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Membrane lipid perturbation modifies the set point of the temperature of heat shock response in yeast

(Δ^9-desaturase/membrane physical state)

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ABSTRACT **Addition of a saturated fatty acid (SFA) induced a strong increase in heat shock (HS) mRNA transcription when cells were heat-shocked at 37°C, whereas treatment with an unsaturated fatty acid (UFA) reduced or eliminated the level of HS gene transcription at 37°C. Transcription of the Δ^9-desaturase gene (*Ole1*) of *Histoplasma capsulatum*, whose gene product is responsible for the synthesis of UFA, is up-regulated in a temperature-sensitive strain. We show that when the L8-14C mutant of *Saccharomyces cerevisiae*, which has a disrupted *Ole1* gene, is complemented with its own *Ole1* coding region under control of its own promoter or *Ole1* promoters of *H. capsulatum*, the level of HS gene transcription depends on the activity of the promoters. Fluorescence anisotropy of mitochondrial membranes of complemented strains corresponded to the different activity of the *Ole1* promoter used. We propose that the SFA/UFA ratio and perturbation of membrane lipoprotein complexes are involved in the perception of rapid temperature changes and under HS conditions disturbance of the preexisting membrane physical state causes transduction of a signal that induces transcription of HS genes.**

We investigated the possibility that, in *Saccharomyces cerevisiae* and in the dimorphic pathogenic fungus *Histoplasma capsulatum*, the preexisting physical state of membranes controls the temperature at which heat shock (HS) gene expression occurs.

Cellular responses to HS occur over a wide range of temperatures—i.e., from 4°C in Antarctic fish (1) to 95–102°C in hyperthermophilic bacteria (2). The HS response is characterized by transcriptional activation of HS genes (3, 4) after binding of the heat shock factor (HSF) to a heat shock element. Despite extensive research concerning the mechanism or mechanisms that regulate activation of HS gene expression, no conclusion has been reached concerning the nature of a "primary sensor" that detects elevated temperatures, or how the signal or signals are transferred to the nucleus (5, 6). Experimental data clearly show that HS gene transcription is induced by phosphorylation of HSF (7–10). The question still remains as to the initial event after a temperature shift up. In many organisms, the temperature at which HS occurs is not genetically determined but depends on the temperature at which the organism lives (11, 12). A complex system that adjusts to fluctuations in the external temperature and that senses an abrupt increase above the tolerable temperature range must be operative in organisms.

We propose that some forms of heat injury are due to a reorganization of lipid protein complexes of the membrane at the time of HS. It is well established that many of the physiological responses to environmental changes are caused directly by modifications of membrane lipid structures, which affect either the overall membrane physical state or specific protein and lipid domains (13, 14). During seasonal or evolutionary temperature adaptation (homeoviscous adaptation), the conservation of a particular state of membrane physical state and the preservation of a fluid or nonrigid condition following temperature changes are critical for a wide spectrum of membrane-associated protein functions (15). Responses of cellular membranes to temperature changes depend largely on the degree of unsaturation of fatty acids (FA) of membrane lipids (16, 17).

Using yeast as experimental models, we predicted that if a primary heat shock sensor (HSS) responsible for monitoring abrupt temperature variations is membrane-associated, modification of membrane fluid state and/or loss of bilayer integrity when the temperature exceeds the physiological range (during HS) should influence the level of expression of HS genes by setting the temperature at which optimal HS response occurs.

In this study, we complemented a mutant of *S. cerevisiae* that has a disrupted *Ole1* gene with plasmids containing the homologous and heterologous promoters of the *Ole1* gene. The complemented strains assembled membranes with different FA composition and membrane physical state and showed a different threshold temperature of HS gene transcription. 1,6-Diphenyl-1,3,5-hexatriene (DPH) fluorescence anisotropy showed that changes in microviscosity of mitochondrial membrane also occurred in an internal organelle. The physical state of mitochondrial membranes was measured, because these membranes are obtained with higher purity than other cellular membranes. Coupling of mitochondrial ATPase activity changed when FA were added. In the complemented strains, changes in coupling were similar to the changes observed during HS both in *S. cerevisiae* and *H. capsulatum* (18, 19).

