Alterations in cellular lipids may be responsible for the transient nature of the yeast heat shock response

Mahua T. Chatterjee, Seunath A. Khalawan and Brendan P. G. Curran

Author for correspondence: Brendan P. G. Curran. Tel: +44 171 775 3013. Fax: +44 181 983 0973.
e-mail: B.Curran@qmw.ac.uk

School of Biological
Sciences, Queen Mary and
Westfield College, Mile
End Road, London E1 4NS,
UK

The stress-sensing systems leading to the cellular heat shock response (HSR) and the mechanism responsible for the desensitizing of this response in stress-acclimated cells are largely unknown. Here it is demonstrated that there is a close correlation between a 3 °C increase in the temperature required for maximal activation of a heat-shock (HS)-inducible gene in *Saccharomyces cerevisiae* and an increase in the percentage of cellular unsaturated fatty acids when cells are subjected to extended periods of growth at 37 °C. The latter occurs with the same kinetics as HS gene down-regulation during a prolonged HS and is reversed by reacclimation to growth at 25 °C. The transient nature of the HS may therefore be due to a lipid-mediated decrease in cellular heat sensitivity. Further evidence that unsaturated fatty acids desensitize cells to heat, with a resultant down-regulation of the HSR, is provided by demonstrating a 9 °C increase in the temperature required for maximal induction of this HS-inducible gene in cells containing high levels of unsaturated fatty acids assimilated during anaerobic growth at 25 °C.

Keywords: *Saccharomyces cerevisiae*, lipids, transient heat shock response

INTRODUCTION

The yeast *Saccharomyces cerevisiae*, in common with all living organisms examined to date, possesses a set of heat shock (HS) genes. In yeast these are induced in cells exposed to a sublethal HS, and many are also induced by any one of a number of other stressing agents (for reviews, see Mager & Ferreira, 1993; Parsell & Lindquist, 1993). HS gene induction is dependent upon the activation of a HS transcription factor (Sorger & Pelham, 1988; Wiederrecht *et al.*, 1988) bound to HS elements (HSEs) (Pelham & Bienz, 1982) in the promoters of these genes (for review, see Sorger, 1991), but the trigger responsible for HS transcription factor activation is unknown. The classical view of the HS response (HSR) is that stressing agents cause the accumulation of denatured proteins in the cell with a resultant induction of the genes responsible for HS protein production (for review, see Parsell & Lindquist, 1993). An alternative view is that the temperature-sensing mechanism(s) is(are) intimately associated with membrane structure

and function. This is supported by a number of independent observations: plasma-membrane ATPase activity affects HS protein synthesis in yeast (Panaretou & Piper, 1990); yeast thermotolerance increases with HS (Coote *et al.*, 1994); and the activity of the lipid-modifying $\Delta 9$ -desaturase enzyme affects the temperature for optimal HS gene expression in yeast (Carratu *et al.*, 1996). These two views are not necessarily mutually exclusive (e.g. some cytoplasmic activities could be affected by heat-induced changes to membrane proteins and lipids) but the precise mechanism responsible for transducing heat stress into the intracellular message resulting in HS transcription factor activation has yet to be elucidated.

The HSR is generally transient in all organisms. At sublethal temperatures there is a significant diminution of the response within 1 h of induction and an eventual return to normal protein synthesis (Miller *et al.*, 1979; Slater & Craig, 1987; Sorger, 1990, 1991). This indicates that the temperature-sensing mechanism becomes desensitized by prolonged exposure to heat. Conversely, the temperature-sensing mechanism in yeast cells is rendered more sensitive in the presence of alcohols, the alcohol concentration required to elicit this increased sensitivity decreasing with increasing alcohol hydro-

Abbreviations: HS, heat shock; HSE, heat shock element; HSR, heat shock response.

We dedicate this paper to Mary Curran and to the memory of Séan Curran.

phobicity (Curran & Khalawan, 1994). Neither of these observations lend support to a model of HS gene activation that involves the general thermal denaturation of cellular proteins as the trigger, but instead points to a sensor with a hydrophobic, dynamic nature that permits it to become differentially sensitive to the same temperature.

