Lack of correlation between trehalose accumulation, cell viability and intracellular acidification as induced by various stresses in *Saccharomyces cerevisiae*

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A *pma1-1* mutant of *Saccharomyces cerevisiae* with reduced H⁺-ATPase activity and the isogenic wild-type strain accumulated high levels of trehalose in response to a temperature upshift to 40 °C and after addition of 10% ethanol, but only modest levels in response to a rapid drop in external pH and after addition of decanoic acid. There was, however, no correlation between the absolute levels of trehalose in the stressed cells and their viability. All these treatments induced a significant decrease in intracellular pH, and surprisingly, this decrease was very similar in both strains, indicating that intracellular acidification could not be the triggering mechanism for trehalose accumulation in response to stress. A careful investigation of metabolic parameters was carried out to explain how trehalose accumulated under the four different stress conditions tested. No single and common mechanism for trehalose accumulation could be put forward and the transcriptional activation of *TPS1* was not unequivocally related to trehalose accumulation. Another finding was that a *pma1-1* mutant exhibited a two- to threefold greater capacity to accumulate trehalose than the isogenic wild-type. This enhanced disaccharide synthesis could be attributed to a twofold higher trehalose-6-phosphate synthase activity, together with a fourfold higher content of intracellular UDP-Glc. In addition, this mutant showed 1.5-fold higher levels of ATP compared to the wild-type. The various stress treatments studied showed that a drop in intracellular pH does not correlate with trehalose accumulation. It is suggested that plasma membrane alteration could be the physiological trigger inducing trehalose accumulation in yeast.

Keywords: trehalose, intracellular pH, stress, H⁺-ATPase, *Saccharomyces cerevisiae*

INTRODUCTION

Large amounts of trehalose are accumulated in *Saccharomyces cerevisiae* shifted to temperatures exceeding the normal growth range (Attfield, 1987; Hottiger *et al.*, 1987). Besides heat, other stress agents such as ethanol, freezing-thawing and dehydration can induce trehalose accumulation (Attfield, 1987; Hottiger *et al.*, 1987). Since in most cases a close correlation between trehalose content and stress resistance of the cells has been

observed (Panek & Panek, 1990; Wiemken, 1990), it has been proposed that trehalose acts as a protectant of the structure of cell membranes and proteins under conditions that deplete intracellular water (Wiemken 1990; Van Laere, 1989; Crowe *et al.*, 1984). In addition, a role for trehalose in preventing ethanol-induced leakage from cells has been proposed (Mansure *et al.*, 1994).

However, conflicting data on the relevance of trehalose in stress resistance has appeared in the literature. Nwaka *et al.* (1994, 1995) showed that a *nth1* mutant defective in neutral trehalase was even less thermotolerant than the wild-type control despite its high content of trehalose in response to a heat stress. It was also reported that

Abbreviations: Glc6P, glucose 6-phosphate; pH_i, intracellular pH; Tre6P synthase, trehalose-6-phosphate synthase.

accumulation of trehalose was not required for the acquisition of thermotolerance in glucose-derepressed strains (Gross & Watson, 1996). In contrast, Van Dijck *et al.* (1995) confirmed the correlation between trehalose content and stress resistance in yeast growing on non-fermentable carbon sources, and De Virgilio *et al.* (1994) further illustrated this correlation by using a *tps1* mutant deficient in trehalose-6-phosphate synthase (Tre6P synthase, EC 2.4.1.15). Clearly, the role of trehalose in resistance to stress is still an open question.