MATERIALS AND METHODS

Organisms and Culture Conditions. Both a temperature-sensitive strain (Downs) and a temperature-tolerant strain (G217B) of *H. capsulatum* were grown as described (20). *S. cerevisiae* L8-14C mutant strain (*mata*, *Ole1Δ::LEU2*, *ura3–52*, *his4*; ref. 21) was used for complementation studies. L8-14C strain was grown in either complete or minimal medium supplemented with 0.5 mM palmitoleic acid (16:1) and 0.5 mM oleic acid as described (21).

Measurement of Oxygen Consumption and Coupling of Oxidative Phosphorylation. Oxygen consumption and cou-

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Abbreviations: HS, heat shock; HSP, heat shock protein; HSF, heat shock factor; HSS, heat shock sensor; SFA, saturated fatty acid(s); UFA, unsaturated fatty acid(s); FA, fatty acid(s); DPH, 1,6-diphenyl-1,3,5-hexatriene; totRNA, total RNA.

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pling of oxidative phosphorylation were determined as described (18, 19, 22).

FA Determination. Total lipids of logarithmically growing mycelia of *H. capsulatum* and of mitochondrial membranes of *S. cerevisiae* were extracted according to Bligh and Dyer (23). The various polar lipid classes were then separated by two-dimensional thin layer chromatography according to Rouser *et al.* (24). After visualization with 0.05% 8-anilino-1-naphthalene sulfonic acid in 50% methanol, the FA were subjected to methanolysis. The resultant methyl esters were quantified using a Hewlett–Packard gas-liquid chromatograph (HP3396A) equipped with a capillary column (SP2230), a flame ionization detector, and an HP3396A integrator.

Isolation of Mitochondrial Membrane. *S. cerevisiae* mitochondria were isolated according to Daum *et al.* (25) with modifications. Yeast cells (500 ml) were spun 10 min at 3500 rpm in a GSA rotor and washed with ultrapure distilled H_2O. Cells were slowly shaken at 22°C for 20 min in a volume of 20 ml/40 mM 2-mercaptoethanol/0.25 M EDTA. Cells were then washed with 40 ml of cold 1.08 M sorbitol/50 mM citric acid/0.15 M potassium phosphate buffer (pH 5.8). Protoplasts were obtained by treating cells for 2 hr at 28°C in the above buffer (3 ml) containing 1.4 mg lyticase (Sigma). The suspension was centrifuged, and protoplasts were washed twice in cold 0.7 M sorbitol/1 mM EDTA/0.2% bovine serum albumin/60 mM Tris·Cl, (pH 7.2). Protoplasts were broken by French press. Unbroken cells were sedimented, and supernatant was spun at 13,000 rpm in a SW-41 Ti (Beckman) rotor for 30 min. The sediment was collected and resuspended in the same buffer and spun again at 3500 rpm for 30 min. The resultant supernatant was spun for 30 min at 13,000 rpm. The pellet was then resuspended in 20 mM potassium phosphate buffer (pH 7.2), and protein concentration was determined.

Microviscosity Determination of Mitochondrial Membrane. Microviscosity of broken yeast mitochondrial membranes was determined using DPH fluorescence anisotropy. Briefly, the OD_{366} of mitochondrial membranes was adjusted with 20 mM potassium phosphate (pH 7.2) to 0.07 in 3-ml volume. Two microliters of 0.2 mM DPH was added, and then the mixture was incubated for 30 min at 30°C. Steady-state fluorescence measurements were carried out with a Quanta Master QM-1 T-format luminescence spectrometer (Photon Technology International, Princeton, NJ). Fluorescence anisotropy was calculated as described (26). All measurements were done in triplicate.

Northern Blot Analysis. Total RNA (totRNA) was purified, and Northern blot analyses were performed as described (20). Filters were hybridized with 10^7 cpm of ^{32}P-labeled *H. capsulatum hsp82* and *hsp70* genes labeled by multipriming to a specific activity of 10^9 cpm/μg (20, 27). The amount of totRNA loaded on gels was determined by spectrophotometry and by scanning an ethidium bromide gel containing the same volume of totRNA used for the Northern blot. The ethidium bromide gel was photographed, and the picture was captured with Video Image (Scion, Frederick, MD) and analyzed with COLLAGE 2.1 (Fotodyne, New Berlin, WI) software. Filters that were probed with HS genes were also hybridized with a 190-nucleotide fragment of a *H. capsulatum* 18S rRNA gene (28) to ensure that the same amount of RNA was on filters (not shown). The exposed film was scanned and intensity of bands was measured by the COLLAGE 2.1 software. In addition, the filter, after hybridization, was stained for 5 min in 5% acetic acid for 5 min in 0.04% methylene blue in 0.5 M sodium acetate followed by washing with water. The intensity of rRNA bands was further quantified to ensure that the same amount of RNA loaded on the gel had been uniformly transferred to the filter.

