Microbiology (2000), **146**, 877–884 Printed in Great Britain

Cellular lipid composition influences stress activation of the yeast general stress response element (STRE)

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The heat inducibility of the yeast heat-shock response (HSR) pathway has been shown to be critically dependent on the level of unsaturated fatty acids present in the cell. Here the inducibility by heat or salt of the independently regulated general stress response (GSR) pathway is shown to be affected in the same way. An increase in the percentage of unsaturated fatty acids in heat- or salt-acclimated cells correlated with a decrease in the induction of a general stress-response-promoter-element (STRE)-driven reporter gene by either stress. Despite inducing reporter gene expression, sorbic acid treatment did not confer salt cross-tolerance on the cells. This failure correlated with a failure to increase the percentage of unsaturated fatty acids in the cells, suggesting that GSR pathway induction, in the absence of lipid changes, is insufficient for the induction of cross-tolerance. Cells grown with fatty acid supplements under anaerobic conditions provided further evidence for a potential role for lipids in the acquisition of stress resistance. These cells contained different fatty acid profiles depending on the fatty acid supplement supplied, exhibited differential sensitivity to both heat and salt stress, but had not undergone STRE induction. These results suggest that heat- and salt-stress induction of the GSR are sensitive to the level of unsaturated fatty acids present in the cell and that stress cross-tolerance may be a lipid-mediated phenomenon. Given that an increased level of unsaturated fatty acids also down-regulates heat induction of the HSR pathway, these observations lead to the provocative hypothesis that lipid modifications, rather than HSR or GSR pathway induction, are a major contributor to the induced heat and salt tolerance of yeast cells.

Keywords: lipids, yeast, general stress response, STRE

INTRODUCTION

Saccharomyces cerevisiae has two major independently regulated stress responses: the heat-shock response (HSR) is induced when cells are exposed to a sublethal heat shock. It is also induced by a number of other stressing agents such as alcohol, heavy metals and anoxia (reviewed by Mager & De Kruijff, 1995). The general stress response (GSR) is induced by a wider variety of stressing agents including heat, osmotic stress, oxidative stress, nitrogen starvation, alcohol, sorbate and low external pH (reviewed by Ruis & Schüller, 1995).

Regulation of these systems is well understood at the DNA level. Induction of the HSR system requires the activation of a specific heat-shock transcription factor (HSTF; Sorger & Pelham, 1988; Wiederrecht *et al.*, 1988), which in turn activates an evolutionarily conserved set of genes – heat shock genes – via a specific promoter sequence, the heat-shock element (HSE; Pelham & Bienz, 1982). Genes in the GSR system contain a different general stress-response promoter element (STRE) which binds transcription factors encoded by two homologous and functionally redundant genes, *MSN2* and *MSN4* (Martinez-Pastor *et al.*, 1996).

This paper is dedicated to my parents Sandhya and Samir.

Abbreviations: GSR, general stress response; HSE, heat-shock element; HSR, heat-shock response; HSTF, heat-shock transcription factor; STRE, general stress response promoter element.

STREs or STRE-like sequences have been identified in the promoter region of a large number of genes including *DDR2* (Kobayashi & McEntee, 1990, 1993), *CTT1* (Wieser *et al*., 1991; Marchler *et al*., 1993), *HSP12* (Varela *et al*., 1995), *HSP104*, *TPS2*, *GSY2* and *GPH1* (reviewed by Ruis & Schüller, 1995). Activation of this system has been implicated in the acquisition of cross-tolerance to other stressing agents (Marchler *et al*., 1993; Martinez-Pastor *et al*., 1996; Winderickx *et al*., 1996).

Despite our detailed understanding of how these two systems are regulated at the DNA level, little is known about how heat is detected by cells and how sublethal increases in temperature activate these pathways. The accumulation of denatured proteins is commonly regarded as the trigger for stress-response activation (reviewed by Parsell & Lindquist, 1993), but a number of independent observations identify the plasma membrane as being critically involved in the transduction of heat stress into a biological signal (Thomas *et al*., 1978; Panaretou & Piper, 1990; Coote *et al*., 1994; Curran & Khalawan, 1994; Carratu *et al*., 1996). Previous work from this laboratory revealed that cellular lipids play a major role in determining the sensitivity of the HSR to stress. The temperature required for maximal heat shock activation of a HSR reporter gene varied from 40 °C to 49 °C depending on the level of unsaturated fatty acid present in the cell and an increase in the percentage of unsaturated fatty acids in heat-stressed cells occurred with kinetics that could explain the transient nature of the HSR pathway (Chatterjee *et al*., 1997).