The yeast cell membrane is hydrophobic and dynamic, its lipid composition changing to adapt to the prevailing conditions (Hunter & Rose, 1972; Suutari *et al.*, 1990; Lloyd *et al.*, 1993). In this study, we have monitored changes in the percentages of cellular unsaturated fatty acids during a sublethal HS and have demonstrated that the thermal inducibility of a HS-sensitive reporter gene is closely correlated with the percentage of unsaturated fatty acids present in the cell. This correlation is further substantiated by demonstrating an increase of up to 9 °C in the optimal activation temperature for the HSR in cells supplemented with high levels of unsaturated fatty acids under anaerobic growth metabolism. These results suggest that the transient nature of the HSR may be a manifestation of a lipid-mediated desensitization of the heat stress signal transduction mechanism. They are consistent with the recent findings of Carratu *et al.* (1996), which suggest that thermal stress is transduced into a cellular signal at the level of the membrane.

METHODS

Strains. *Saccharomyces cerevisiae* strain DBY747 (MATa *leu2-112 leu2-3 ura3 his3 trp1*) was transformed to uracil prototrophy with the HS expression vector GA1695-HSE1 (a kind gift from Dr P. Sorger, University of California, USA) and the resulting transformant DBY747-HSE1 was used in this study. GA1695-HSE1 contains a single HSE sequence inserted in a disabled CYC1 promoter fused to the *lacZ* gene (Sorger & Pelham, 1987). β -Galactosidase is not expressed at detectable levels in DBY747-HSE1 under normal conditions.

Culture conditions

Aerobic growth. DBY747-HSE1 was grown to exponential growth phase (2×10^6 cells ml⁻¹) in liquid minimal medium made to the specification of the formula of Wickerham (1951) supplemented with methionine (20 mg l⁻¹), histidine (20 mg l⁻¹), leucine (30 mg l⁻¹) and tryptophan (20 mg l⁻¹).

Cells were either grown at 25 °C and acclimated in a 37 °C shaking water-bath for 16 h (during which time cells were diluted with fresh selective medium to maintain exponential growth phase) or grown to exponential phase at 37 °C and acclimated in a 25 °C shaking water-bath for 16 h (during which time cells were diluted as described above). Samples were analysed for lipid and temperature profiles of HSE-*lacZ* induction at the specified time points.

Anaerobic growth. DBY747-HSE1 was grown on minimal medium plates to the specification of the original formula of Wickerham (1951) with the above amino acid supplements plus ergosterol (5 mg l⁻¹) and either Tween 80 (500 μ l l⁻¹) or linoleic acid (300 μ g l⁻¹) as specified. 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 DBY747-HSE1 (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 selective medium, to an OD₆₀₀ of 0.05–0.10 (Cecil spectrophotometer), harvested by centrifugation at 3000 r.p.m. for 3 min in an MSE bench-top centrifuge, washed twice with distilled water and resuspended in selective medium. Samples were taken for lipid and HSR profiles as specified below.

Reversion procedure. Colonies were washed from linoleic-acid-supplemented plates after 4 d anaerobic growth into selective medium to an OD₆₀₀ of 0.05–0.10. The cells were harvested by centrifugation at 2000 g for 3 min in an MSE bench-top centrifuge, washed with distilled water and resuspended in selective medium. The cell suspension was acclimated in a shaking 25 °C water-bath for 16 h, during which time cells were diluted with selective medium to maintain exponential growth phase. Samples were analysed for lipid and HSR profiles at the specified time points as described below.

Determination of temperature profiles of HSE-*lacZ* induction.

Aliquots (10 ml) of exponentially growing cells were placed in 20 ml Wickerham's liquid minimal media pre-heated to the stated temperature (in 250 ml conical flasks) in shaking water-baths and subjected to a 10 min HS. 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 previously (Curran & Khalawan, 1994). One representative set of experimental results is presented for each temperature profile of HSE-*lacZ* expression in Figs 1(b), 2(b), 3(b) and 4(b). The absolute level of β -galactosidase varied between experiments but these profiles were reproduced in repeated separate experiments.