Construction of Heterologous *S. cerevisiae Ole1* Gene/*H. capsulatum Ole1* Promoters. *S. cerevisiae Ole1* gene contained in YEp352 plasmid (gift of C. E. Martin, Nelson Biological Laboratory, Rutgers University, Piscataway, NJ), was used to complement *S. cerevisiae* L8-14C strain. *S. cerevisiae Ole1* coding region (2.4 kb) containing 30 additional nucleotides before the ATG was removed from plasmid YEp352/OLE4.8 (21) by *Bcl* I digestion and inserted in plasmid YEp352 in the *Bam*HI site. *H. capsulatum* G217B *Ole1* promoter was prepared from plasmid pUC19 by *Pst* I digestion (872 nucleotides) (29) and inserted in *Pst* I site of YEp352. *H. capsulatum* Downs *Ole1* promoter was prepared from plasmid pUC19 by *Pst* I digestion (868 nucleotides) (29) and inserted in *Pst* I site of YEp352.

Complementation of *S. cerevisiae* L8-14C Mutant Strain with Heterologous *Ole1* Constructs. L8-14C was transformed with the three different constructs using the lithium acetate methods. Recombinants were selected first on plates containing yeast nitrogen base without amino acids and 2% glucose supplemented with amino acids and unsaturated fatty acids (UFA) without uracil. Colonies were collected and plated on plates containing the growth medium but not UFA.

RESULTS

FA Composition of Downs and G217B Strains of *H. capsulatum* and Effect of FA Supplementation on HS Gene Transcription. Total FA composition of mycelia of *H. capsulatum* strains showed that the temperature-tolerant G217B strain contained more saturated fatty acids (SFA) than the temperature-sensitive Downs (data not shown), and it had a higher SFA/UFA ratio.

All organisms synthesize only SFA, while UFA are produced by the action of microsomal desaturases (30). The gene product of Δ^9-desaturase is the major enzyme in animal and yeast cells for converting SFA into UFA and catalyzes the insertion of a double bond in palmitoyl- and stearoyl-CoA (30). We previously cloned the *Ole1* gene both from the Downs (GenBank accession number X85963) and G217B (X85962) strains (29). Whereas the *Ole1* gene is transcribed at comparable levels in both strains at 37°C, at 25°C, it is highly expressed in the Downs strain but not detectable in the G217B strain (29). Our results, based on sequence and deletion analysis of promoter constructs of the two cloned genes, suggest that major differences exist among certain regions that are responsible for the differential expression (refs. 28, 31, and 32; unpublished data).

To test the hypothesis that transcription of HS genes is influenced by rapid perturbation of lipid membrane organization, we have measured the level of HS gene expression in mycelial isolates of *H. capsulatum* incubated with different FA or benzyl alcohol. Furthermore, because we had shown that under stress conditions heat shock proteins (HSPs) protect mitochondrial ATPase activity, we also measured this membrane-bound enzymatic activity in the presence of FA and benzyl alcohol.

We previously demonstrated that maximal mRNA transcription of cloned *hsp70* (27) and *hsp82* genes (33) as well as HSP induction (34) occurs at 34°C in the temperature-sensitive Downs strain, whereas in the temperature-tolerant G217B strain, they occur at 37°C. Furthermore, induction of thermotolerance induces higher levels of HS mRNA transcription at the corresponding higher temperatures (19). Using the *hsp82* gene as a probe, mycelia heat-shocked for 1 hr at 37°C after incubation for 30 min at 25°C with 0.1, 0.3, and 0.5 mM palmitic acid induced 2.7, 2.9, and 3.1 times more hsp82 mRNA, respectively, than control cells (Fig. 1). Similar results were obtained with 0.1–0.5 mM stearic acid (data not shown).

Thus, addition of SFA rescues HS gene transcription at nonpermissive temperature as does the induction of thermotolerance. We believe that the effect of SFA rigidifying the membrane shifts the temperature of HS transcription in the Downs strain from 34 to 37°C. Possibly, HSPs rigidify membranes during thermotolerance and mimic the effect of a structure richer in SFA, allowing optimal transcription at 37°C.