Here we provide evidence that lipid composition plays a similar role in STRE-mediated activation by both heat and salt stress. Evidence is also provided that implicates lipid changes as a mechanism of stress cross-tolerance. These results collectively do not totally exclude the direct thermal denaturation of general cellular proteins as the mechanism of induction of two major stress-sensing systems by heat, but suggest that a more important trigger(s) lies with cellular lipids and their associated proteins.

METHODS

Strains. *Saccharomyces cerevisiae* strain GG18 (MATa *leu2 ura3 his3 trp1 ade8 cta1-2* P*CTT1*-18/7/*LEU2–lacZ*) was obtained from Dr P. Piper (University College London, UK). The strain was originally prepared by integrative transformation of strain GA74-6A with the pLS9 derivative pCTT1-18/7x, as described by Schüller *et al*. (1994). GG18 msn2msn4 (produced by Martinez-Pastor *et al*., 1996 and isogenic except for the disruption of *MSN2* and *MSN4*) was used as a control in all of these experiments but as it did not express *β*-galactosidase under any of these conditions, the data are omitted from the results.

Culture conditions

Aerobic growth. GG18 was grown to exponential phase in liquid-rich YEPD medium: 2·0 % (w/v) bactopeptone, 2·0 % (w/v) glucose, 1·0 % (w/v) yeast extract (2·0 %, w/v, agar was added for solid medium). Cells were grown overnight in a shaking water bath at 25 °C, 25 °C in the presence of 250 mM sodium chloride, 25 °C in the presence of 10 mM sorbic acid, or 37 °C.

Anaerobic growth. GG18 was grown on rich YEPD medium plates, in the presence of ergosterol (5 mg l^{-1}) and Tween 80 (500 µl l^{-1}), oleic acid (300 µg l^{-1}) or linoleic acid (300 µg l^{-1}). Plates were prepared under anaerobic conditions by inserting Petri dishes containing molten agar into an Oxoid anaerobic jar, previously purged with nitrogen, and adding two sachets of Oxoid Anaerogen anaerobic atmosphere generation system. The plates were removed the next day, inoculated by spreading approximately 500 cells from an exponentially growing liquid culture of GG18-STRE (2×10^6 cells ml^{-1}) and incubated at 25 °C under the anaerobic conditions described above. Four days later the small colonies that appeared were washed into rich YEPD medium to an OD_{600} of 0·35–0·45, harvested by centrifugation at 2000 ***g*** for 3 min in a bench-top centrifuge, washed twice with distilled water and resuspended in rich YEPD medium. Samples were taken for lipid and/or heat-shock-response, salt-shock-response or sorbic-acid-shock-response profiles as specified.

Determination of temperature profiles of STRE–*lacZ* induction. Aliquots of 10 ml exponentially growing cells were added to flasks containing 20 ml liquid YEPD medium, preheated to the stated temperature (in 250 ml conical flasks) in shaking water baths and subjected to a 10 min heat shock. The flasks were then placed in a 25 °C shaking water bath for a further 50 min to allow *β*-galactosidase expression from the induced transcripts. *β*-Galactosidase activity was measured from a total cell extract as described below.

Determination of salt and sorbic acid profiles of STRE–*lacZ* induction. Aliquots of 10 ml exponentially growing cells were added to 20 ml aliquots of liquid YEPD medium (in 250 ml conical flasks) at 25 °C in a shaking water bath. Sodium chloride or sorbic acid was added to the stated concentrations and cells were subjected to 10 min salt shock or sorbic acid shock. The cells were washed twice in YEPD medium and resuspended into 30 ml fresh YEPD medium. The flasks were then placed in a 25 °C shaking water bath for a further 50 min to allow *β*-galactosidase expression from the induced transcripts. *β*-Galactosidase activity was measured from a total cell extract as described below.

One representative set of experimental results (from at least three replicates) is presented for each temperature, salt and sorbic acid profile of STRE–*lacZ* expression. The absolute level of *β*-galactosidase varied between experiments but these profiles were reproduced in repeated separate experiments.

