

# Heat-shock response in a yeast *tps1* mutant deficient in trehalose synthesis

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## Abstract

Exponential cells of the *Saccharomyces cerevisiae tps1* mutant underwent a rapid loss of viability upon a non-lethal heat exposure (from 28 to 42°C). However, a further more severe heat stress (52.5°C 5 min) induced an increase in the fraction of viable cells. This mutant can not synthesize trehalose either at 28°C or at 42°C due to the lack of a functional trehalose-6P synthase complex. In control experiments carried out with the wild-type W303-1B, heat-stressed exponential phase cultures grown on YPgal at 28°C acquired thermotolerance to a higher extent than identical cultures grown on YPD, although in both cultures the level of stored trehalose was negligible. These data suggest that the bulk of trehalose accumulated in yeast upon mild heat treatments is not sufficient to account for the acquisition of thermotolerance.

**Key words:** Heat shock; Trehalose; Thermotolerance; *Saccharomyces cerevisiae*; Trehalase; Trehalose-6P synthase

## 1. Introduction

Thermotolerance has been widely investigated in yeasts in connection with trehalose metabolism [1–5]. *Saccharomyces cerevisiae* cells growing exponentially on glucose (28–30°C) accumulate an enormous amount of trehalose (an up to 4- to 5-fold increase) when subjected to heat-shock treatments (37–42°C). This accumulation plays a protective role in the cells during their further exposition to higher temperatures (>50°C) [1,4,5]. Conclusive genetic and biochemical data have provided a close correlation between the intracellular levels of trehalose and the degree of acquired thermotolerance [4–6]. The capacity of the cell to withstand other stress conditions, such as hyperosmotic shock and dehydration, is also dependent on massive synthesis of trehalose (reviewed in [7]). A similar role has been demonstrated in certain bacteria, which are able to store large concentrations of trehalose as the compatible solute in response to a variety of osmotic stresses (reviewed in [8]).

Trehalose appears to act by preventing the deleterious effect caused by heating on membranes and proteins [5,9,10]. The disaccharide substitutes water and binds to the polar head groups of phospholipids, preserving the properties of a hydrated membrane [9]. Recent findings also show that trehalose added at physiological concentrations efficiently increases the thermal stability of yeast proteins *in vitro* against several heat-shock treatments, as well as reducing the formation of heat-induced protein aggregates [10].

Nevertheless, the direct relationship between changes in trehalose accumulation and reversible acquisition of thermotolerance has recently been challenged by utilizing deletion mutants of the heat-shock protein HSP104

(*hsp104Δ*) and neutral trehalase (*nth1Δ*) [11,12]. Both strains were heat-shock sensitive, despite the fact that they exhibited normal levels of trehalose. In this study, I have analyzed the thermotolerance response in a *S. cerevisiae tps1* (formerly *cif1*) mutant [13], which is deficient in the small 56 kDa subunit of the T-6P synthase complex [14,15] and is therefore unable to synthesize endogenous trehalose.

## 2. Materials and methods

### 2.1. Yeast strains and culture conditions

The following *S. cerevisiae* strains were used: wild-type W-303-1B, Mat  $\alpha$  *ade2-1 his3-11,15 ura3-1 leu2 trp1-1 TPS1*, and the isogenic *tps1* mutant WDC-3A, Mat  $\alpha$  *ade2-1 his3-11,15 ura3-1 leu2 trp1-1 tps1::HIS3*. They were kindly provided by Dr. C. Gancedo (Instituto de Investigaciones Biomédicas, Madrid, Spain). The cells were grown at 28°C with shaking in 2% peptone, 1% yeast extract and 2% glucose (YPD) or 2% galactose (YPgal).

### 2.2. Heat-shock treatment and determination of thermotolerance

When 200 ml of the cultures reached an  $OD_{600} = 1.0$ – $1.3$  units (about  $1.1$ – $1.5 \times 10^7$  cells/ml) they were divided into two flasks of 100 ml each and placed into a shaking water bath at 28°C. After 15 min one flask was transferred to another water bath at 42°C.