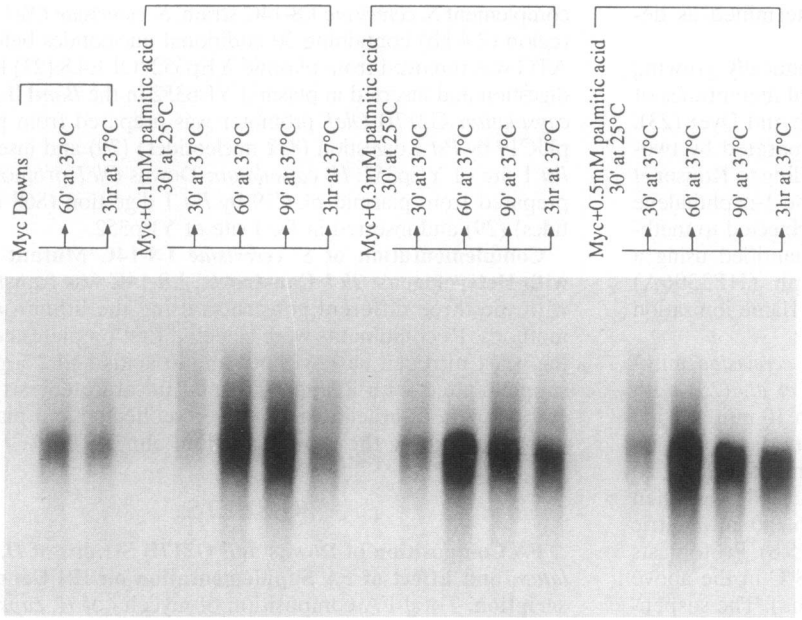

FIG. 1. Northern blot of totRNA purified from the temperature-sensitive Downs strain in the presence and absence of palmitic acid. Fifteen micrograms of RNA were loaded on the gel.

A similar shift in maximal temperature of HS response is obtained in the normal G217B strain at 40°C when it is incubated with SFA (data not shown).

Conversely, incubation of G217B mycelia with increasing concentrations of oleic acid reduced HS gene transcription at 37°C, with virtually no transcription detectable at 1 mM (Fig. 2). The presence of transcription at 25°C in cells treated with oleic acid is expected if one considers that a sufficient amount of UFA can change the fluid state of the membrane at room temperature, causing induction of HS gene transcription even in the absence of a HS. The reason no transcription is detectable at 37°C may be that addition of oleic acid and the concomitant increase of temperature to 37°C cause overfluidification of membrane, and possibly, disruption of the "primary complex" involved in detecting temperature increase. Similar results were obtained with the G217B strain under HS condition with 0.1 mM benzyl alcohol (a membrane-perturbing agent), which has an effect on the physical state of membranes similar to those observed with UFA (Fig. 3). RNA filters, were also probed with *hsp70* gene with identical results and with a fragment of *H. capsulatum* 18S ribosomal DNA to demonstrate that the addition of FA and benzyl alcohol had no effect on RNA synthesis (data not shown). The filters were stained after hybridization to check that the same amount of RNA was loaded in each lane.

Effect of Fatty Acid Supplement on Mitochondrial ATPase Activity. Previous respiratory studies on the coupling capacity of G217B and Downs strains of *H. capsulatum* showed that within 5 min after the temperature change from 25 to 37°C, respiration of the Downs strain is completely uncoupled, whereas with the thermotolerant G217B strain, uncoupling of respiration occurs at 40°C (19, 35). In addition, we showed that a strong correlation exists between the temperature necessary to uncouple respiration and the level of HS gene expression (19). An HSP-dependent thermotolerant state protects oxidative phosphorylation at 37°C in Downs and at 40°C in G217B and G222B strains (19). A similar effect was observed in *S. cerevisiae* (18).