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 μ l 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) containing 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 μ l hexane for GLC analysis. Fatty acids were identified by their retention time relative to that of known standards on a packed column (2 m $\times$ 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 from two separate experiments is presented in each figure in Results. The range is less than 10%.

RESULTS

There is an increase in cellular fatty acid unsaturation and in the temperature needed to elicit a maximal HSR during prolonged heat stress

The percentage of cellular unsaturated fatty acids increased by 9% when cells grown at 25 °C were shifted to 37 °C for 1 h. It increased by a further 12% to 66% after 3 h and remained constant thereafter. This 21% increase in unsaturated fatty acids was the result of a decrease in C18:0 (18%) and increases in C16:1 (7%) and C18:1 (14%) (Fig. 1a). The cells grown at 25 °C and 37 °C were differentially sensitive to HS. β -Galactosidase was minimally induced from the HS expression vector when cells grown at 25 °C were exposed to 38 °C, this level increasing slightly at 39 °C, peaking at 40 °C and declining to zero at 44 °C. In contrast, cells grown at 37 °C had a lower sensitivity to heat: β -galactosidase was minimally induced between 38 °C and 40 °C (the temperature at which cells grown at 25 °C responded maximally), and the maximal response shifted to 43 °C (Fig. 1b).

Fig. 1. (a) Percentage of individual fatty acids present in cells grown at 25 °C and exposed to 37 °C for 1, 3 and 16 h; the mean values from two separate experiments are shown (range less than 10%). (b) Profile of HS-induced β -galactosidase expression from cells grown at 25 °C and at 37 °C for 16 h. □, 0 h; ■, 16 h.

Fig. 2. (a) Percentage of individual fatty acids present in cells acclimated to 37 °C and after growth at 25 °C for 2, 4, 6 and 16 h; the mean values from two separate experiments are shown (range less than 10%). (b) Profile of HS-induced β -galactosidase expression at the same time points. ●, 0 h; ○, 2 h; ▲, 4 h; □, 6 h; ◆, 16 h.

The percentage of unsaturated fatty acids and the temperature of the maximal HSR decline concomitantly as cells grown at 37 °C reacclimatize to growth at 25 °C

The relationship between the degree of fatty acid unsaturation in the cell and the temperature of maximum HSR was maintained when cells grown at 37 °C were reacclimated to growth at 25 °C (Fig. 2a, b). Neither the percentage of unsaturated fatty acids present (66%) nor the temperature required for maximal HS induction (43 °C) changed significantly during the first 2 h. The percentage of unsaturated fatty acids fell by 10% to 56% over the next 2 h, however, with the cells then responding maximally to heat stress at 42 °C. These values fell to 47% and 40 °C, respectively, over the next 2 h and remained relatively constant thereafter. The changes in the individual fatty acids were the reverse of what had occurred during the upshift in temperature. There were increases in C16:0 and C18:0 of 2% and 17%, respectively, whereas C16:1 and C18:1 both decreased by 10% (Fig. 2a). The lipid and HSR profiles did not differ significantly after 16 h at

Fig. 3. (a) Percentage of individual fatty acids in cells grown at 25°C under anaerobic conditions in the presence of Tween 80 or linoleic acid; the mean values from two separate experiments are shown (range less than 10%). $\square$, C16:0; $\boxtimes$, C16:1; $\square$, C18:0; $\blacksquare$, C18:1; $\blacksquare$, C18:2. (b) Profile of HS-induced β -galactosidase expression for the same cells. $\square$, Tween 80; $\blacksquare$, linoleic acid.

25°C from those of cells grown at 25°C , where the percentage of unsaturated fatty acids was 45% and the activation temperature for the maximal HSR was 40°C (compare Fig. 2a, b to Fig. 1a, b).