To determine thermotolerance, aliquots of both cultures (1 ml) were collected in prewarmed Eppendorf tubes, placed at 52.5°C for 5 min and quickly cooled in ice. The samples were appropriately diluted with sterile water and plated in triplicate on solid YPD or YPgal. Colonies were scored after incubation for 3–5 days at 30°C. In each case, the percentage of viability was referred to control samples from the same culture without exposure to 52.5°C (100% viability).

### 2.3. Enzymatic assays

Cell-free extracts in 25 mM MES, pH 7.1, were obtained and neutral trehalase was measured as described previously [16]. T-6P synthase activity was determined by the discontinuous procedure described elsewhere [17] except that the temperature of the assay was raised to 40°C.

### 2.4. Analytical procedures

Intracellular trehalose was extracted at 4°C with 5% trichloroacetic acid and estimated according to the anthrone method. Confirmation of its identity was performed by gas chromatography using the trimethylsilyl derivatization technique including commercial trehalose (Merck)

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as an external standard. Protein was measured according to Lowry et al. [18] with bovine serum albumin as the standard.

### 3. Results

#### 3.1. Thermotolerance and trehalose content in the wild-type strain W303-1B

Because the *tps1* mutant (allelic to *cif1*, *fdp1*, *byp1*, *glc6* or *ggs1*) can not grow on glucose or fructose [13,19], galactose was used as an alternative carbon source. Initial experiments were carried out in wild-type cells to analyze the degree of viability vs. the trehalose level after mild heat treatments in cultures grown on YPD and YPgal media.

W303-1B cells pregrown at 28°C on YPgal showed a higher capacity for survival (about 20%) when subjected to a thermal stress (52.5°C for 5 min) with respect to that observed in identical cells pregrown on YPD (Fig. 1A). After a shift to 42°C and subsequent heat treatment, the percentage of viability increased in parallel in both cultures and became practically similar within 90 min of incubation (Fig. 1A).

Exponential cells at 28°C contained low levels of trehalose after growth either with glucose or galactose (Fig. 1B). In YPD cultures, the change to 42°C induced a marked increase in the trehalose content (Fig. 1B) which mirrored the rise in viability exhibited by these cells (Fig. 1A). The pattern of trehalose synthesis in YPgal cultures followed the same trend, but the actual content of treha-

lose was lower (Fig. 1B). This might be due to a minor concentration of the biosynthetic substrates of T-6P synthase (UDP-glucose and glucose-6P) in galactose than in glucose [20]. Therefore, in this case, there is not an exact correlation between acquisition of thermotolerance and trehalose storage upon sublethal heat-shock treatment.

#### 3.2. Thermotolerance response in the *tps1* mutant

Cells of the *tps1* strain shifted from 28 to 42°C and further incubated at this temperature for various periods, rapidly lost viability (Fig. 2). However, aliquots harvested from the culture incubated at 42°C and submitted to a thermal shock (52.5°C, 5 min) (Fig. 3A) displayed a progressive increase in cellular viability, which was more evident when compared to the same samples obtained from the culture at 28°C (Fig. 3A). Recently, similar results were achieved with another *tps1* strain growing on maltose instead of galactose [6]. Determination of trehalose performed in cells maintained at 28°C showed that *tps1* cells did not accumulate detectable amounts of this disaccharide (Fig. 3B). Since it has been proposed that another ADPG-dependent T-6P synthase activity is present in *S. cerevisiae* [21], I tested whether this pathway could be operative during the heat-shock process. However, no significant levels of trehalose were measurable at 42°C either (Fig. 3B). Gas chromatography determinations confirmed the lack of intracellular trehalose in the *tps1* mutant (data not shown).