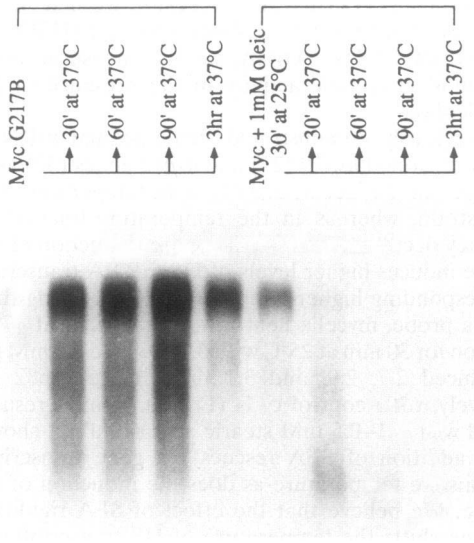

FIG. 2. Northern blot of totRNA purified from the temperature-tolerant G217B strain in the presence and absence of oleic acid. Fifteen micrograms of RNA were loaded on the gel.

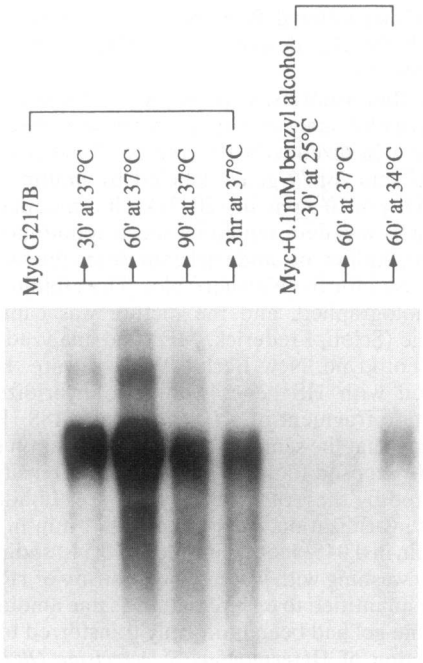

FIG. 3. Northern blot of totRNA purified from temperature-tolerant G217B strain in the presence and absence of benzyl alcohol. Fifteen micrograms of RNA were loaded on the gel.

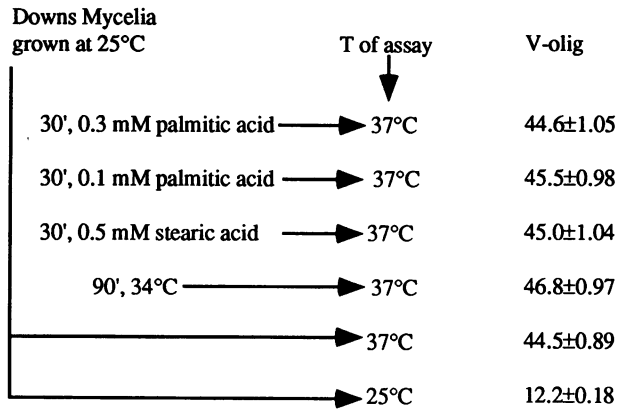

FIG. 4. Coupling of oxidative phosphorylation of mycelia of Downs at 25°C and after HS and in the presence of palmitic and stearic acids. Additions were oligomycin (olig; 5 μg/ml) and Cl-CCP (carbonyl cyanide *m*-chlorophenylhydrazone; 10 μg/ml). Rates of oxygen uptake are expressed as μl O_2/hr/mg dry weight of cells and represent the average of at least five independent measurements, and they include standard errors.

We show that protection of mitochondrial ATPase is obtained by treating Downs mycelia at 25°C for 30 min with 0.3 palmitic acid (+26%) or with 0.5 mM stearic acid (+74%) when cells were directly exposed to 37°C (Fig. 4). A contrary effect was obtained when G217B mycelia, whose cell respiration is normally coupled at 37°C, were incubated with 2 mM oleic acid, resulting in loss of coupling capacity of mitochondrial ATPase at 37°C (Fig. 5). However, concentrations between 0.5 and 1 mM oleic acid, while sufficient to alter HS gene transcription (Fig. 1), did not significantly affect ATPase activity. This effect is may be due to a different rate of FA diffusion into cell compartments and differences in mitochondrial versus cell membrane composition (13). ATPase activity of G217B, which is normally uncoupled at temperatures above 40°C, was rescued if mycelia were first incubated with 0.5 mM palmitic acid (Fig. 5) or stearic acid (data not shown).

Complementation of *S. cerevisiae* L8-14C, *Ole1*Δ::*LEU2*. Using *S. cerevisiae* L8-14C, *Ole1*Δ::*LEU2*, we tested whether insertion of the homologous *Ole1* sequence under control of the normal (*S. cerevisiae*) G217B or up-regulated Downs promoters could influence the level of HS gene transcription as a result of modification in membrane FA composition.

