JOURNAL OF BACTERIOLOGY, July 1994, p. 3895–3902
0021-9193/94/$04.00+0

Vol. 176, No. 13

Trehalose-6-P Synthase Is Dispensable for Growth on Glucose but Not for Spore Germination in *Schizosaccharomyces pombe*

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Received 15 February 1994/Accepted 19 April 1994

Trehalose-6-P inhibits hexokinases in *Saccharomyces cerevisiae* (M. A. Blázquez, R. Lagunas, C. Gancedo, and J. M. Gancedo, FEBS Lett. 329:51–54, 1993), and disruption of the *TPS1* gene (formerly named *CIF1* or *FDP1*) encoding trehalose-6-P synthase prevents growth in glucose. We have found that the hexokinase from *Schizosaccharomyces pombe* is not inhibited by trehalose-6-P even at a concentration of 3 mM. The highest internal concentration of trehalose-6-P that we measured in *S. pombe* was 0.75 mM after heat shock. We have isolated from *S. pombe* the *tps1*$^+$ gene, which is homologous to the *Saccharomyces cerevisiae TPS1* gene. The DNA sequence from *tps1*$^+$ predicts a protein of 479 amino acids with 65% identity with the protein of *S. cerevisiae*. The *tps1*$^+$ gene expressed from its own promoter could complement the lack of trehalose-6-P synthase in *S. cerevisiae tps1* mutants. The *TPS1* gene from *S. cerevisiae* could also restore trehalose synthesis in *S. pombe tps1* mutants. A chromosomal disruption of the *tps1*$^+$ gene in *S. pombe* did not have a noticeable effect on growth in glucose, in contrast with the disruption of *TPS1* in *S. cerevisiae*. However, the disruption prevented germination of spores carrying it. The level of an RNA hybridizing with an internal probe of the *tps1*$^+$ gene reached a maximum after 20 min of heat shock treatment. The results presented support the idea that trehalose-6-P plays a role in the control of glycolysis in *S. cerevisiae* but not in *S. pombe* and show that the trehalose pathway has different roles in the two yeast species.

Glycolysis is the backbone of carbohydrate metabolism in yeast cells. Control of this pathway in *Saccharomyces cerevisiae* has been mainly attributed to phosphofructokinase and pyruvate kinase, the enzymes catalyzing two of the three physiologically irreversible steps of glycolysis (for a review, see reference 17). However, data in the literature indicate that control mechanisms should exist at the level of sugar transport, sugar phosphorylation, or both. Mammalian hexokinases are inhibited by glucose-6-P (10), but hexokinases from *S. cerevisiae* are not inhibited by this glycolytic intermediate. Blázquez et al. (5) found that trehalose-6-P inhibits the hexokinases of *S. cerevisiae* and ascribed an important role to this inhibition in the control of yeast glycolysis. Their idea was based on the phenotype exhibited by mutants from *S. cerevisiae* impaired on trehalose-6-P synthesis as a result of a mutation in the *TPS1* gene encoding trehalose-6-P synthase. These *tps1* mutants (originally named *fdp1* [45] and *cif1* [35]) do not grow on glucose (19, 35, 45). Addition of glucose to *tps1* mutants does not elicit an increase of cyclic AMP concentration but produces an immediate depletion of ATP (19) and an accumulation of several glycolytic intermediates (19, 35, 45). These results suggested that the product of *TPS1* or a metabolite produced by it in some way regulated the rate of the initial steps of glycolysis (19). The inhibition of hexokinase by trehalose-6-P reported by Blázquez et al. (5) provided experimental support for this idea. If trehalose-6-P is so critical for the control of glycolysis, it could be predicted that the phenotype of *tps1* mutants from a yeast strain whose hexokinase were insensitive to trehalose-6-P would be different from that observed in the corresponding *S. cerevisiae* mutants. Although trehalose-6-P inhibits hexokinases from different origins (5), we found that the enzyme from the fission yeast *Schizosaccharomyces pombe* is not inhibited by trehalose-6-P. We therefore isolated a homolog of the *S. cerevisiae TPS1* gene in *S. pombe* and produced a chromosomal disruption of it. We show in this report that disruption of this gene in *S. pombe* does not affect growth in glucose but prevents germination of spores carrying the disruption.