The response to heat shock of the enzymes involved

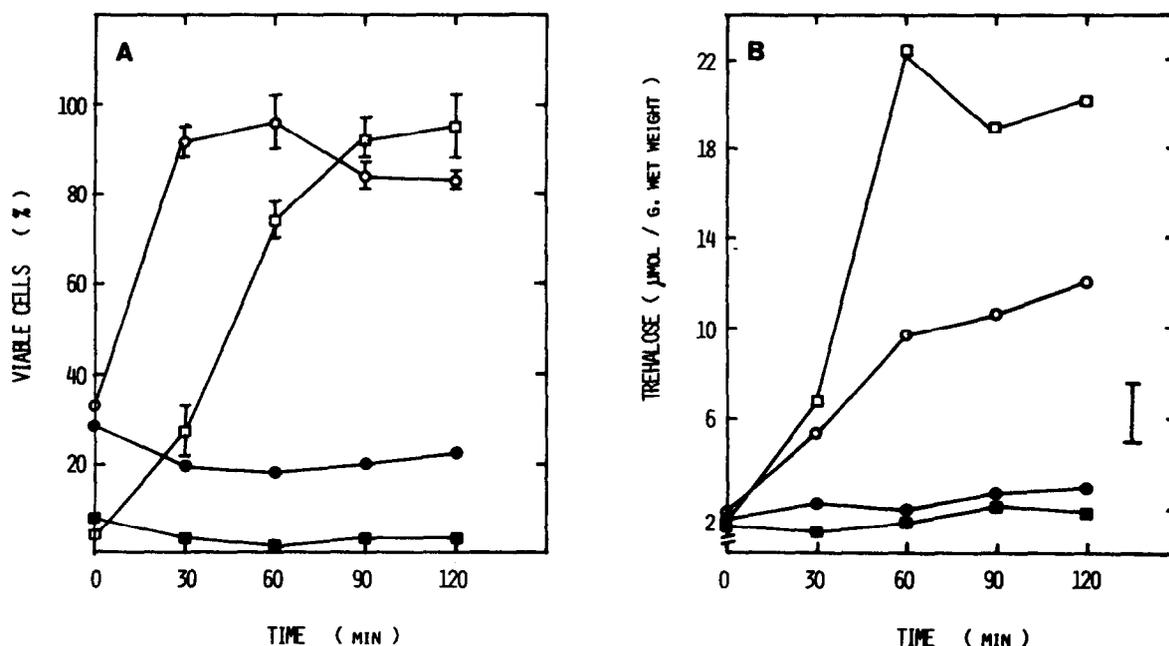


Fig. 1. Acquisition of thermotolerance (A) and trehalose accumulation (B) in *S. cerevisiae* wild-type cells grown in YPD (squares) or YPgal (circles) liquid media. Log-phase W303-1B cells were subjected to a preconditioning heating at 42°C for various periods. Induction of thermotolerance was carried out at 52.5°C for 5 min and expressed as the percentage of survival respect to an identical, untreated sample (100%). Filled symbols, cells incubated at 28°C; (open symbols, cells incubated at 42°C. Error bars in (A) represent the S.D. of 3 measurements. Error bars from the data at 28°C were omitted for the sake of clarity, but the S.D. was lower than 8%. The error bar at the right in (B) represents the maximal S.D.