In yeast, trehalose content is determined by the balance between the activity of the trehalose synthase complex encoded by at least four genes, *TPS1*, *TPS2*, *TPS3* and *TSL1* (Thevelein & Hohmann, 1995; Reinders *et al.*, 1997) and that of the neutral trehalase, encoded by *NTH1* (Kopp *et al.*, 1993). The dramatic accumulation of trehalose in cells growing exponentially in glucose induced by temperature upshifts (Hottiger *et al.*, 1987) was ascribed to a transcriptional activation of *TPS1* and *TPS2* genes (Bell *et al.*, 1992; Vuorio *et al.*, 1993), whereas Winkler *et al.* (1991) considered more likely this rapid synthesis was as a result of a dramatic increase in the concentration of UDP-Glc and glucose 6-phosphate (Glc6P), the substrates of Tre6P synthase. On the other hand, Neves & François (1992) proposed that the accumulation of trehalose upon heat shock was due to changes in kinetic properties of Tre6P synthase and trehalase induced by temperature. These controversies have recently been reinvestigated by Parrou *et al.* (1997) who showed that both transcriptional activation of *TPS1* and temperature activation of Tre6P synthase participates in the phenomenon, and that the predominance of the first effect over the second is actually temperature-dependent.

Although the regulation of trehalose metabolism in yeast seems to be well understood (Thevelein & Hohmann, 1995; François *et al.*, 1997), a remaining and intriguing question concerns the initial cellular signal(s) which triggers induction of trehalose synthesis under various types of stressful conditions. Since many of the activators which induce trehalose accumulation are also known to cause perturbations of the intracellular pH (pH_i) (Pampulha & Loureiro-Dias, 1989; Coote *et al.*, 1994), a possible role for internal acidification of the cell as triggering trehalose accumulation can be suggested. This is reinforced by the observation that the acquisition of thermotolerance is enhanced in cells having an acidic external environment (Coote *et al.*, 1991).

To verify this hypothesis, we used a *pma1-1* mutant strain with a reduced H^+ -ATPase activity (Van Dyck *et al.*, 1990). Since the pH_i in yeast is largely controlled by the activity of the membrane-bound H^+ -ATPase (Serrano, 1988), this mutant should be more sensitive to treatments which cause perturbations of pH_i . Thus, we argued that if the induction of trehalose is mediated by cytoplasmic acidification, then a *pma1-1* mutant should be more sensitive to treatments which lower the pH_i , and hence should accumulate more trehalose.

We actually found no correlation between a drop in pH_i and the capacity of cells to induce *TPS1* and to accumulate trehalose. Instead, it is shown that a *pma1-1* mutant accumulated more trehalose than the isogenic wild-type not because of a pH homeostasis problem, but because a mutation at the *PMA1* locus resulted in an increased Tre6P synthase activity together with higher levels of Tre6P synthase substrates.

METHODS

Yeast strains and culture conditions. Strains of *S. cerevisiae* Σ 1278b and MG2132 were kindly provided by Professor A. Goffeau (Catholic University of Louvain, Belgium). MG2132 is a *pma1-1* mutant strain (decreased H^+ -ATPase activity, around 40% of the parental activity), isogenic to the wild-type strain Σ 1278b (Van Dyck *et al.*, 1990). Strain JLP87-8A (*MATa leu2 his3 URA3::TPS1-lacZ*) was used for transcriptional activation of *TPS1* in response to stress treatments. Yeast cells were grown aerobically at 25 °C with orbital shaking (250 r.p.m.) to mid-exponential phase (1.6 mg dry wt ml⁻¹) on YPD medium as previously described (Alexandre *et al.*, 1994).

Stress conditions. Ethanol and decanoic acid shock were carried out by addition of 10% (v/v) ethanol and 6 mg undissociated decanoic acid l⁻¹ (the toxic compound is the undissociated form), respectively, to mid-exponential-phase cultures (at this point of growth, the pH of the medium was 5). Heat shock treatment was performed by transferring exponential-phase cultures growing at 25 °C to a water bath set at 40 °C. The pH shock was accomplished by decreasing the medium pH from 5 to 3.5 with concentrated HCl. All stress treatments lasted 1 h, during which time samples were taken for measuring pH_i , trehalose and activity of trehalose metabolizing enzymes (see below), and for estimating viable cells by plating suitable cellular dilutions onto YPD agar and incubating the plates at 28 °C for 24–48 h.