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MATERIALS AND METHODS

Yeast strains and growth conditions. *S. pombe* PB003 (*h*$^+$ *ade6-M216 leu1-32 ura4-D18*) and its isogenic strain PB004 (*h*$^-$ *ade6-M210 leu1-32 ura4-D18*) were used in this work. Strains PBU13 (*h*$^+$ *ade6-M216 leu1-32 ura4-D18 tps1*::*ura4*$^+$) and PBL17 (*h*$^+$ *ade6 leu1-32 ura4-D18 tps1*::*LEU2*) were constructed as part of this work (see below). Diploid PB341 was obtained from a cross between PB003 and PB004. PB342 is identical to strain PB341 but carries in one chromosomal copy an interruption of *tps1*$^+$ (see below). The *S. cerevisiae* strains used were W303-1A (*MAT***a** *ade2-1 his3-11,15 ura3-1 leu2-3,112 trp1-1*) (44), CJM221 (*MAT***a** *ade2-1 his3-11,15 ura3-1 leu2-3,112 trp1-1 tps1*::*HIS3*) (4), and CJM019 (*MAT*α *hxk1 HXK2 glk1*). *S. pombe* was grown either in YES medium (0.5% yeast extract supplemented with the appropriate auxotrophic requirements with 3% glucose or gluconate as the carbon source) or in EMM medium as described by Moreno et al. (34). *S. cerevisiae* was grown at 30°C with 2% glucose or galactose as the carbon source either on rich medium (1% yeast extract, 1% peptone) or on minimal medium (yeast nitrogen base and the appropriate auxotrophic requirements). Mating, sporulation, and tetrad analysis of *S. pombe* were performed as described by Moreno et al. (34).

Bacterial strains and plasmids. *Escherichia coli* TG1 and DH5α were used for transformations and preparation of plasmid DNAs. *E. coli* JM103 was used for M13 propagation (33).

Plasmids YEp351 and YEp352 (21) were used for subcloning. Plasmid pMR8 (see Fig. 2a) was obtained from a clone of a genomic library of *S. pombe* by hybridization with an adequate DNA probe (see below). To construct episomal plasmid pMR82 carrying the *tps1*$^+$ from *S. pombe*, the 4.8-kb *Bgl*II-*Pvu*II fragment from pMR8 was inserted into YEp351 digested with *Bam*HI and *Sma*I. The same fragment was inserted into pRS315 (43) that had been digested with *Bam*HI and *Sma*I to produce the centromeric plasmid pMR85. An interruption of *tps1*$^+$ with *LEU2* was constructed as follows. A 4.8-kb *Xba*I-*Kpn*I fragment from plasmid pMR82 was inserted into pIB18 (pUC18 with the polylinker of pRS316) (43) digested with *Xba*I and *Kpn*I to produce plasmid pMR821. A 4.1-kb *Pst*I fragment from YEp13 (6) containing the *LEU2* gene was inserted into plasmid pMR821 digested with *Pst*I to give plasmid pMR826. Plasmid pMR828 with an interruption of *tps1*$^+$ with *ura4*$^+$ was obtained by digestion of plasmid pMR821 with *Hin*dIII, elimination of the 2.3-kb fragment, and insertion of a 1.8-kb *Hin*dIII fragment from pREP4 (31) containing the *ura4*$^+$ gene. Plasmid pMR86 carries the *tps1*$^+$ gene in an episomal vector and was constructed by digesting pREP4 with *Bam*HI and *Sma*I and inserting the 4.6-kb *Bgl*II-*Pvu*II fragment from plasmid pMR8. pMB14 is an episomal plasmid carrying gene *TPS1* from *S. cerevisiae* (19).

DNA manipulations. Recombinant DNA manipulations were done by standard techniques (41). DNA probes were labelled as described by Feinberg and Vogelstein (16). Total yeast RNA was extracted as described by Elder et al. (15) and fractionated by electrophoresis on a 1.3% formaldehyde agarose gel. Nucleic acids were transferred to nylon membranes as instructed by the manufacturer. A genomic library derived from *S. pombe* in vector YEp13 (40) was kindly provided by S. Moreno (Salamanca, Spain).