The temperature of maximal HSR induction is affected by fatty acids assimilated during anaerobic growth

The absolute requirement of anaerobically growing yeast cells for an external source of unsaturated fatty acid was exploited to produce cells with fatty acid profiles that were significantly different from one another and from cells grown under aerobic conditions at 25°C . The percentage of unsaturated fatty acids in cells supplemented with Tween 80 was 66%. The cells had 12% more C18:1 and 18% less C18:0 than aerobically grown cells. They also contained 4% C18:2, which aerobically growing cells completely lack (compare Figs 1a and 3a). There was minimal induction of β -galactosidase activity from these cells at 39°C . This increased rapidly as the temperature was raised beyond 41°C with

Fig. 4. (a) Percentage of individual fatty acids present in linoleic-acid-supplemented cells after growth at 25°C under aerobic metabolism for 0, 2, 4, 6 and 16 h; the mean values from two separate experiments are shown (range less than 10%). $\square$, C16:0; $\boxtimes$, C16:1; $\square$, C18:0; $\blacksquare$, C18:1; $\blacksquare$, C18:2. (b) Profile of HS-induced β -galactosidase expression at the same time points. $\blacklozenge$, 0 h; $\circ$, 2 h; $\blacktriangle$, 4 h; $\square$, 6 h; $\bullet$, 16 h.

peak induction occurring at 45°C (see Fig. 3b). Cells supplemented with linoleic acid (C18:2) contained 73% unsaturated fatty acids, 41% of which was linoleic acid (Fig. 3a), and required a significantly higher temperature for maximum induction of the HS reporter gene. There was minimal induction of β -galactosidase between 39°C and 41°C , and the level of induction increased slowly between 41°C and 45°C and finally peaked at 49°C (Fig. 3b).

The percentage of unsaturated fatty acids and the temperature of maximal HSR induction decrease as linoleic-acid-containing cells return to aerobic growth

There was a 29% decrease in lipid unsaturation and the activation temperature for the maximal HSR reverted to normal when linoleic-acid-supplemented cells were returned to aerobic growth in the absence of the supplement over a 6 h period (Fig. 4a, b). Initially these cells contained 70% unsaturated fatty acids (Fig. 4a) (39% C18:2; 22% C16:1; 9% C18:1; 21% C16:0; and 9%

C18:0) and responded maximally to heat at 49 °C (Fig. 4b). For the first 2 h of aerobic growth in the absence of linoleic acid there was no significant change in either the lipid or HSR profiles but after 4 h the percentage of lipid saturation had fallen by 15% to 55% with a 36% decrease in C18:2, a 20% increase in C16:1, a 2% increase in C18:1 and a 16% increase in C18:0 (Fig. 4a). At this point the cells responded maximally to heat at 45 °C (Fig. 4b). After 6 h growth in the absence of linoleic acid, the percentage of unsaturated fatty acids decreased by a further 14% to 41%: there were further increases in C18:0 (12%) and C18:1 (4%) and a further decrease in C16:1 (19%) (Fig. 4a). These cells had a greater responsiveness to heat with a HSR maximum of 41 °C (Fig. 4b). Neither the lipid nor the HS induction profiles changed significantly thereafter (Fig. 4a, b)

DISCUSSION

The classical model of the HSR, that thermal denaturation of cellular proteins triggers HS gene induction (for a review, see Mager & Ferreira, 1993), has difficulty explaining why the plasma-membrane ATPase activity affects the response (Panaretou & Piper, 1990), why certain chemicals inhibit the response (Cheng & Piper, 1994), how hydrophobic alcohols decrease the threshold temperature of HS gene activation (Curran & Khallawan, 1994) and how changes in osmotic pressure induce HS gene expression (Varela *et al.*, 1992; Lewis *et al.*, 1995). It also fails to explain why HS genes are only transiently expressed during a sublethal HS (Miller *et al.*, 1979; Slater & Craig, 1987; Sorger, 1990, 1991) – continuous heat stress should give rise to continual protein denaturation and hence continual HS gene activation.

This is the first paper to show, not only that there is a correlation between an increase in the percentage of unsaturated fatty acids in the cell and a desensitization of the temperature-sensing mechanism of *S. cerevisiae*, but that the increase in unsaturated fatty acid levels in cells exposed to a long-term heat stress occurs with kinetics consistent with those reported for the down-regulation of HS gene induction which manifests as the transient HSR (Miller *et al.*, 1979; Slater & Craig, 1987; Sorger, 1990, 1991). Cellular unsaturated fatty acids increased by 9% within 1 h of the temperature upshift, by a further 12% within 3 h and remained constant thereafter (Fig. 1a). Yeast cells grown at 25 °C had 45% unsaturated fatty acids and peak β -galactosidase induction at 40 °C whereas cells grown at 37 °C had 64% unsaturated fatty acids and peak induction at 43 °C (Fig. 1b). These results suggest that the transience of the HSR may be due to the desensitization of the signal leading to HS gene induction during a prolonged sublethal HS because an increase in the percentage of unsaturated fatty acids present in the cell results in the deactivation of the initial trigger.