Measurement of *β*-galactosidase activity from total cell extracts. Cells were harvested by centrifugation at 2000 ***g*** for 3 min in an MSE bench-top centrifuge. The pellets were washed with distilled water, resuspended in 1 ml ice-cold Z buffer (0·05 M sodium phosphate pH 7·0, 0·01 M potassium chloride, 1 mM magnesium sulphate, 0·05 M *β*-mercaptoethanol) and transferred to 1·5 ml Eppendorf tubes. These tubes were spun at 13000 r.p.m. in an MSE microfuge for 1·5 min and the supernatants discarded. Glass beads (0·4 mm; Sigma) equal in volume to the cell pellet, were added to the tubes and 20 µl Z buffer was also added. The cells were disrupted by whirlimixing the samples for 1 min, on six occasions and cooling them on ice for 1 min between each burst of treatment. A further 80 µl Z buffer was added to each sample, whirlimixed briefly and spun for 5 s at 13000 r.p.m. in an MSE Microcentaur microfuge. The *β*-galactosidase activity and protein concentration in the supernatants were measured: *β*-galactosidase activity was monitored by the hydrolysis of

orthonitrophenol ONPG to *o*-nitrophenol + galactose: 20 µl supernatant was added to 1 ml solution of 0·2 ml Z buffer, 0·2 ml ONPG (2 mg ml^{-1} in water), 0·6 ml distilled water, and incubated for 35 min at 37 °C. Absorbance readings were taken at 420 nm. *β*-Galactosidase activity was expressed in units defined as the change in absorption at 420 nm in 35 min (mg protein)$^{-1}$. The total protein content in the supernatants was estimated by adding 2 µl supernatant to 1 ml of a solution of 0·2 ml Bio-Rad protein reagent, 0·8 ml distilled water and incubated for 20 min at 25 °C. Absorbance readings were taken at 600 nm and measured against a BSA protein standard curve.

Determination of the cellular lipid profile. A modification of a previously described procedure (Hossack & Rose, 1976) was used. Cells were harvested from 60 ml cultures by centrifugation (2000 ***g*** for 3 min at 25 °C), washed twice with distilled water and the final pellet was resuspended in 400–500 µl distilled water. This suspension was added to a mortar, rapidly frozen in liquid nitrogen and ground with acid-washed sand. After the addition of 3 ml chloroform/methanol (2:1, v/v) and a few crystals of butylated hydroxytoluene, the lipids were extracted at 40 °C for 1 h. The upper layer was removed, the lower layer was washed by adding 2 ml methanol/water (1:1, v/v) and the upper layer was again removed. The remaining organic layer was evaporated to dryness in a stream of nitrogen gas and fatty acid methyl esters were prepared by refluxing the residue at 80–90 °C for 1 h in 5 ml concentrated sulphuric acid/toluene/methanol (1:10:20, by vol.). The methyl esters were extracted into 2 ml hexane, evaporated to dryness in a stream of nitrogen gas and dissolved in 20 µl hexane for GLC analysis. Fatty acids were identified by their retention time relative to that of known standards on a packed column (2 m × 2 mm) containing 10% CP-SIL 58 packing on chromosorb WHP 100–120 mesh (w/w). The relative percentage of each fatty acid was calculated by dividing the area underneath the peak by the total peak areas of both saturated and unsaturated fatty acids. The mean value and range from at least two separate experiments are presented in each figure. Although the ranges of individual fatty acids varied by up to 20% over a series of repeated experiments, the ranges of total saturated and unsaturated fatty acids never exceeded 10%.

