fermentable carbon source and with a high respiratory rate were intrinsically more resistant to

heat (Hou *et al.*, 1991; Sanchez *et al.*, 1992; Elliot & Futcher, 1993) and H_2O_2 (Hou *et al.*, 1991) than the corresponding cells grown on a fermentable carbon source.

It should be pointed out that the relationship of intrinsic or induced tolerance and growth phase with respect to studies on stress tolerance is crucial and is one which has not been adequately addressed in the past. For example, for yeast cells growing on glucose as a carbon source, the term stationary phase is generally taken to mean the point in the growth curve corresponding to exhaustion of glucose. For the purposes of the present studies we have adopted this definition for stationary phase. However, many yeasts are capable of further growth on ethanol, the end-product of glucose fermentation. In this case, the point corresponding to glucose exhaustion should be more precisely termed diauxic lag phase. This is followed by respiratory growth on ethanol (respiratory phase), and the point at which ethanol is exhausted is true stationary phase. Recent studies from this laboratory have demonstrated marked differences during growth in the response of yeast cells to a wide range of environmental stresses including heat and freeze-thaw (Lewis *et al.*, 1993b).

In the context of the present study, anaerobically grown cells were essentially obtaining energy via glycolysis, whereas the aerobically grown cells, although subject to catabolite repression, were obtaining energy via glycolysis and respiration. In keeping with the above discussion, aerobically grown cells were intrinsically more stress tolerant than anaerobically grown cells. This may in part be due also to induction of antioxidant enzyme systems, which is the subject of further study in our laboratory.

Higher levels of tolerance to heat stress could be induced by prior heat shock (37 °C, 30 min) in aerobic and anaerobic cells (Fig. 2). While induced thermotolerance has been reported previously for aerobic yeast cells (reviewed by Watson, 1990), the demonstration that thermotolerance may be induced in anaerobically grown *S. cerevisiae* is a novel finding. Induced thermotolerance followed the same trends as for intrinsic tolerance, in that tolerance increased as cells grew into stationary phase, and aerobic cells were the most resistant, followed in order by anaerobic unsupplemented, $C_{18:1}$ -supplemented and $C_{18:3}$ -supplemented anaerobic cells.

Similar trends were noted for induction of tolerance to H_2O_2 stress (Fig. 5). Increased tolerance to 20 mM H_2O_2 could be induced in all cells by a mild H_2O_2 shock (0.1 mM, 30 min). The induced tolerance was also growth-phase dependent. It is interesting to note that induced tolerance in anaerobic unsupplemented and $C_{18:1}$ -supplemented cells approached the level of induced tolerance of aerobic cells, with $C_{18:3}$ -supplemented cells again the most sensitive. That a prior mild peroxide shock should lead to peroxide tolerance has been demonstrated in this report for *S. cerevisiae* and also recently by others (Collinson & Dawes, 1992; Jamieson, 1992). Similarly, in *N. crassa*, pretreatment with sublethal doses of H_2O_2 induces tolerance to lethal doses (Kapoor *et al.*, 1990). It is noteworthy that in *E. coli* (Demple & Halbrook, 1983;

Christman *et al.*, 1985), *Sal. typhimurium* (Christman *et al.*, 1985; Winquist *et al.*, 1984) and *B. subtilis* (Murphy *et al.*, 1987), exposure of cells to mild peroxide stress induces tolerance to high levels of peroxide.

The relationship between tolerance to heat and H_2O_2 was also examined. While a mild heat shock was found to induce tolerance against both lethal temperature and H_2O_2 challenges (Fig. 6), pretreatment with low levels of H_2O_2 induced tolerance against a lethal level of H_2O_2 but not a lethal temperature challenge. Studies by us (Steels *et al.*, 1991; this report) and others (Collinson & Dawes, 1992; Jamieson, 1992) have shown heat-shock acquisition of peroxide tolerance in *S. cerevisiae*, as has been found for *N. crassa* (Kapoor & Lewis, 1987a). However, Christman *et al.* (1985) reported that in *Sal. typhimurium* a prior heat shock did not result in peroxide tolerance. Interestingly, in *N. crassa*, mild peroxide shock also induces thermotolerance (Kapoor *et al.*, 1990). By contrast, this report and that of Collinson & Dawes (1992) showed that yeast cells do not acquire thermotolerance following a mild peroxide shock, although Jamieson (1992) recently reported a small increase in thermotolerance. In *Sal. typhimurium*, a mild peroxide shock was found to induce thermotolerance (Christman *et al.*, 1985).