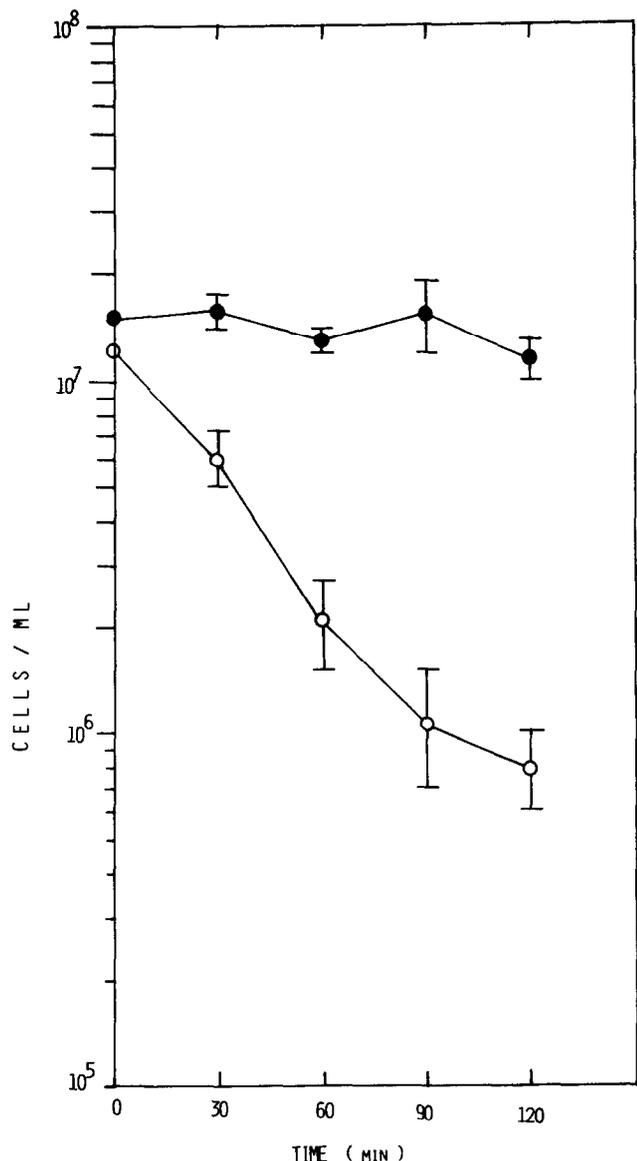


Fig. 2. Effect of the non-lethal pretreatment at 42°C on cell viability in *tps1::HIS3*. Log-phase cultures pregrown at 28°C were maintained at this temperature (●), or transferred to 42°C (○). The values represent the mean  $\pm$  S.D. of three measurements.

in trehalose metabolism was also examined. In galactose-grown W303-1B cells, basal T-6P synthase displayed values somewhat higher than those recorded in YPD (Fig. 4A) [17,22]. This is probably due to the fact that the T-6P synthase complex is subjected to glucose repression [13,22], which is partially alleviated in the presence of galactose. However, the enzyme could still be activated after transfer of the cultures to 42°C (Fig. 4A). As expected, T-6P synthase in the *tps1* mutant was barely detectable under both experimental conditions.

On the other hand, an increase in neutral trehalase upon heat-shock exposition was observed in *tps1* cells,

although to a lower extent than that recorded in the wild-type cells (Fig. 4B). This result is consistent with the observation that although *tps1* exhibits a greatly reduced glucose-induced cAMP signal [13,19], the pathway for cAMP synthesis remains intact [13].

#### 4. Discussion

Trehalose, considered for a long time as a reserve carbohydrate in yeasts [23], has recently been rediscovered as an efficient protectant of cell integrity against a variety of stresses [5,7]. In this context it is well documented that *S. cerevisiae* proliferating cells submitted to a temperature shift (from 28–30°C to 37–45°C) accumulate trehalose which, in turn, results in an increase in the fraction of survivors during a further more severe heat stress (> 50°C) [1,4,5]. Cellular resistance to dehydration [24] or noxious chemicals [3] was also dependent on the concentration of trehalose. Nevertheless, other agents (i.e. ethanol or *N*<sup>6</sup>-(2-isopentenyl)adenosine) are able to induce the thermotolerant state without a concomitant accumulation of trehalose [25]. Moreover, when the deletion mutants *hsp104Δ* and *nth1Δ* were submitted to heat stress (50°C for 10–20 min), they showed low thermotolerance, although the concentration of trehalose was normal (*hsp104Δ*) or even higher (*nth1Δ*) compared with the respective parent strains [11,12]. If we assume the existence of a common mechanism for producing a tolerant viable state under a wide range of circumstances, this would indicate that the trehalose stored during the preconditioning temperature shift may be only part of a general thermoprotective response.