Depending on the activity of the *Ole1* promoter used, the FA profile of mitochondrial membranes of the different transformants changed dramatically (Table 1). In particular, the level of palmitic acid of the strain complemented with the up-regulated *Ole1* promoter (Downs) dropped to about half of the values of corresponding transformant containing the G217B promoter. Simultaneously, the level of mono-UFA increased in the constructs containing the Downs promoter, particularly in the oleic acid fraction. Similar changes of FA profile were obtained at the level of individual phospholipids. No significant alteration was found in the ratio of membrane lipid classes (data not shown).

DPH, which is known to partition into the hydrophobic core of the membrane bilayer, was used to study order and dynamics in biomembranes (15). The level of changes in microviscosity measured in the mitochondrial membranes reflected the tendencies of the alterations detected in FA composition of the different transformants. The most dramatic of the microviscosity changes revealed that while the presence of the up-regulated *Ole1* promoter decreased the total SFA/UFA ratio, membrane anisotropy increased markedly over the entire temperature range investigated (Fig. 6).

HS Expression in Complemented *S. cerevisiae* Strain. Fig. 7 shows that different *Ole1* promoters in *S. cerevisiae* L8-14C dictate different levels of HS gene expression. As determined by quantitative analysis with COLLAGE 2.1 software, when the homologous *Ole1* promoter was used, a normal HS response was measured with maximal transcription at 42°C. Similarly, HS expression was measured when the *Ole1* promoter from *H. capsulatum* G217B strain was used (0.9 and 1.01 times at 36° and 42°C), respectively. However, when the Downs *Ole1* promoter was used, there was a marked decrease in HS gene transcription (80% decrease at 36°C and >20% at 42°C). Thus, depending on the construct used and on the resultant physical state of the membrane, the level of HS gene transcription changed accordingly.

DISCUSSION

HS genes are rapidly and transiently induced as a response to abrupt increases in temperature and other conditions of stress (36). While it has been demonstrated that HS genes are activated by phosphorylation of HSF (37), the event that initiates the cascade that triggers phosphorylation and the reason each cell induces HS at its own specific temperature have not yet been elucidated.

The models so far proposed imply the existence of a protein that is "activated" each time there is a 6–8°C shift above the growth temperature of the organism. Alternatively, a large number of "temperature sensors" would be required, each detecting the specific ΔT necessary for HSF phosphorylation. For example, insolubilization of specific proteins has also been pro-

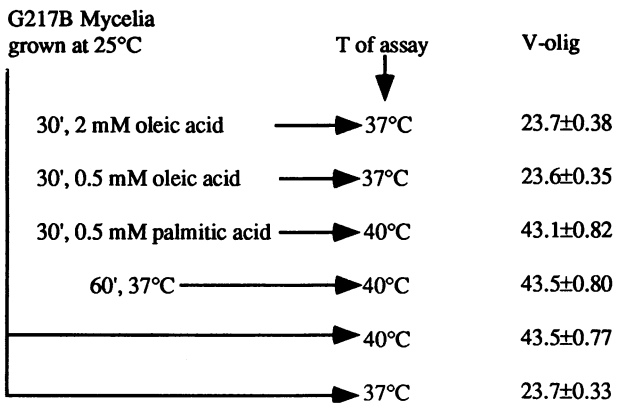

FIG. 5. Coupling of oxidative phosphorylation of mycelia of G217B at 25°C and after HS and in the presence of palmitic and oleic acids. Additions were oligomycin (olig; 5 μg/ml) and Cl-CCP (defined in Fig. 4; 10 μg/ml). Rates of oxygen uptake are expressed as μl O_2/hr/mg dry weight of cells and represent the average of at least five independent measurements, and they include standard errors.

Table 1. FA composition and SFA/UFA ratios

Constructs	% of total fatty acids				SFA/UFA
	16:0	16:1	18:0	18:1	
Ole1-p	23.3	44.1	5.0	27.2	0.39
G217B-p	27.0	43.9	7.0	22.1	0.52
Downs-p	14.3	47.9	4.5	33.3	0.23

FA composition and SFA/UFA ratios of mitochondrial total membrane lipids isolated from *S. cerevisiae* L8-14C *Ole1*::*LEU2* mutant strain complemented as described in text. Values represent the average of six independent measurements, with variation of <1%.

posed to be the primary sensor for HS gene activation (10). However, for all nonhomeothermic organisms living in a changing environment, we must assume that insoluble proteins must modify their physical state in order to sense the new higher temperature necessary to induce HS, or that distinct sets of proteins denaturing at different temperature are present in the cells and operate at different temperatures (38).