Tolerance to the heat and oxidative stresses in *S. cerevisiae* is related, as evidenced by the protection against oxidative stress by heat shock. It is possible that mild oxidative shock induces only a subset of responses induced by heat shock, and therefore is not protective against heat stress. This is consistent with our finding that peroxide shock did not induce synthesis of heat-shock proteins (Steels, unpublished observation). While Collinson & Dawes (1992) found that H_2O_2 induced some proteins, none of the classical heat-shock proteins were induced. In eukaryotic micro-organisms, there are few data concerning the function of stress proteins in oxidative stress. In *N. crassa*, the hsp70 group of stress proteins includes at least seven polypeptides (Kapoor & Lewis, 1987b), two of which are constitutively synthesized and elevated in response to a heat shock and another two which are apparently induced only on a heat shock or on H_2O_2 treatment of cells. Exposure of *N. crassa* cells to low levels of H_2O_2 for 1 h induced the synthesis of a subset of hsp70 stress proteins as well as a specific oxidative responsive protein, designated OSP80 (Kapoor & Lewis, 1987b). In addition, it was demonstrated in *N. crassa* that heat shock induced the synthesis of peroxidase activity (Kapoor & Lewis, 1987a).

In the present study, increased levels of TBARS were observed, indicative of malondialdehyde formation, in yeast cells subjected to a heat or oxidative stress (Table 1). Although the TBA test is not specific to malondialdehyde and its derivatives, it is an approximate measure of lipid oxidation and has been widely used as an index of lipid damage. In cells grown anaerobically and stressed with H_2O_2 , the amount of TBARS correlated with the degree of membrane lipid unsaturation. Cell survival correlated inversely with levels of TBARS. When cells were exposed to a mild heat shock or H_2O_2 shock, levels of TBARS

increased (Table 2). When this was followed by a lethal stress, levels of TBARS increased to levels similar to those produced by the stress alone, although higher tolerance was induced. This indicates that although cellular damage was evident, protective or repair mechanisms were induced to increase survival of the stresses. Damage limitation seems to have occurred, since the shock/stress levels of TBARS were lower than the sum of individual shock and stress components (Table 2). We are tempted to conclude that cells subjected to heat or oxidative stress may share a common mechanism of induced synthesis of oxygen-derived free radicals, leading to membrane lipid damage. The more unsaturated the membrane lipids the higher the degree of lipid and cell damage.

This hypothesis may be supported by observations about the temperature dependence of the solubility of oxygen in aqueous and membrane compartments. Increasing the temperature from 25 °C decreases the solubility of oxygen in media and cells. Using the equations provided by Hitchman (1978) it was calculated that in our experiments there would be a decrease in oxygen solubility of 21 % from 25 °C (211 mM O_2) to 37 °C (167 mM), and a 47 % decrease at 52 °C (111 mM). In contrast to the situation in aqueous solution, Smotkin *et al.* (1991) found that in liquid-crystalline phase phospholipid vesicles, oxygen solubility was about four times that in aqueous solution, and that as the temperature rose from 25 °C to 40 °C, partitioning of oxygen into membranes increased. Thus as the temperature increases, oxygen solubility in aqueous environments decreases, while relative solubility in membranes increases. This may lead to localized high concentrations of oxygen within membranes, which may correlate with a rapid rise in reactive oxygen-derived species as a result of temperature upshifts. This analysis is somewhat simplified and other factors must be taken into account, for example that oxygen gradients may exist within cells (Halliwell & Gutteridge, 1989).

Thus the changes in oxygen solubility noted above provide an attractive explanation for increased free-radical damage as a result of heat shock under aerobic conditions. In addition to generating local high concentrations of oxygen, increased temperatures themselves could be expected to lead to increased free-radical generation. The latter temperature effect would be especially important in cells grown under low oxygen tensions. The common features of cellular responses to heat and oxidative stress may therefore be due to common mechanisms of damage caused by free radicals.

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