This increase in the temperature of maximal HSR induction can be directly related to the decreased sensitivity of the HS gene activation system. The HS

reporter gene was minimally induced at 40 °C (the temperature of maximal induction for cells grown at 25 °C) for cells grown at 37 °C (see Fig. 1b). Their HSR profile returned to normal over a 6 h period, as the percentage of unsaturated fatty acids fell by 25%, when these cells were reacclimated to growth at 25 °C (see Fig. 2a, b).

These results, which complement the findings of Carratu *et al.* (1996), who demonstrated that $\Delta 9$ -desaturase activity affected the sensitivity of the HSR, were further substantiated by demonstrating an increase of 9 °C in the HSR maximum in cells supplemented with high levels of unsaturated fatty acids during anaerobic growth (Fig. 3a, b). Linoleic-acid-supplemented cells containing 73% unsaturated fatty acids, 41% of which was the supplement, displayed maximal HSR at 49 °C. Within 6 h of aerobic growth in the absence of the fatty acid the cellular lipid profile approximated to that of cells grown aerobically at 25 °C and the temperature of maximal HSR had fallen to 41 °C (Fig. 4a, b). The fatty acid and HS profiles of linoleic-acid-supplemented cells were not simply artifacts of anaerobic growth because Tween-80-supplemented cells grown in the same anaerobic jar, on plates containing the same concentration of ergosterol, had a lower level of unsaturated fatty acids (65%) and had a HSR maximum of 45 °C, 4 °C lower than the linoleic-acid-supplemented cells (see Fig. 3a, b).

That a HS expression vector was differentially induced at the same temperature by cells containing different levels of unsaturated fatty acids suggests that the direct thermal denaturation of intracellular proteins is unlikely to be the initial trigger for the induction of the HSR. Instead, given that the lipid composition of the plasma membrane is a reflection of total cellular lipids (Thomas *et al.*, 1978), that the plasma-membrane ATPase has been implicated in cellular heat tolerance (Panaretou & Piper, 1990; Piper, 1993; Coote *et al.*, 1994) and that an ionophore can cause concomitant changes in intracellular pH and HS gene induction (Weitzel *et al.*, 1987), a more likely candidate is a fluctuation in the electrochemical gradient across the plasma membrane.

By demonstrating a correlation between an increase in fatty acid unsaturation and an increase in the temperature required for HS gene induction, this study lends strong support to the idea that a primary temperature sensor exists at the level of the membrane. Furthermore, by demonstrating that the temperature-sensing mechanism is reset in stress-acclimated yeast cells and that the percentage of unsaturated fatty acids increases in these cells with the kinetics reported for HS gene down-regulation during a prolonged heat stress, the transient nature of the HSR can be explained by a lipid-mediated decrease in cellular heat sensitivity.

ACKNOWLEDGEMENTS

We thank Dr Virginia Bugeja for helpful discussions, Alan Scott for expert technical assistance with lipid analysis and Rebecca Burbidge for performing preliminary experiments.