Genomic DNA from *S. pombe* was obtained as described by Hoffman and Winston (23). Transformation of *S. pombe* was done as described by Moreno et al. (34). *S. cerevisiae* was transformed as described by Ito et al. (25). Interruption of genomic *tps1*$^+$ was performed either with the 9.6-kb *Pvu*II-*Pvu*II fragment from plasmid pMR826 or with the 4.5-kb *Xba*I-*Kpn*I fragment from plasmid pMR828.

Isolation of the *tps1*$^+$ gene from *S. pombe*. On the basis of the sequence similarity of the *TPS1* gene from *S. cerevisiae* with stretches of an open reading frame of unknown function in *Methanobacterium thermoautotrophicum* (36), the following degenerated oligonucleotides coding for two common regions were prepared: 5′ GAAGCTTGA(G/A)CA(C/T)CC(C/G/T/A)GA(G/A)T(G/T)(C/G/T)C(G/A)(C/G/T/A)GG(C/G/T/A)AA(G/A)GT 3′ and 5′ CGGATCCAC(C/G/T/A)A(G/T/A)(G/A)TTCAT(C/G/T/A)CC(G/A)TC 3′. They were used as primers in a PCR with genomic DNA from *S. pombe* in the following conditions: 5 μg of genomic DNA, 100 μM each primer, 250 μM each deoxynucleotide, and 2.5 U of *Taq* polymerase (Boehringer Mannheim) in a final volume of 0.1 ml. The samples were run through five cycles of the following program: 2 min at 94°C to denature, 1 min at 48°C to anneal, and 1 min at 72°C for synthesis. After this, 30 cycles of 1 min of denaturation at 91°C, 1 min of annealing at 52°C, and 1 min of elongation at 72°C were performed in a TR2 Hybaid thermocycler. Amplification products were separated by agarose electrophoresis and ligated into M13 digested with *Hin*dIII and *Bam*HI. A 250-bp DNA fragment with 70% sequence identity with a region of the *S. cerevisiae TPS1* gene was isolated. Using this fragment as a probe, we screened an *S. pombe* genomic bank by colony filter hybridization as described by Grunstein and Hogness (20).

Sequencing was performed by the dideoxy-chain termination method (42). Sequences were derived from both strands. Computer analyses were carried out on a Digital DECStation 5000/200 workstation, using the University of Wisconsin Genetics Computer Group software (11).

Sugar phosphorylation and trehalose-6-P synthase assay. Extracts were obtained by disruption of cells with glass beads as described by Blázquez et al. (5). Phosphorylation of fructose was measured as described by Gancedo et al. (18). Trehalose-6-P synthase activity was assayed as described by Argüelles et al. (1), with the addition of 5 mM fructose-6-P to the assay mixture as specified by Londesborough and Vuorio (28). Protein was assayed as described by Lowry et al. (29), with bovine serum albumin used as a standard.

Trehalose and trehalose-6-P determination. Trehalose was extracted from 50-mg yeast samples in 2 ml of boiling water as described by Kienle et al. (27) and assayed with commercial trehalase (Sigma, St. Louis, Mo.) by the following procedure. Fifty-microliter aliquots of the extracts were incubated for 1 h at 30°C in a total volume of 200 μl with 100 μl 25 mM sodium acetate (pH 5.5) and 0.1 U of trehalase. Glucose was then assayed with hexokinase as described by Bergmeyer (3).

Trehalose-6-P was extracted and determined as described by Blázquez et al. (5).

Heat shock. Exponentially growing cells (about 5 mg/ml) were shifted for different time periods to 42°C. After the appropriate times, 50 mg of cells was poured over 30 ml of ice-cold water and immediately harvested by centrifugation. The cell pellets were frozen and maintained at −70°C until they were used.

Nucleotide sequence accession number. The sequence of the *tps1*$^+$ gene has been entered in the EMBL data bank under accession number Z29971.