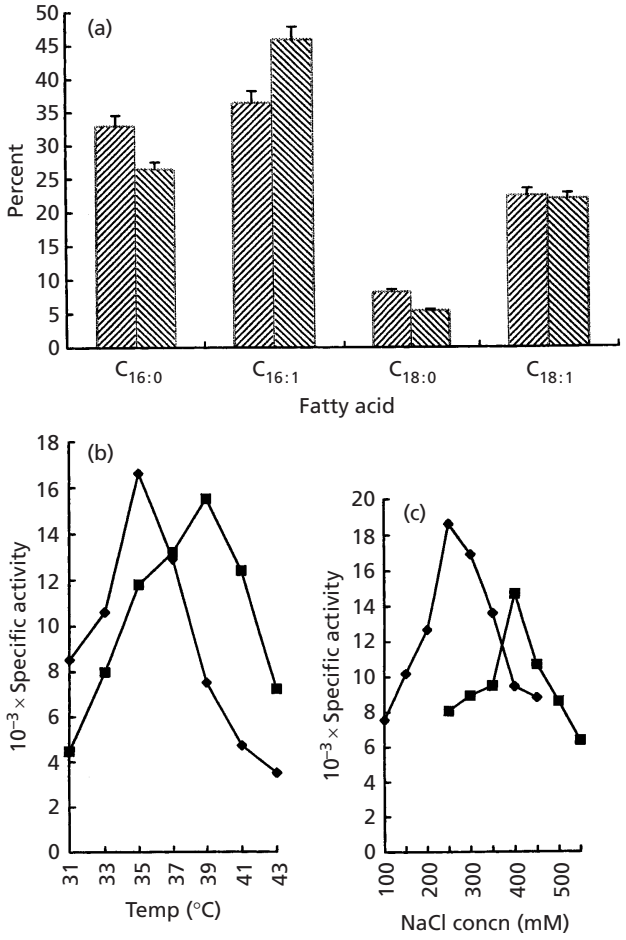

Fig. 1. (a) The percentage of individual fatty acids present in cells grown at 25 °C (left-hand bar of each pair) and 37 °C (right-hand bar); the mean and range from two separate experiments are shown. (b) The profile of heat-shock-induced *β*-galactosidase expression from cells grown at 25 °C (◆) and at 37 °C (■) for 16 h. (c) The profile of salt-shock-induced *β*-galactosidase expression from cells grown at 25 °C (◆) and at 37 °C (■) for 16 h.

RESULTS

A decreased STRE response to heat and salt correlates with an increase in the percentage of cellular unsaturated fatty acids in heat-stress-acclimated cells

There was a 9% increase in the percentage of cellular unsaturated fatty acids in heat-stress-acclimated cells. This comprised a 9·5% increase in $C_{16:1}$ and a 0·5% decrease in $C_{18:1}$ with respect to the percentages found in 25 °C-grown cells (Fig. 1a).

There was also a significant decrease in both heat and salt sensitivity of the STRE-inducible reporter gene in 37 °C-grown cells (Fig. 1b, c). There was a 4 °C increase in the temperature required to induce maximal *β*-galactosidase synthesis and a 150 mM increase in the salt concentration required for maximal *β*-galactosidase induction from the STRE-driven expression system, as compared to 25 °C-grown cells.

In 25 °C-grown cells, the peak heat-induced response occurred at 35 °C, the level of response declining to less than half this maximal value at 39 °C. In contrast, cells grown at 37 °C had a low level of response at 35 °C, peak induction at 39 °C and less than half this maximal value at 43 °C (Fig. 1b).

There was minimal induction of *β*-galactosidase with the lowest level of sodium chloride tested (150 mM) in 25 °C-grown cells. Induction increased slightly at 200 mM, peaked at 250 mM and decreased to less than half this value at 400 mM sodium chloride. In contrast, cells acclimated at 37 °C had minimal induction of *β*-galactosidase at the level of osmotic stress (250 mM) causing maximal response in 25 °C grown cells, there being a shift in the peak response concentration to 400 mM sodium chloride. The level of response fell at the maximum sodium chloride concentration subsequently tested (550 mM) (see Fig. 1c). Decrease in both the heat- and salt-shock responsiveness of the STRE-mediated pathway in heat-stressed cells correlated

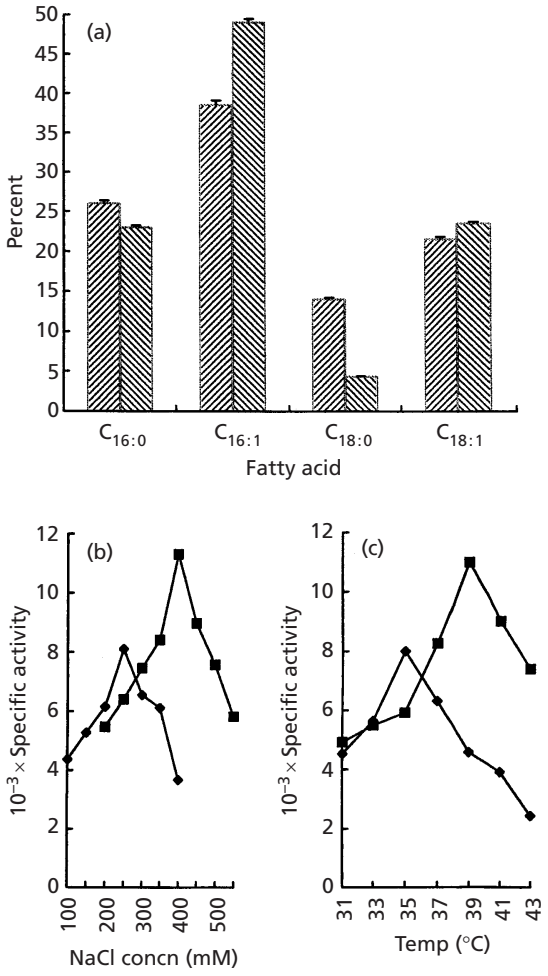

Fig. 2. (a) The percentage of individual fatty acids present in cells grown at 25 °C in the presence (right-hand bar of each pair) and absence (left-hand bar) of 250 mM NaCl; the mean and range from two separate experiments are shown. (b) The profile of salt-shock-induced *β*-galactosidase expression from cells grown at 25 °C with (■) and without (◆) salt-stress acclimation. (c) The profile of heat-shock-induced *β*-galactosidase expression from cells grown at 25 °C with (■) and without (◆) salt-stress acclimation.

therefore with an increase in the percentage of cellular unsaturated fatty acids.