Analytical procedures. Cell extracts for enzymic measurements were obtained by vortexing yeast cells (about 20 mg dry wt) into 0.5 ml 25 mM ice-cold HEPES buffer, pH 7.1, containing 1 mM PMSF, 2 mM EDTA and 100 mM KCl, with 1 g glass beads (0.5 mm diameter) for six periods of 30 s, with 30 s intervals on ice after each period. The extracts were centrifuged for 5 min at 700 g, and the supernatants were clarified by a second centrifugation at 10000 g for 15 min. Tre6P synthase was assayed by measuring the formation of UDP as described by Vandercammen *et al.* (1989), except that the temperature of the enzymic assay was set at 45 °C. Trehalase was assayed according to François *et al.* (1984).

Transcriptional activation of *TPS1* was determined by measuring the β -galactosidase activity from a *TPS1-lacZ* gene fusion as described previously (Parrou *et al.*, 1997).

For trehalose determination, yeast cells (10–20 mg dry wt) were quickly collected by filtration and washed twice with ice-cold water. The yeast-cake was dropped into 3 ml 0.25 M Na₂CO₃ and incubated for 2 h at 90 °C. The alkaline suspension was further treated for trehalose assay as described elsewhere (Parrou *et al.*, 1997). The glucose liberated by trehalase digestion was determined by the glucose oxidase method.

Metabolite extraction was performed essentially as described by Gonzalez *et al.* (1997). Briefly, a 5 ml sample was sprayed into 26 ml of cold solution containing 60% (v/v) methanol and 70 mM HEPES, pH 7.5 (methanol buffer solution), kept at

–70 °C in a dry ice/ethanol bath. The mixture was allowed to cool for 3 min and was then centrifuged at 5000 *g* for 5 min in a Sorvall RC5B centrifuge set at –10 °C. Metabolites from cell pellets were extracted in 8 ml of a solution of 75% (v/v) boiling absolute ethanol containing 70 mM HEPES, pH 7.5. After evaporation of ethanol, the pellet was resuspended in 2 ml cold water and used for metabolite measurements as described by Bergmeyer (1986) by coupling appropriate enzyme assays with fluorimetric detection of NAD(P)H. Emission was measured at 450 nm after excitation at 350 nm using a fluorescence spectrophotometer (Hitachi F-2000).

pH_i measurements. These were conducted over the 60 min of stress treatments, which were carried out exactly as described above. To measure changes in pH_i, exponentially growing cells were harvested by centrifugation, washed once with sterile water and resuspended at 1 mg dry weight ml⁻¹ in 0.1 M phosphate buffer, pH 5, containing 2 mM MgCl₂, 10 mM KCl, 2 mM CaCl₂ and 1% glucose. The pH of the buffer corresponded to the pH of the culture at the time it was harvested. At different times during the stress treatment, an aliquot (3 ml) of the cell suspension in phosphate buffer was incubated for 5 min at 25 °C with 10 µM fluorescein diacetate and then placed in a cuvette of a Hitachi-F2000 spectrofluorimeter to record the fluorescein intensity at 520 nm after excitation at 490 nm and 435 nm. The values of pH_i were read from a calibration curve prepared according to Slavik (1982).

RESULTS

Effect of *pma1-1* mutation on pH_i in response to various stresses

Changes in pH_i were monitored in yeast cells subjected to four types of stress, namely a temperature shift from 25 to 40 °C, ethanol and decanoic acid shocks and a drop in external pH. As shown in Fig. 1, the pH_i of the *pma1-1* strain before treatment (6.2 ± 0.2) was comparable to that of its isogenic parent (6.3 ± 0.2). These values agree with those given in the literature (Slavik, 1982; Haworth & Fliegel, 1993; Pena *et al.*, 1995; Coote *et al.*, 1994). Contrary to expectation, the stress treatments induced a similar drop in pH_i in both the wild-type and in the *pma1-1* mutant, with the decanoic acid stress being least and the pH shock being the most effective, resulting in a drop of 0.8 pH units. However, a slight difference between the wild-type and the mutant could be seen with regard to the kinetics of pH_i drop, as the decrease in pH_i upon ethanol and pH shock was faster in the control strain than in the *pma1-1* mutant, whereas the converse situation was found for temperature shock.