The results presented here suggest that with reference to the role of trehalose, a distinction should be made between the non-lethal heat treatment and the subsequent thermal stress. Indeed, trehalose acts as a cellular protectant during the mild heat exposure, since cultures of a trehalose-deficient mutant (*tps1*) quickly die when shifted from 28°C to 42°C (Fig. 2). However, it appears to be less evident that trehalose can, by itself, account for the resistance to the severe heat stress. Exponential wild-type cultures grown on YPgal showed higher survival after a 52.5°C exposure than the same cultures grown on YPD (Fig. 1A), whereas the level of trehalose at 28°C was very low in both cases (Fig. 1B). In addition, the percentage of viability upon transfer to 42°C tended to be similar (Fig. 1A), whereas YPD-grown cultures displayed a higher accumulation of trehalose than YPgal-grown cultures (Fig. 1B). Furthermore, cells of the *tps1* mutant preincubated at 42°C and then subjected to 52.5°C acquired a certain degree of thermotolerance (about 30% increase in cellular viability) (Fig. 3A; [6]), despite the fact that this strain is unable to synthesize trehalose either at 28°C or 42°C (Figs. 3B and 4). Measurements of neutral trehalase (Fig. 4) are consistent with

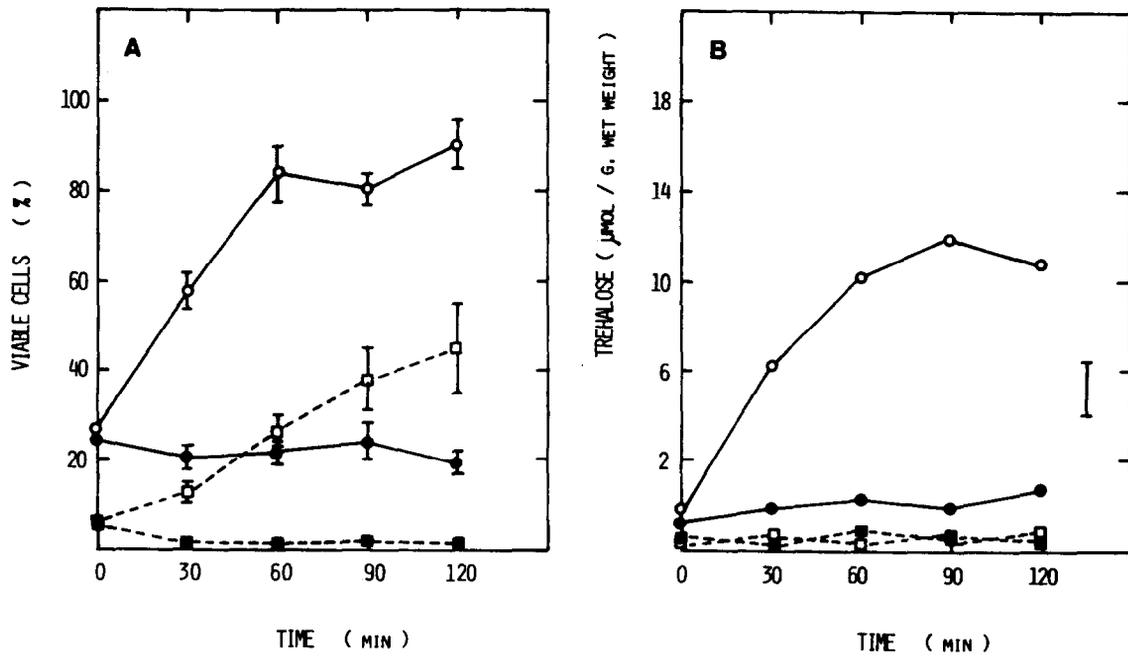


Fig. 3. Acquisition of thermotolerance (A) and trehalose accumulation (B) in the *tps1::HIS3* strain. Log-phase cultures were grown in YPgal at 28° C (filled symbols). At time zero, half of the wild-type culture and the *tps1* mutant were transferred to 42° C (open symbols). Circles, wild-type W303-1B; squares, *tps1* mutant. For other details see Fig. 1.

the idea that the pathway for cAMP synthesis is operative in this mutant [13,19].