Decrease in STRE-mediated responsiveness may be related to lipid composition

The correlation between changes in cellular lipid composition and cellular stress sensitivity was maintained in cells that had been exposed to a long-term salt stress. Analysis of the lipid composition of cells that had been acclimated to 250 mM sodium chloride revealed a 12·5 % increase in cellular unsaturated fatty acids with respect to cells grown in the absence of salt. This comprised a 10·5 % increase in $C_{16:1}$ and a 2·0 % increase in $C_{18:1}$ (Fig. 2a). These changes correlated with a 150 mM increase in the salt concentration required to induce peak *β*-galactosidase expression and a 4 °C increase in the temperature required to induce peak *β*-galactosidase expression, as compared with non-stressed cells (Fig. 2, c).

β-Galactosidase was minimally induced at a salt concentration of 100 mM in unacclimated cells. The level of induction increased gradually as the salt concentration was raised to 150 mM, peaked at 250 mM sodium chloride and fell to less than half of this level at 400 mM sodium chloride. In contrast, minimal levels of *β*-galactosidase were induced at 250 mM sodium chloride in salt-stress-acclimated cells (the concentration of peak response in non-acclimated cells) and the peak response had shifted to 400 mM sodium chloride. Lower levels of *β*-galactosidase were induced at 450 mM and the level of response fell to less than half the peak value at 550 mM (Fig. 2b).

The temperature of maximum heat-shock-induced STRE activity also changed in salt-stress-acclimated cells. *β*-Galactosidase was minimally induced at 31 °C in cells grown in the absence of salt acclimation. The level of enzyme induction increased slightly as the temperature was raised to 33 °C with peak induction at 35 °C and low levels of induction at 39 °C. In contrast, salt-stress-acclimated cells had low levels of *β*-galactosidase induction at 31, 33 and 35 °C. Enzyme induction increased rapidly as the temperature was raised beyond 35 °C with peak induction at 39 °C. Enzyme induction was significantly decreased from the peak value at 43 °C (Fig. 2c).

Thus not only is there an increase in the percentage of unsaturated fatty acids in heat- or salt-stressed cells, but a corresponding decrease in the STRE responsiveness of these cells to either stress.

Sorbic acid stress does not induce lipid changes or result in a decrease in STRE responsiveness

An analysis of sorbic acid stressed cells also supported the notion that lipid changes were intimately involved in cellular stress sensitivity.

STRE-driven *β*-galactosidase activity was induced when cells were exposed to 10 mM sorbic acid. Long-term exposure to the same concentration of this stressing agent failed, however, to induce any significant increase in the percentage of unsaturated fatty acids present in the cells (see Fig. 3a). This treatment also failed to confer either tolerance to higher doses of the same stress (data not shown) or cross-tolerance to a salt stress. Induction of *β*-galactosidase activity was maximal at 250 mM sodium chloride in both cases and the salt-induced enzyme expression profiles were very similar (see Fig. 3b).

These results suggest that STRE induction *per se* does not decrease the STRE-mediated responsiveness of cells. However, sorbic acid's inability to confer stress desensi-