RESULTS

Effect of trehalose-6-P on *S. pombe* hexokinase. Trehalose-6-P inhibits the hexokinases from *S. cerevisiae* and other organisms competitively with the sugar substrate (5). However, we did not find inhibition of the enzyme from *S. pombe* even when 3 mM trehalose-6-P was used (Fig. 1a). For comparison, the inhibition of hexokinase II of *S. cerevisiae* by trehalose-6-P is shown in Fig. 1b. The internal concentration of trehalose-6-P was determined in *S. pombe* cells harvested at the exponential and stationary phases of growth and after heat shock (Table 1), and the highest value measured was 0.75 mM. Since trehalose-6-P plays an important role in the control of glycolysis in *S. cerevisiae* (5), we examined the effects of the disruption of the gene encoding trehalose-6-P synthase in *S. pombe*.

Isolation and sequence of the *tps1*$^+$ gene from *S. pombe*. We isolated from *S. pombe* by PCR (see Materials and Methods) a DNA fragment of 250 bp that gave a translated sequence with 78% identity with a region of the trehalose-6-P synthase from *S. cerevisiae*. Using this fragment as a probe, we screened an *S. pombe* genomic bank by colony filter hybridization and found a positive clone that carried pMR8 (Fig. 2a). A strain of *S. cerevisiae* unable to grow on glucose because of a disruption of the *TPS1* gene was transformed with pMR8, and transformants were selected on galactose; all transformants grew on glucose. Subcloning of pMR8 yielded the centromeric plasmid pMR85 and the episomal plasmid pMR82 (see Materials and Methods). Both complemented a *tps1* mutation in *S. cerevisiae* even though the *S. pombe* gene was expressed from its own promoter. The generation times in rich glucose medium were 130 min for the strain transformed with the episomal plasmid and 225 min for the strain transformed with the centromeric plasmid, compared with 100 min for the wild type. Trehalose-

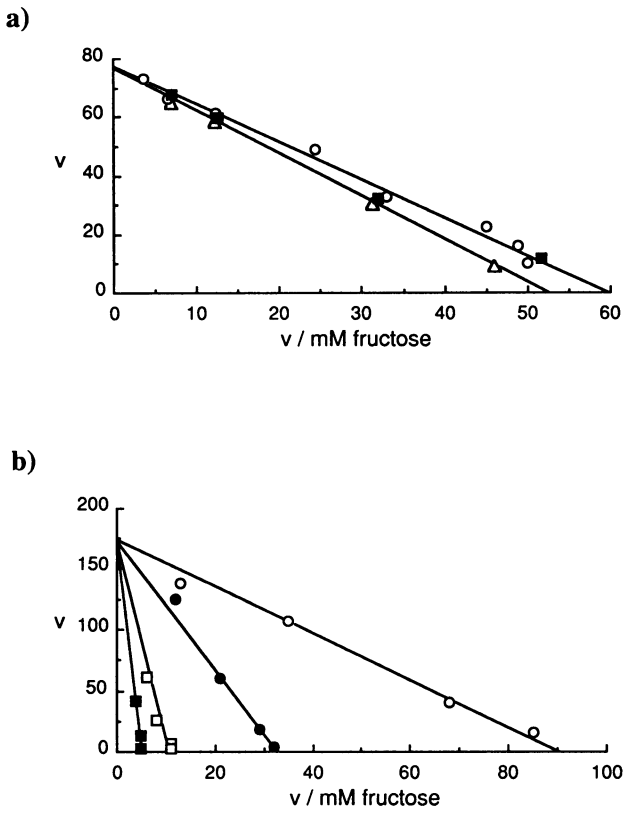

FIG. 1. Effects of trehalose-6-P on the hexokinases from *S. pombe* and *S. cerevisiae*. Phosphorylation of fructose by crude extracts was assayed as described in Materials and Methods. (a) *S. pombe* PB003; (b) *S. cerevisiae* CJM019 expressing only hexokinase II. Concentrations of added trehalose-6-P: ○, none; ●, 0.1 mM; □, 0.4 mM; ■, 1 mM; △, 3 mM. V is expressed in nanomoles per minute per milligram of protein.

6-P synthase activity could be measured in the transformants, although its level was lower than in the wild type (Table 1). Also, the ability to synthesize trehalose and to increase its level in stationary phase and in response to heat shock was recovered in the transformed strains, although the values reached were lower than those measured in the wild type (Table 1). We conclude, therefore, that we have isolated a gene encoding trehalose-6-P synthase in *S. pombe* that is homologous to the *S. cerevisiae TPS1* gene; this gene has been termed *tps1*$^+$.