In addition to trehalose, other factors synthesized during the sublethal heat exposition have to be directly in-

involved in the acquisition of thermotolerance. An obvious candidate would be the set of heat-shock proteins (HSPs) (recently reviewed in [26]). Their involvement in the heat-shock response was previously questioned because,

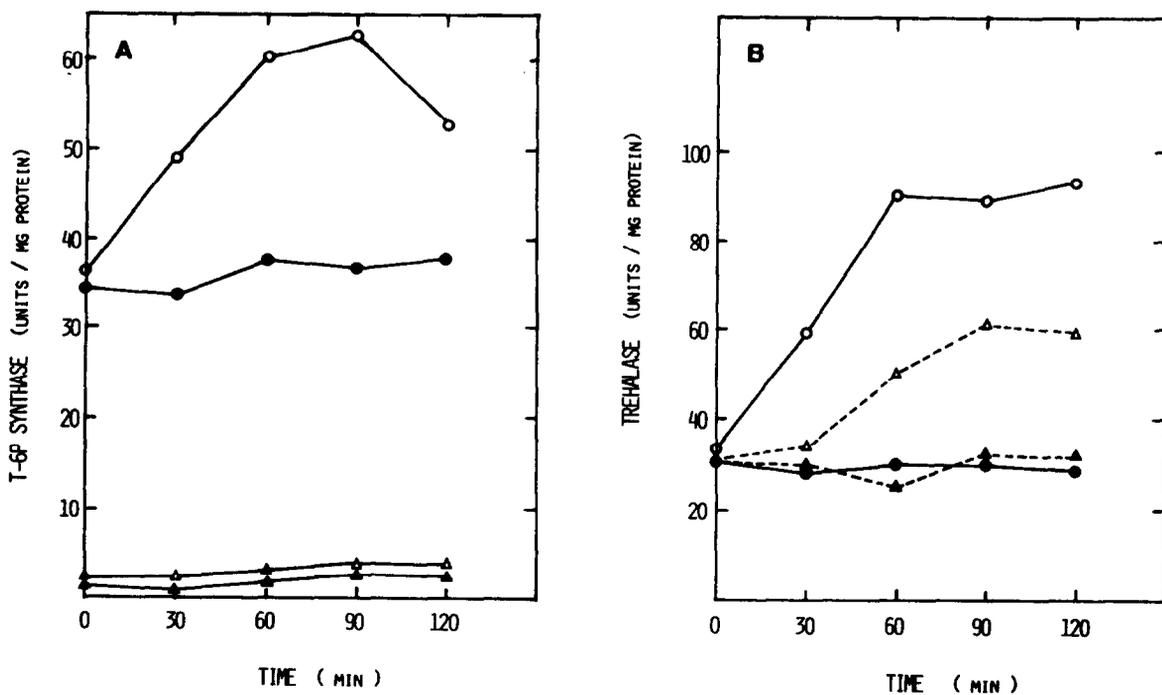


Fig. 4. Changes in T-6P synthase (A) and trehalase (B) activities in the *tps1::HIS3* strain. Log-phase cultures from the wild-type W303-1B or the *tps1* mutant were grown in YPgal at 28° C (filled symbols). At time zero, half of each culture was transferred to 42°C (open symbols). Circles, wild-type; triangles, *tps1* mutant.

under certain conditions, thermotolerance was induced in the absence of HSP synthesis [27]. However, a direct effect on the induced thermotolerance has been demonstrated in the case of HSP 104 [28]. Different lines of evidence have led to the proposal of a protective cooperation between some HSPs and trehalose: (i) trehalose accumulation occurs in parallel with HSP synthesis in mutants altered in the RAS-cAMP pathway [4]; (ii) the activity (or level of expression) of several HSPs negatively modulates the actual content of trehalose during the heat-shock stress and/or heat-shock recovery [20,29]; (iii) the genes *TPS1* and *TPS2*, which encode two subunits of the T-6P synthase complex, belong to the group of HSPs [6,13,14].