Dietz and Somero (11) have shown that in eurythermal goby fishes, *hsp90* gene is induced at 28°C for 20°C-acclimated fish, and at 24°C for 10°C-acclimated fish, whereas in summer-acclimated fish (30°C), *hsp90* transcription occurs between 36°C and 38°C. These authors suggested that the threshold temperature at which expression of HS genes occurs is not entirely genetically determined but correlates with normal body temperature and is thus subjected to acclimatization. Furthermore, it has been shown in mouse liver that HSF1 binds to heat shock element at 42°C (37°C being the organ temperature), but the same HSF1 binds at 36°C in the mouse testis (30°C being the organ temperature), suggesting that the same HSF is not activated in response to absolute temperature but rather in response to a change to relative temperature (12).

We propose that a primary target in the biological perception of temperature is the membrane, in which a HSS is present and whose activation following HS represents the first step of the signal transduction pathway that leads to HSF phosphorylation. Such cascade would be similar to others described in many prokaryotic and eukaryotic cells in which extracellular signals affect the activity of a protein kinase cascade (39). We have shown that we can modulate HS gene transcription and ATPase activity by addition of FA or benzyl alcohol. In the Downs strain, in which the optimal HS response occurs at 34°C, exposure of mycelia to SFA at 25°C induced a strong increase in HS gene transcription when cells were shifted to 37°C. Such increase is similar to that measurable when thermotolerance is induced (19). In the G217B strain, which has a maximal HS response at 37°C, addition of 0.5–1 mM oleic acid to mycelia drastically reduced HS gene transcription when cells were shifted to 37°C. Therefore, to

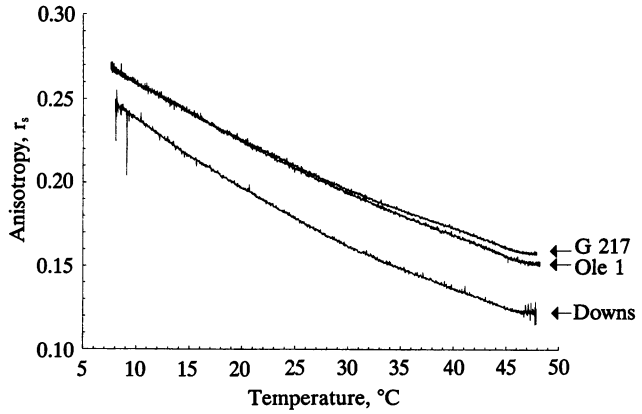

FIG. 6. Membrane anisotropy of isolated mitochondrial membranes of *S. cerevisiae*. Anisotropy changes of membrane were determined by DPH fluorescence over a temperature range of 8–48°C.

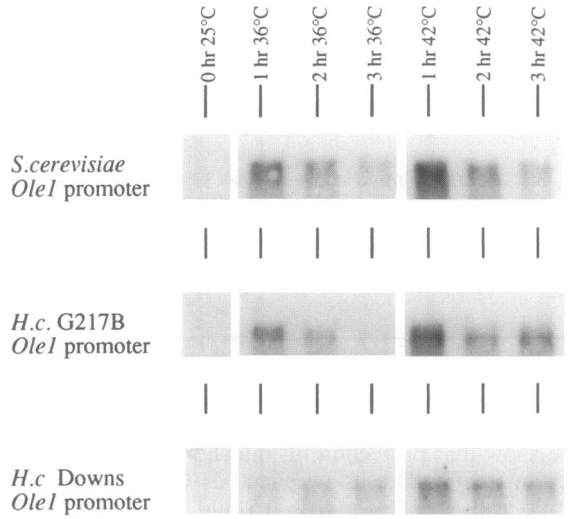

FIG. 7. Northern blots of HS mRNA of complemented *S. cerevisiae* L8-14C *Ole1*::*LEU2* mutant strain. HS transcription was measured using the *hsp82* gene of *S. cerevisiae*.

predict the effect of a FA on HS response, one must consider the temperature at which the experiment is performed in relation to the membrane SFA/UFA ratio.