Effect of *pma1-1* mutation on trehalose levels and cell survival in response to various stresses

The trehalose content of the mutant strain MG2132 (*pma1-1*) and of its isogenic parent strain Σ1278b was measured after 60 min incubation under the four different stress conditions (Table 1). Both strains accumulated high levels of trehalose in response to temperature or ethanol shock, but the content of trehalose was significantly higher in the *pma1-1* mutant than in the control strain. Stress induced by decanoic acid or by a drop in the medium pH also caused a slight but definite

Fig. 1. Effect of various stress conditions on pH_i in exponential-phase glucose-grown cells of *S. cerevisiae* wild-type (a) and *pma1-1* mutant (b) strains. ×, Ethanol shock; ■, pH shock; ▲, decanoic acid shock; ◆, heat shock; ○, control. Data are the means of three independent experiments. Error bars indicate the standard deviation.

increase in trehalose levels, and this effect was even more significant in the *pma1-1* mutant. A similar rise in trehalose levels in response to ethanol and decanoic acid stress and after a drop in pH was obtained in a *nth1* mutant defective in neutral trehalase (results not shown). In contrast, lack of trehalase resulted in a much higher increase in trehalose levels after a temperature upshift to 40 °C (see also Parrou *et al.*, 1997).

The viability of wild-type and *pma1-1* strains was estimated after 60 min incubation under the various stress conditions. For both strains, there was no clear correlation between their trehalose content and cell survival (Table 1). In particular, a lower viability was recorded upon incubation of the cells in the presence of ethanol despite a higher trehalose content, and this reduced survival was even more pronounced in the *pma1-1* mutant, indicating that the resistance of cells to high ethanol concentrations is independent of trehalose levels but may require normal H⁺-ATPase activity.

Effect of stress conditions on the activity of Tre6P synthase and trehalase, and on a *TPS1-lacZ* gene fusion

Trehalase and Tre6P synthase were measured in crude extracts of wild-type and *pma1-1* mutant cells in an attempt to explain how trehalose accumulated to different extents under the different stress conditions investigated, and how a mutation in *PMA1* resulted in an enhanced trehalose synthesis efficiency.

Table 1. Trehalose concentration and percentage cell survival of exponentially growing wild-type $\Sigma 1278b$ and mutant MG2132 *S. cerevisiae* before and after 60 min stress

Growth and stress conditions are described in Methods. Data are means $\pm$ standard deviation of four independent experiments.

		Stress treatment:				
		Control	Heat shock	Ethanol shock	Decanoic acid shock	pH shock
Wild-type ($\Sigma 1278b$)	Trehalose ($\mu\text{g mg}^{-1}$)	0.2 ± 0.05	31.7 ± 4.1	5.2 ± 1.9	0.7 ± 0.2	0.4 ± 0.1
	Viable cells (%)	98 ± 5	74 ± 4	60 ± 6	73 ± 7	75 ± 2
Mutant (MG2132)	Trehalose ($\mu\text{g mg}^{-1}$)	0.5 ± 0.5	40.7 ± 9.5	15.1 ± 1.1	1.9 ± 0.4	1.5 ± 0.1
	Viable cells (%)	98 ± 5	81 ± 12	40 ± 5	70 ± 10	72 ± 12

Fig. 2. Tre6P synthase activity of exponentially growing cells of *S. cerevisiae* wild-type (a) and $pma1-1$ mutant (b) strains after 30 min (open bars) and 60 min (black bars) stress treatment. Data are the means of three independent experiments. Error bars indicate the standard deviation.