The sequence of the DNA isolated from *S. pombe* comprising the *tps1*$^+$ gene is shown in Fig. 3. A putative TATA element could be located 202 bp upstream of the translation initiation codon. The *tps1*$^+$ gene encodes a protein of 479 amino acids, of which 65% are identical and 71% are similar with those of the trehalose-6-P synthase of *S. cerevisiae*. The amino acid sequence showed also great similarity with that of an open reading frame of unknown function from *M. thermoautotrophicum* (36), which likely is a trehalose-6-P synthase, with the trehalose-6-P synthase from *E. coli* (EMBL accession number X69160), and with a sequence from *Kluyveromyces lactis* recently reported and termed *GGS1* (30) (Fig. 4). Significant similarities were also found with the sequences of three other *S. cerevisiae* proteins: trehalose-6-P phosphatase, the product of the gene *TPS2* (12); the product of *TSL1*, a protein possibly implicated in trehalose synthesis (47); and a sequence of unknown function (EMBL accession number M88172).

Effects of the disruption of the *tps1*$^+$ gene. We disrupted *tps1*$^+$ by insertion of *LEU2* (Fig. 2b). Since disruption of *TPS1* results in lack of growth in glucose in *S. cerevisiae* and *S. pombe* does not grow on most alternative carbon sources, we performed the disruption in a diploid strain and grew the disruptants in a glucose medium. Southern blot analysis showed that only one chromosomal copy was disrupted in the diploid (Fig. 2b, lane 1). Germination on glucose or gluconate (22) of spores derived from this diploid strain gave a $2^+:2^-$ segregation for growth, with none of the germinated spores carrying the *LEU2* marker. However, if such a diploid was first transformed with a plasmid carrying *tps1*$^+$, all spores germinated on glucose, and loss of the plasmid by growth in nonselective medium showed that all four spores from each tetrad grew on glucose. In each tetrad, two spores were wild type and two carried the disruption (Fig. 2b, lanes 2 to 5). These results show that *tps1*$^+$ is not an essential gene for growth but is implicated in spore germination in *S. pombe*. In agreement with these results, we found that interruption of the *tps1*$^+$ gene in the

TABLE 1. Trehalose-6-P synthase activity and concentrations of trehalose-6-P and trehalose in *S. cerevisiae* and *S. pombe* strains bearing interruptions of the *TPS1* and *tps1*$^+$ genes[a]

Strain	Relevant genotype	T6P synthase activity (mU/mg of protein)	Concn					
			Exponential		Stationary		Heat shock	
			T6P (mM)	T (nmol/mg [wet wt] of cells)	T6P (mM)	T (nmol/mg [wet wt] of cells)	T6P (mM)	T (nmol/mg [wet wt] of cells)
S. cerevisiae								
W303-1A	*TPS1*	85	0.18	2.0	0.15	18.0	0.20	41.5
CJM221	*tps1::HIS3*	<2	<0.04	0.1	<0.04	0.4	0.04	0.9
CJM221/pMR85	*tps1::HIS3/tps1*$^+$	7	0.16	0.9	0.18	4.2	3.0	10.8
CJM221/pMR82	*tps1::HIS3/tps1*$^+$	13	0.17	0.9	0.19	5.8	3.1	10.7
S. pombe								
PB003	*tps1*$^+$	59	0.43	2.0	0.20	60.6	0.75	77.1
PBU-13	*tps1::ura4*$^+$	<2	0.05	0.6	<0.04	0.5	0.05	1.1
PBL-17	*tps1::LEU2*	<2	ND	0.2	ND	0.7	ND	0.9
PBL-17/pMB14	*tps1::LEU2/TPS1*	7	ND	6.8	ND	29.7	ND	11.1

[a] The yeast strains were grown in rich glucose medium except PBL-17 and PBL-17/pMB14, which were grown in minimal glucose medium, and CJM221, which was grown in galactose, and harvested at the indicated phases of growth. Heat shock was performed on exponentially growing cells as described in Materials and Methods. Trehalose-6-P (T6P) synthase activity was assayed in samples harvested at the stationary phase of growth as indicated in Materials and Methods. T6P and trehalose (T) were extracted and assayed as described in Materials and Methods. Plasmids pMR85 and pMR82 are, respectively, centromeric and multicopy plasmids carrying the *tps1*$^+$ gene from *S. pombe*. Plasmid pMB14 is a multicopy plasmid carrying the *TPS1* gene from *S. cerevisiae* (see Materials and Methods). ND, not done.