It has been shown that with most membrane-bound enzymes, the activities are dependent on membrane lipid constituents (16, 40). Consistent with the hypothesis that the Downs strain of *H. capsulatum* is a variant with a defect in the regulation of the primary HSS, we have shown that addition of SFA restored normal HS transcription and ATPase activity by modifying membrane microviscosity and the dynamic properties of membrane. A contrasting effect was observed at 37°C when either UFA or benzyl alcohol was added to G217B.

Genetic evidence that the membrane plays a key role in setting the temperature at which HS gene expression occurs is provided by the experiments in which insertion of the homologous *Ole1* coding region under control of different *Ole1* promoters in the *S. cerevisiae* L8-14C mutant (*Ole1*$^-$) modified the SFA/UFA ratio. When the up-regulated promoter of Downs was used to drive desaturase expression in the *S. cerevisiae* L8-14C mutant, a higher level of UFA and lower microviscosity were measured, and was paralleled by a strong decrease in HS gene expression (Fig. 7). Conversely, the presence of the normal G217B *Ole1* promoter produced a higher SFA/UFA ratio, more rigid membranes, and HS gene expression similar to that produced by the *S. cerevisiae* promoter (Fig. 7).

We propose that during HS a primary target is the membrane. As a result of a temporary, moderate temperature increase that is specific for each organism, no or little HS response occurs. However, it is known that a rapid increase in the environmental temperature causes disturbances of membrane architecture (41). We suggest that during HS, when membranes become "hyperfluid" and destabilization of the lamellar phase occurs, such as that demonstrated in heat-induced formation of nonbilayer phase of lipids of thylakoids of chloroplasts (41, 42), the temperature increase may produce a temporary reorganization of lipid membranes, resulting in delocalization of a "factor" embedded in the membrane. The presentation to the cytoplasm of this factor (a kinase?), may elicit a cascade reaction that leads to phosphorylation of HSF. Support of the hypothesis that a physical attribute may affect a kinase activity comes from the recent work by Kamada *et al.* (43) who have shown in *S. cerevisiae* that reduction of extracellular osmolarity induces a very rapid and transient activation of MPK1 kinase by tyrosine phosphorylation. In addition, amphipatic drugs such as CPZ (chlorpromazine) that induce

"membrane stretch" strongly activate MPK1 (mitogen-activated kinase) (43). These authors have postulated that membrane stretch is sufficient to activate the PKC1 (protein kinase C) signaling pathway.

However, other mechanisms such as Ca^{2+} or cAMP-dependent induction may be involved in the signal transduction that activates HSF phosphorylation. A biochemical analysis is thus required to identify the nature of the HSS and the precise role that the ratio SFA/UFA of membrane play in regulating the expression of HS genes.

While membrane perturbation may be the initial sensor during HS and when cells are exposed to other inducers such as ethanol and benzyl alcohol (which are known to be membrane-perturbing agents), we cannot exclude the possibility that other forms of stresses may activate HS transcription through some other primary sensor. Furthermore, it is reasonable to suggest that protection of membrane activities (as shown for mitochondrial ATPase by HSP induction or by an increased SFA/UFA ratio) is achieved in the period immediately following temperature stress either by new HSP synthesis or by rigidification of membranes. Induced HSPs may bind membrane lipoproteins (44) leading to stabilization of membrane topology, which in turn could maintain an appropriate physical state until the temperature is lowered or new lipids are incorporated. Recently, it has been shown that mitochondrial ClpB, a homolog of HSP78, cooperates with matrix HSP70 in the maintenance of mitochondrial functions (45).

It has also been shown that lowering the growth temperature of cyanobacteria leads to a marked increase in the level of Δ^{12}-desaturase (*desA*) transcription (46), and that *in vivo* catalytic hydrogenation of a small pool of plasma membrane FA-activated transcription of *desA* in *Synechocystis* (46) without lowering the temperature. Because the catalytic technique affects only the plasma membrane, this implied that the membrane acts as a sensor and that altered saturation remotely activates a mechanism whose ultimate effect is to enhance *desA* transcription (47, 48).

It is reasonable to speculate that transcriptional regulation of HS and desaturase genes are only two examples of genes whose expressions are directly controlled by changes in the membrane fluid state. It is possible that during physical stresses, embryogenesis, and pathophysiological conditions when membrane remodeling occurs, the physical state and phase of the membrane may act directly in controlling the expression of other genes.

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