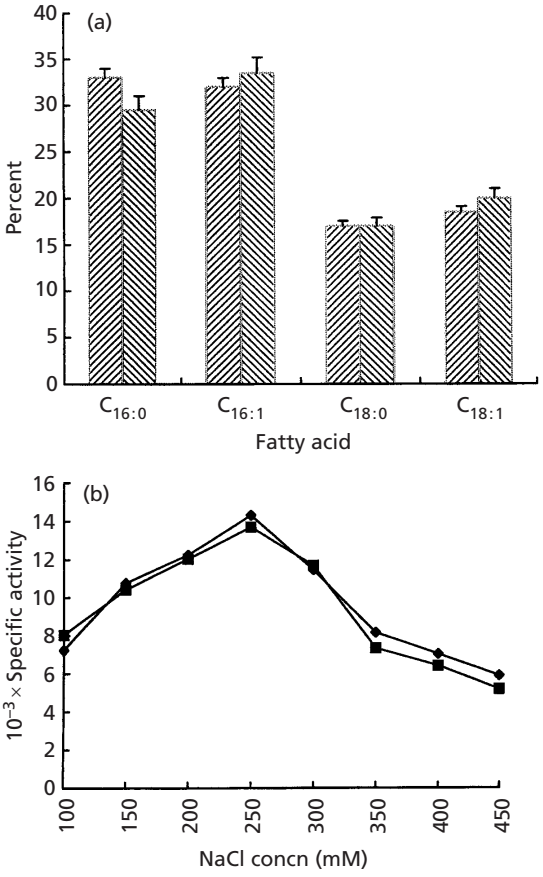

Fig. 3. (a) The percentage of individual fatty acids present in cells grown at 25 °C in the presence (right-hand bar of each pair) and absence (left-hand bar) of 10 mM sorbic acid; the mean and range from two separate experiments are shown. (b) The profile of salt-shock-induced *β*-galactosidase expression from cells grown at 25 °C with (■) and without (◆) sorbic acid stress acclimation.

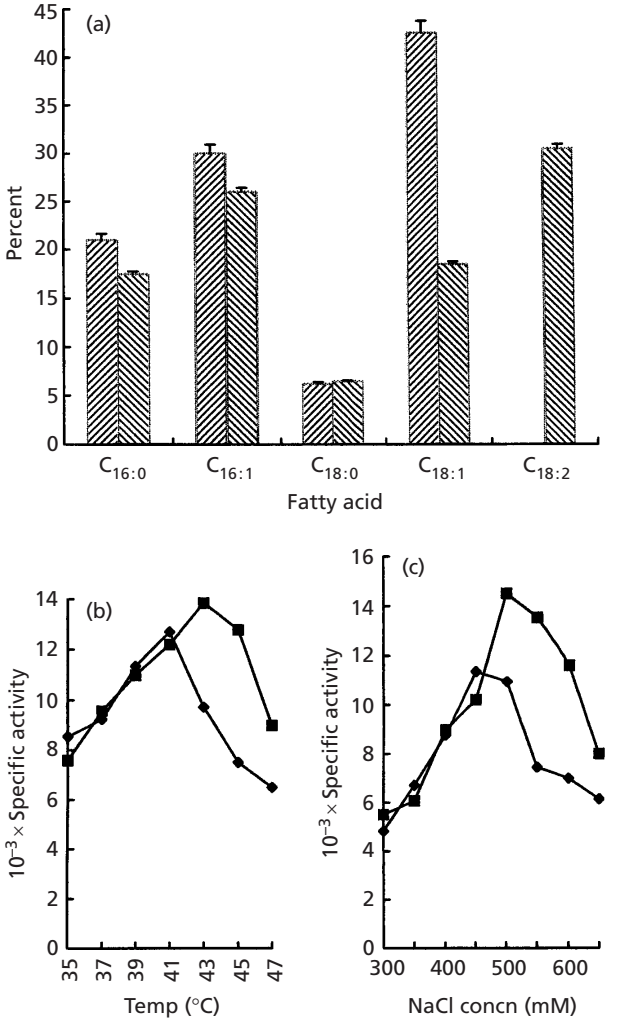

Fig. 4. (a) The percentage of individual fatty acids in cells grown at 25 °C under anaerobic conditions in the presence of Tween 80 (left-hand bar of each pair) or linoleic acid (right-hand bar); the mean and range from two separate experiments are shown. (b) The profile of heat-shock-induced *β*-galactosidase expression from cells grown at 25 °C under anaerobic conditions in the presence of Tween 80 (◆) or linoleic acid (■). (c) The profile of salt-shock-induced *β*-galactosidase expression from cells grown at 25 °C under anaerobic conditions in the presence of Tween 80 (◆) or linoleic acid (■).

tization correlates with its inability to induce lipid changes, thus supporting the notion that stress desensitization may be related to the concomitant lipid alterations associated with GSR pathway induction by other inducing agents such as heat or salt.

Lipid changes induce a decrease in STRE responsiveness in the absence of STRE induction

Further evidence is provided for this in Fig. 4 where cells grown under anaerobic conditions in the presence of different fatty acids are shown to have changes in their lipid profiles, the level of stress cross-tolerance and in the inducibility of the STRE-mediated response as compared with control cells.

The percentage of cellular unsaturated fatty acids in cells supplemented with Tween 80 was 13·5% higher than that found in aerobically grown cells at 25 °C. This comprised a 6·5% decrease in $C_{16:1}$ and a 20·0% increase in $C_{18:1}$ [compare Fig. 4a (Tween 80) with Fig. 1a (25 °C)]. The fatty acid profile of cells supplemented with linoleic acid was significantly different from that of cells supplemented with Tween 80 (see Fig. 4a): these cells contained 30·5% linoleic acid, which was completely absent from the lipid profile of cells supplemented with Tween 80, 4·0% less $C_{16:1}$ and 24·0% less $C_{18:1}$.