As shown in Fig. 2, the activity of Tre6P synthase in exponential cultures of the $pma1-1$ strain was about twofold higher than that in the isogenic control strain. This difference was even more marked after 60 min

incubation at 40 °C, since activity was increased by sixfold in the $pma1-1$ mutant but by only fourfold in the control strain. In contrast, addition of 10% ethanol to exponential-phase cells of wild-type and $pma1-1$ mutant strains growing on glucose caused a twofold reduction in the activity of this enzyme, whereas stress induced by a drop in the pH of the medium and by decanoic acid had no effect on Tre6P synthase. As shown in Fig. 3, assays of neutral trehalase did not reveal any significant differences between the wild-type and the $pma1-1$ mutant, and as reported previously (Neves & François, 1992; De Virgilio *et al.*, 1991), a temperature upshift to 40 °C caused a twofold increase in trehalase activity after 60 min of incubation (Fig. 3). We also observed that addition of 10% ethanol caused a 30–50% reduction in trehalase activity in the two yeast strains.

We also investigated the effect of different stresses on transcriptional expression of *TPS1* by means of β -galactosidase activity from a *TPS1-lacZ* gene fusion to determine whether a transcriptional activation of *TPS1* was accompanied by changes in Tre6P synthase activity. Contrary to expectation, there was no transcriptional activation of *TPS1* upon transfer of cells from 25 to 40 °C (Table 2). This result is, however, in agreement with those previously reported by Parrou *et al.* (1997), who showed that transcriptional induction of 'stress-related' genes was optimal at 37 °C and then dropped to zero at temperatures above 40 °C. In contrast, a drop in pH caused a two- to threefold transcriptional activation of *TPS1* although there was no increase in Tre6P synthase activity (see Fig. 2a). Table 2 shows also that the expression of *TPS1* was not affected by stress induced by ethanol and decanoic acid.

Changes in metabolite levels in response to stress

We next determined the intracellular concentration of UDP-Glc and Glc6P, which are substrates of Tre6P synthase, and of ATP, which is involved in pH homeostasis through its hydrolysis by H⁺-ATPase, to complete the explanation of how trehalose is accumulated to

Fig. 3. Trehalase activity of exponentially growing cells of *S. cerevisiae* wild-type (a) and *pma1-1* mutant (b) strains after 30 min (open bars) and 60 min (black bars) stress treatment. Data are the means of three independent experiments. Error bars indicate the standard deviation.

Table 2. Effect of pH shock, decanoic acid shock, temperature upshift and ethanol shock on a *TPS1-lacZ* gene fusion in *S. cerevisiae*

Exponential-phase cells growing on glucose were subjected to the different stresses as described in Methods. Data are means of three independent experiments. Activity is expressed in $\text{nmol min}^{-1} (\text{mg protein})^{-1}$.

Time (min)	Control	Heat shock	Ethanol shock	Decanoic acid shock	pH shock
15	16	20	9	11	30
30	23	18	10	13	58
60	20	19	11	18	75

different extents under the different stress conditions. As shown in Figs 4 and 5, the levels of UDP-Glc and ATP in the *pma1-1* mutant growing on glucose were 4 and 1.5-fold higher, respectively, than those in the wild-type strain, whereas levels of Glc6P were the same in the two

Fig. 4. Changes in the concentrations of Glc6P (a), UDP-Glc (b) and ATP (c) in exponential-phase cultures of *S. cerevisiae* $\Sigma 1278b$ (wild-type) induced by various stress conditions. $\times$, Ethanol shock; $\blacksquare$, pH shock; $\blacktriangle$, decanoic acid shock; $\blacklozenge$, heat shock; $\circ$, control. Data are means of three independent experiments. Error bars indicate the standard deviation.

strains (Figs 4, 5). When the two yeast strains were subjected to a temperature upshift to 40°C , the concentration of Glc6P decreased by fourfold within 5 min whereas that of UDP-Glc increased, the rise in this metabolite being greater in the wild-type than in the mutant. Levels of ATP, which were 1.5-fold greater in the mutant strain than in the wild-type, were not significantly affected by this treatment (Figs 4c, 5c). Stress induced by addition of decanoic acid or by a pH drop did not cause significant changes in the intracellular levels of UDP-Glc or Glc6P in the wild-type strain, whilst these stresses resulted in a slight decrease in UDP-

Fig. 5. Changes in the concentrations of Glc6P (a), UDP-Glc (b) and ATP (c) in exponential-phase cultures of *S. cerevisiae* MG2132 (*pma1-1*) induced by various stress conditions. ×, Ethanol shock; ■, pH shock; ▲, decanoic acid shock; ◆, heat shock; ○, control. Data are means of three independent experiments. Error bars indicate the standard deviation.