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## References

- [1] Hottiger, T., Boller, T. and Wiemken, A. (1987) FEBS Lett. 220, 113–115.
- [2] Hottiger, T., Schmutz, P. and Wiemken, A. (1987) J. Bacteriol. 169, 5518–5522.
- [3] Attfield, P.V. (1987) FEBS Lett. 225, 259–263.
- [4] Hottiger, T., Boller, T. and Wiemken, A. (1989) FEBS Lett. 255, 431–434.
- [5] Wiemken, A. (1990) A. van Leeuwenhoek 58, 209–217.
- [6] De Virgilio, C., Hottiger, T., Dominguez, J., Boller T. and Wiemken, A. (1994) Eur. J. Biochem. 219, 179–186.
- [7] Van Laere, A. (1989) FEMS Microbiol. Rev. 63, 201–210.
- [8] Strom, A.R. and Kaasen, I. (1993) Mol. Microbiol. 8, 205–210.
- [9] Crowe, J.H., Crowe, L.M. and Chapman, D. (1984) Science 223, 701–703.
- [10] Hottiger, T., De Virgilio, C., Hall, M.N., Boller, T. and Wiemken, A. (1994) Eur. J. Biochem. 219, 187–193.
- [11] Winkler, K., Kienle, I., Burgert, M., Wagner, J.C. and Holzer, H. (1991) FEBS Lett. 291, 269–272.
- [12] Nwaka, S., Kopp, M., Burgert, M., Deuchler, I., Kienle, I. and Holzer, H. (1994) FEBS Lett. 344, 225–228.
- [13] González, M.I., Stucka, R., Blázquez, M.A., Feldmann, H. and Gancedo, C. (1992) Yeast 8, 183–192.
- [14] Bell, W., Klaasen, P., Ohnacker, M., Boller, T., Herweijer, M., Schoppink, P., Van der Zee, P. and Wiemken, A. (1992) Eur. J. Biochem. 209, 951–959.
- [15] Vuorio, E., Kalkkinen, N. and Londesborough, J. (1993) Eur. J. Biochem. 216, 849–861.
- [16] Argüelles, J.C., Mbonyi, K., Van Aelst, L., Vanhalewyn, M., Jans, A. and Thevelein, J.M. (1990) Arch. Microbiol. 154, 199–205.
- [17] Argüelles, J.C., Carrillo, M.D., Vicente, J., Garcia-Carmona, F. and Gacto, M. (1993) Curr. Genet. 23, 382–387.
- [18] Lowry, O., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
- [19] Van Aelst, L., Hohmann, S., Bulaya, B., de Koning, W., Sierkstra, L., Neves, M.J., Luyten, K., Alijo, R., Ramos, J., Cocceti, P., Martegani, E., Magalhaes-Rocha, M., Lopes-Brandao, R., Van Dijk, P., Vanhalewyn, M., Durnez, P., Jans, A. and Thevelein, J.M. (1993) Mol. Microbiol. 8, 927–943.
- [20] Hottiger, T., De Virgilio, C., Bell, W., Boller, T. and Wiemken, A. (1992) Eur. J. Biochem. 210, 125–132.
- [21] Paschoalin, V.M., Silva, J.T. and Panek, A.D. (1989) Curr. Genet. 16, 81–87.
- [22] François, J., Neves, M.J. and Hers, H.G. (1991) Yeast 7, 575–587.
- [23] Thevelein, J.M. (1984) Microbiol. Rev. 48, 42–59.
- [24] Eleutherio, E.C.A., de Araujo, P.S. and Panek, A.D. (1993) Biochim. Biophys. Acta 1156, 263–266.
- [25] Coote, P.J., Cole, M.B. and Jones, M.V. (1991) J. Gen. Microbiol. 137, 1701–1708.
- [26] Craig, E.A., Gambill, B.D. and Nelson, R.J. (1993) Microbiol. Rev. 57, 402–414.
- [27] Barnes, C.A., Johnston, G.C. and Singer, R.A. (1990) J. Bacteriol. 172, 4352–4358.
- [28] Sanchez, Y., Taulien, J., Borkovich, K.A. and Lindquist, S. (1992) EMBO J. 11, 2357–2364.
- [29] Piper, P.W. (1993) FEMS Microbiol. Rev. 11, 339–356.