REFERENCES

- Carratu, L., Franceschelli, S., Pardini, C. L., Kobayashi, G. S., Horvath, I., Vigh, L. & Maresca, B. (1996). Membrane lipid perturbation modifies the set point of the temperature of heat shock response in yeast. *Proc Natl Acad Sci USA* **93**, 3870–3875.
- Cheng, L. & Piper, P. W. (1994). Weak acid preservatives block the heat shock response and heat-shock-element-directed *lacZ* expression of low pH *Saccharomyces cerevisiae* cultures, an inhibitory action partially relieved by respiratory deficiency. *Microbiology* **140**, 1085–1096.
- Coote, P. J., Jones, M. V., Seymour, I. J., Rowe, D. L., Ferdinando, D. P., McArthur, A. J. & Cole, M. B. (1994). Activity of plasma membrane H⁺-ATPase is a key physiological determinant of thermotolerance in *Saccharomyces cerevisiae*. *Microbiology* **140**, 1881–1890.
- Curran, B. P. G. & Khalawan, S. A. (1994). Alcohols lower the threshold temperature for the maximal activation of a heat shock expression vector in the yeast *Saccharomyces cerevisiae*. *Microbiology* **140**, 2225–2228.
- Hossack, J. A. & Rose, A. H. (1976). Fragility of plasma membranes in *Saccharomyces cerevisiae* enriched with different sterols. *J Bacteriol* **127**, 67–75.
- Hunter, K. & Rose, A. H. (1972). Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. *Biochim Biophys Acta* **260**, 639–653.
- Lewis, J. G., Learmonth, R. P. & Watson, K. (1995). Induction of heat, freezing and salt tolerance by heat and salt shock in *Saccharomyces cerevisiae*. *Microbiology* **141**, 687–694.
- Lloyd, D., Morrell, S., Carlsen, H. N., Degn, H., James, P. E. & Rowlands, C. C. (1993). Effects of growth with ethanol on fermentation and membrane fluidity of *Saccharomyces cerevisiae*. *Yeast* **9**, 825–833.
- Mager, W. H. & Ferreira, P. M. (1993). Stress response of yeast. *Biochem J* **290**, 1–13.
- Miller, M. J., Xuong, N. & Geiduschek, E. P. (1979). A response of protein synthesis to temperature shift in the yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **76**, 5222–5225.
- Panaretou, B. & Piper, P. W. (1990). Plasma-membrane ATPase action affects several stress tolerances of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* as well as the extent and duration of the heat shock response. *J Gen Microbiol* **136**, 1763–1770.
- Parsell, D. A. & Lindquist, S. (1993). The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu Rev Genet* **27**, 437–496.
- Pelham, H. R. B. & Bienz, M. (1982). A synthetic heat shock promoter element confers heat-inducibility on the herpes-simplex virus thymidine kinase gene. *EMBO J* **1**, 1473–1477.
- Piper, P. W. (1993). Molecular events associated with the acquisition of heat tolerance in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **11**, 1–11.
- Slater, M. R. & Craig, E. A. (1987). Transcriptional regulation of an Hsp70 heat shock gene in the yeast *Saccharomyces cerevisiae*. *Mol Cell Biol* **7**, 1906–1916.
- Sorger, P. K. (1990). Yeast heat shock factor contains separable transient and sustained response transcriptional activators. *Cell* **62**, 793–805.
- Sorger, P. K. (1991). Heat shock factor and the heat shock response. *Cell* **65**, 363–366.
- Sorger, P. K. & Pelham, H. R. B. (1987). Purification and characterization of a heat-shock element binding protein from yeast. *EMBO J* **6**, 3035–3041.
- Sorger, P. K. & Pelham, H. R. B. (1988). Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. *Cell* **54**, 855–864.
- Suutari, M., Liukkonen, K. & Laakso, S. (1990). Temperature adaptation in yeast: the role of fatty acids. *J Gen Microbiol* **136**, 1469–1474.
- Thomas, S., Hossack, J. A. & Rose, A. H. (1978). Plasma-membrane lipid composition and ethanol tolerance in *Saccharomyces cerevisiae*. *Arch Microbiol* **117**, 239–245.
- Varela, J. C. S., Van Beekvelt, C., Planta, R. J. & Mager, W. H. (1992). Osmostress-induced changes in yeast gene expression. *Mol Microbiol* **6**, 2183–2190.
- Weitzel, G., Pilatus, U. & Rensing, L. (1987). The cytoplasmic pH, ATP content and total protein synthesis rate during heat shock inducing treatments in yeast. *Exp Cell Res* **170**, 64–79.
- Wickerham, L. J. (1951). United States Department of Agriculture Technical Bulletin, no. 1029.
- Wiederrecht, G., Seto, D. & Parker, C. S. (1988). Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. *Cell* **54**, 841–853.

Received 9 December 1996; revised 18 April 1997; accepted 27 May 1997.