Glc in the *pma1-1*, and in a progressive increase in ATP concentration in both strains. In contrast, addition of 10% ethanol to exponential-phase cells of both the wild-type and the *pma1-1* mutant was quite different from the other treatments as it caused a strong increase in both Glc6P and UDP-Glc levels without significantly affecting ATP concentration.

DISCUSSION

The initial mechanisms triggering trehalose accumulation upon exposure of cells to various types of stress factors are still unknown. Because a large drop in

external pH has been reported to cause stress to yeast cells (Coote *et al.*, 1991), we started with the idea that an immediate decrease in the pH_i could be a common denominator of all stress conditions and, moreover, that this pH change could be the intracellular event triggering trehalose induction. With this hypothesis in mind, we considered the use of a mutant with a reduced H^+ -ATPase activity since several studies have shown that plasma membrane ATPase cannot be ignored as a factor affecting tolerance to various stresses (Panaretou & Piper, 1990; Coote *et al.*, 1991). In addition, H^+ -ATPase consumes large quantities of cellular ATP in pH homeostasis (Serrano, 1988). Interestingly, we found that ATP levels in the *pma1-1* bearing a reduced H^+ -ATPase activity were 1.5-fold higher than those in the wild-type, suggesting that ATP turnover might be controlled by H^+ -ATPase. However, and contrary to expectation, the *pma1-1* mutant behaved like the wild-type as regards to changes in pH_i in response to the four different stress situations. The absence of any effect of *PMA1* mutation on pH_i may be explained by the buffering capacity of the cells, which prevented drastic changes in pH_i despite a lower H^+ -ATPase activity. Alternatively, it is possible that the activation of H^+ -ATPase which has been reported to occur under these stress conditions (Coote *et al.*, 1991; Eraso & Gancedo, 1987; Rosa & Sá-Correia, 1992; Alexandre *et al.*, 1994, 1996) led to sufficient H^+ -ATPase activity in the *pma1-1* mutant to counteract proton influx induced by stress. Therefore, it would be worthwhile to repeat these experiments using a mutant strain defective in the process of H^+ -ATPase activation.

Contrary to our initial hypothesis, the data presented in this work did not reveal any correlation between trehalose accumulation and cytoplasmic acidification. An alternative hypothesis regarding the possible physiological trigger inducing trehalose accumulation could be an alteration of the plasma membrane by stress. There is some research which favours this suggestion since it has been shown that stress induced by a temperature upshift, addition of ethanol and salt, or freezing-thawing yeast cells alters the plasma membrane (Thomas *et al.*, 1978; Beaven *et al.*, 1982; Rose, 1989), and in all these treatments there was an accumulation of trehalose (Van Laere, 1989; Mager & Varela, 1993; Coote *et al.*, 1994). Moreover, it has recently been proposed that yeast cells sense osmotic stress via mechanosensitive ion channels present in their plasma membrane. These channels could function in sensing changes in turgor pressure (Brewster *et al.*, 1993). However, this correlation between membrane alteration and trehalose accumulation might be purely coincidental.

Whilst we do not yet have a clear idea about the initial event triggering trehalose accumulation in response to stress, we can more easily explain how this putative initial event affected trehalose metabolism under the four different stress conditions investigated. The induction of trehalose by a temperature upshift has been studied in great detail (Hottiger *et al.*, 1987; Wiemken, 1990; Bell *et al.*, 1992; Vuorio *et al.*, 1993; Piper, 1995;