There was differential heat-shock and salt-shock responsiveness of the STRE in cells supplemented with different fatty acids. Cells supplemented with Tween 80 had minimal induction of *β*-galactosidase at 35 °C; this increased rapidly as the temperature was raised beyond 37 °C and peaked at 41 °C [6 °C higher than that found

for aerobically grown cells at 25 °C: compare Fig. 4b (Tween 80) and Fig. 1b (25 °C)]. Cells supplemented with linoleic acid required a further 2 °C increase in temperature to maximally induce the STRE reporter gene [8 °C higher than that found for aerobically grown cells at 25 °C: compare Fig. 4b (linoleic acid) with Fig. 1b (25 °C)].

The salt sensitivity was also differentially affected. Cells supplemented with Tween 80 had maximal β-galactosidase expression at 450 mM NaCl with a lower level of induction at 500 mM (Fig. 4c). Cells supplemented with linoleic acid required a further 50 mM increase in salt concentration from that of cells supplemented with Tween 80 to maximally induce the STRE reporter gene (Fig. 4c). These values are 200 mM and 250 mM higher, respectively, than those found for aerobically grown cells at 25 ° [compare Fig. 4c with Fig. 1c (25 °C)].

These results therefore correlate an increase in the percentage of unsaturated fatty acids present in the cell with a decrease in the responsiveness of the STRE to heat or salt stress. Moreover, they strongly suggest that lipid composition can affect the sensitivity of the STRE response to these agents even in the absence of stress induction.

DISCUSSION

The sensing mechanisms leading to the activation by heat of the two major stress-transduction systems in yeast are largely unknown. Many of the HSR genes encode proteins that are involved in protein folding and therefore, not unreasonably, the accumulation of denatured proteins is commonly regarded as the trigger for activation of the HSR (reviewed by Parsell & Lindquist, 1993). A number of independent observations, however, identify the plasma membrane as being critically involved in the transduction of heat stress into a biological signal (Thomas *et al*., 1978; Panaretou & Piper, 1990; Coote *et al*., 1994; Curran & Khalawan, 1994; Carratu *et al*., 1996). We have recently demonstrated that an HSE-driven expression vector is differentially induced at the same temperature in cells containing different levels of unsaturated fatty acids. Furthermore, we have demonstrated that the increased percentage of unsaturated fatty acids in yeast cells during heat shock for 4 h at 37 °C is sufficient to desensitize the HSR pathway to heat, thus providing a possible explanation for the transient nature of this response (Chatterjee *et al*., 1997).

If, as these results strongly suggest, yeast cells are less sensitive to heat stress when they contain high levels of unsaturated fatty acids in their membranes, then the other major heat-sensing system – the STRE-mediated response – should also show decreased heat sensitivity under the same conditions. This study reveals that this is indeed the case. Heat-stress-acclimated cells contained 9% more unsaturated fatty acids than cells grown at 25 °C and required a 4 °C higher temperature to induce peak β-galactosidase induction from an STRE-driven reporter gene (see Fig. 1a, b). The cellular changes associated with acclimation to heat stress also decreased the sensitivity of the GSR pathway to salt stress. Fig. 1c reveals there was a 150 mM increase in the concentration of salt required to maximally induce the response in cells that had been acclimated to 37 °C. Furthermore, remarkably similar changes occurred in cells that had been acclimated to salt stress. There was a 9% increase in the percentage of unsaturated fatty acids (as had been the case with heat-stressed cells) and an identical decrease in the sensitivity of GSR induction by either salt or heat (compare Figs 2a and 1a, 2b and 1c with 2c and 1b).

Yeast cells develop tolerance against higher doses of the same stress (induced stress resistance) and also cross-tolerance to other stressing agents (Lindquist, 1986; Schüller *et al*., 1994; Martinez-Pastor *et al*., 1996) after exposure to sublethally stressful conditions. Although the reciprocal nature of the decrease in STRE responsiveness observed in heat- or salt-acclimated cells (Figs 1 and 2) supports the currently held view that the acquisition of stress tolerance is related to the activation of the GSR system (Martinez-Pastor *et al*., 1996; Winderickx *et al*., 1996), the results in Figs 3 and 4 suggest that attendant lipid changes may play a significant role in this phenomenon.