Parrou *et al.*, 1997), and results shown here largely confirm that trehalose accumulation resulted from an increased Tre6P synthase activity (Neves & François, 1992; Vuorio *et al.*, 1993). However, we found that the increase in the amount of Tre6P synthase was not accompanied by transcriptional activation of *TPS1*. This discrepancy may be explained by a slight difference in the absolute temperature at which heat shock was carried out with the different strains, since it was shown that a 1 °C difference strongly affected the transcriptional machinery of STRE (stress responsive element)-related genes (Parrou *et al.*, 1997). Alternatively, it can be postulated that the sensitivity of the transcriptional machinery to absolute temperature is strain-dependent. The effect of ethanol in stimulating trehalose accumulation (Odumeru *et al.*, 1993) could be attributed to an increase in the concentration of UDP-Glc and Glc6P, the two substrates of Tre6P synthase, although both trehalase and Tre6P synthase activity were reduced by 30–50% upon incubation in high ethanol concentrations. This latter result contrasted with the observation that addition of 12.5% ethanol to glucose-growing cells induced transcriptional activation of HSE (heat shock element)- and STRE-related genes (Piper, 1995; Schüller *et al.*, 1994), among which *TPS1* harbours 5 STRE elements in its promoters (Varela *et al.*, 1995). This interesting result reinforces the idea that *cis*-acting elements in addition to the STRE consensus (namely CCCCT) are required for effective transcriptional activation of a subset of genes by stress (Varela *et al.*, 1995; Parrou *et al.*, 1997). It can also be emphasized that the rise in Glc6P accompanied by a decrease in fructose 1,6-diphosphate (unpublished data) reflected an inhibition of glycolysis by high levels of ethanol. However, the mechanism of this inhibition is not yet clear. The very modest accumulation of trehalose after a large drop in external pH and the lack of accumulation of this metabolite upon addition of decanoic acid could not be ascribed to the counteracting activity of trehalase since experiments performed with a mutant defective in neutral trehalase gave rise to identical results as those with the wild-type. This result actually did not contradict those of Valle *et al.* (1986) who showed that a drop of medium pH caused activation of trehalase, since these authors used stationary-phase cells in which trehalase was mostly in its inactivated form whereas we used exponential-phase cells in which trehalase was mostly in its activated form. As in the case of *CTT1* (Schüller *et al.*, 1994), a large drop in external pH caused a two- to threefold transcriptional induction of *TPS1*. Since this transcriptional activation was not accompanied by an increase in Tre6P synthase levels, a likely explanation is that gene expression resulted in a non-functional enzyme.

In summary, there is not a single and common way for cells to accumulate trehalose in response to various types of stress. Either an increase in the amount of enzyme, a change in enzyme activity or in the levels of Tre6P synthase substrate, or a combination of two of these parameters could be invoked to explain the

accumulation of trehalose. Furthermore, we have shown that the presence of STRE elements in the promoter of *TPS1* is not enough for that gene to be induced in any stressful condition. This reinforces the idea of the existence of additional *cis*-acting elements needed for the proper response of STRE-related genes to various types of stress (Varela *et al.*, 1995; Parrou *et al.*, 1997).

The use of a strain with reduced H⁺-ATPase activity led to the finding that this mutation affects the capacity of yeast for trehalose synthesis. By a still unknown mechanism, the point mutation at the *PMA1* locus resulted in a 1.5- to 2-fold higher amount of Tre6P synthase without any effect on trehalase levels, and in fourfold higher levels of intracellular UDP-Glc. These two advantages could account for the two- to threefold higher content of trehalose in this mutant compared to the wild-type both under normal growth conditions and in response to stress, owing to the fact that these treatments caused similar metabolic effects in both the *pma1-1* and the wild-type strain. Whatever the mechanism involved, these results clearly show complex interactions between different, and not obviously related, components of the metabolic activity of the cell. They also emphasize the possibility that any genetic alteration in a cell could mimic a stress situation.

In our hands, a higher trehalose content in the *pma1-1* mutant was not accompanied by a greater cell viability as compared to the wild-type $\Sigma 1278b$ strain, either in control conditions or after various stress treatments. Our results are therefore at variance to those of Panaretou & Piper (1990), who used the same strains and reported a higher viability for the *pma1-1* mutant after exposure to 12.5% ethanol. In addition, our results suggest that trehalose can serve as a stress protectant only under a subset of stress conditions, in particular when cells are challenged with high temperatures.

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