Preliminary experiments revealed that the STRE-driven reporter gene was maximally activated when cells were exposed to sorbic acid concentrations of 10–12 mM, yet acclimation of yeast cells to 10 mM sorbic acid failed either to increase the percentage of unsaturated fatty acids in the cells (Fig. 3) or to decrease the responsiveness of the STRE-driven reporter gene to a subsequent salt stress. These results suggest that a decrease in STRE responsiveness does not necessarily arise in STRE-induced cells and that at least in the case of sorbic acid this correlates closely with the absence of an increase in cellular unsaturated fatty acids.

The differential responsiveness of the STRE reporter in cells grown under identical anaerobic conditions but supplied with different fatty acids provides further evidence for this (Fig. 4). Cells grown with a Tween 80 supplement under anaerobic conditions contained 13·5% more unsaturated fatty acids than cells grown in the absence of fatty acid supplements [see Figs 4a (Tween 80) and 1a (25 °C)] and required a 6 °C higher temperature or a 200 mM higher salt concentration to maximally induce the STRE reporter gene [see Figs 4b (Tween 80) and 1b (25 °C), 4c (Tween 80) and 1c (25 °C)]. Cells grown under identical anaerobic conditions but supplemented with linoleic acid had a significantly different fatty acid profile from cells supplemented with Tween 80 (see Fig. 4a). These cells required a 2 °C higher temperature or a 50 mM higher salt concentration to maximally induce the STRE reporter gene (see Fig. 4b, c). Thus the STRE-driven reporter gene is differentially expressed in cells that have different types of fatty acids and these altered response profiles arise without the need for GSR pathway induction. This lipid-correlated decrease in STRE re-

sponsiveness also provides a possible explanation for the heretofore-unexplained phenomenon of how heat and salt confer cross-tolerance on one another.

A number of independent observations implicate membrane perturbations as being involved in heat-stress-induced gene expression (reviewed by Vigh *et al*., 1998). Mutant *Escherichia coli* strains that have a modified lipid composition show altered expression of genes involved in osmoregulation (Inoue *et al*., 1997) and in the unicellular cyanobacter *Synechocystis*, the temperature at which maximal activation of a number of HSR genes occurs decreases when the fluidity of the thylakoids is increased by temperature acclimation or by the addition of benzyl chloride (Horvath *et al*., 1998). Carratu *et al*. (1996) increased the fluidity of the yeast membrane by genetically manipulating the level of the Δ^9-desaturase enzyme and demonstrated that the temperature inducibility of the *hsp70* and *hsp82* genes was altered in yeast cells containing different levels of unsaturated fatty acids. Furthermore, Kamada *et al*. (1995) provided evidence that thermal stress triggers the expression of the yeast *PKC1* gene, by stretching the plasma membrane.

We have previously demonstrated that the heat sensitivity of the yeast HSR pathway is critically dependent on the level of unsaturated fatty acids present in the cell (Chatterjee *et al*., 1997) and here we provide evidence that cellular lipid composition influences stress activation of the STRE. Together, these studies exclude the direct thermal denaturation of general cellular proteins as the trigger for the activation of these major stress-signalling pathways in yeast. Rather, they suggest that heat stress is detected by membrane-linked 'thermostat(s)' whose activation is a consequence not only of the temperature but also of the type and percentage of unsaturated fatty acids present in the cell. Furthermore, given that the percentage of unsaturated fatty acids maximally increases and that HSR genes are down-regulated within 4 h of an initial heat shock, the corollary to this hypothesis is that stress-inducible genes protect living cells during acute stress but that changes in cellular lipids are a major factor by which cells acclimate to long-term heat (or salt) stress.

Thermotolerance is measured as a cell's ability to withstand normally lethal temperatures (reviewed by Piper, 1993). The decreased cellular heat sensitivity, experienced by both the GSR and HSR pathways in cells containing high levels of unsaturated fatty acids, only makes physiological sense in the context of an increase in cellular thermotolerance. Preliminary experiments (data not shown) have revealed that this is indeed the case. Cells that contain high levels of unsaturated fatty acids are able to survive stress regimens that seriously compromise untreated controls and this aspect of cellular stress resistance is currently under investigation. This gives rise to the exciting possibility that it may be possible to engineer both heat and salt resistance into cells by simply modifying the percentage of fatty acids they contain.

ACKNOWLEDGEMENTS

We thank Dr Peter Piper for providing yeast strains and for helpful discussions, Alan Scott for expert technical assistance with lipid analysis and Dr Virginia Bugeja for critically reading the manuscript.

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Received 1 October 1999; revised 9 December 1999; accepted 24 December 1999.