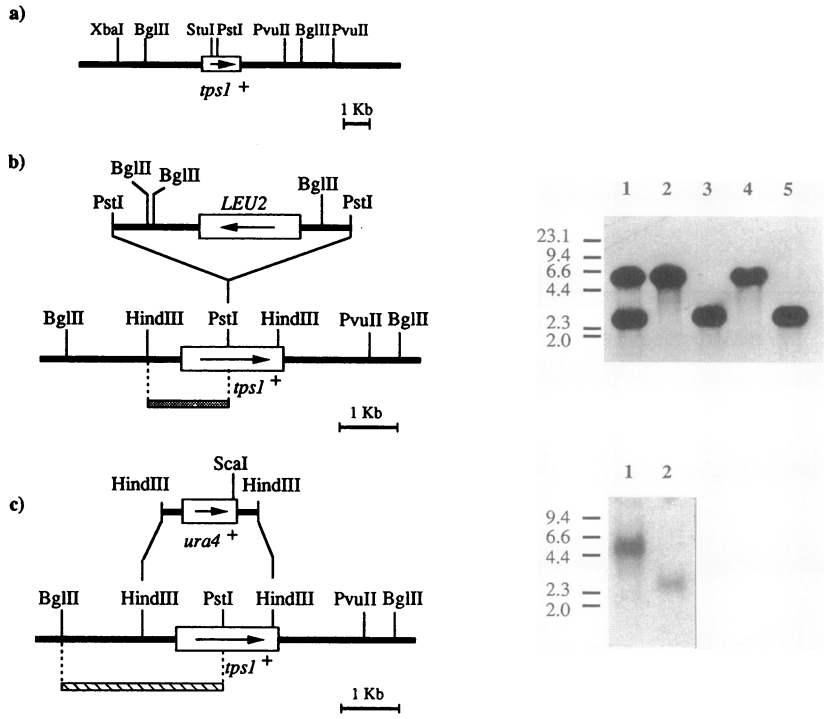

FIG. 2. Structure of the *tps1*$^+$ region from *S. pombe* and Southern blot analysis of *tps1* disruptants. (a) Restriction map of the insert of plasmid pMR8; the region corresponding to the *tps1*$^+$ gene is enclosed in a box. (b) Interruption of *tps1*$^+$ with *S. cerevisiae LEU2* (see Materials and Methods for details of construction). Southern analysis of the interruption is shown at the right. Lane 1, diploid strain PB342 (*tps1*$^+$/*tps1*::*LEU2*); lanes 2 to 5, spores of a tetrad derived from diploid PB342 (see text for details). DNA was digested with *Hin*dIII and probed with the fragment of DNA corresponding to the region indicated by the dotted box. (c) interruption of *tps1*$^+$ with *ura4*$^+$ (details of the construction are given in Materials and Methods). Southern analysis of the interruption is shown at the right. Lane 1, strain PB003 (*tps1*$^+$); lane 2, strain PBU-13 (*tps1*::*ura4*$^+$). DNA was digested with *Bgl*II plus *Sca*I and probed with DNA corresponding to the region indicated by the hatched box. Size of the bands (in kilobases) are indicated at the left of the gels. Horizontal arrows indicate direction of transcription.

haploid *S. pombe* PB003 (Fig. 2c) did not affect growth in glucose medium. The generation times in rich glucose medium of both wild-type and *tps1*$^+$-disrupted strains were 150 min.

Disruption of *tps1*$^+$ had no effect on the ability of cells to conjugate or on the ability to sporulate even when the disruption was present in homozygous condition.

Trehalose synthesis in disrupted *tps1* mutants and expression of *tps1*$^+$ during heat shock. Trehalose accumulates in *S. cerevisiae* as well as in *S. pombe* in stationary-phase cells and after heat shock (38, 46, 48). Disruption of *tps1*$^+$ impaired trehalose accumulation both at the onset of stationary phase and during heat shock (Table 1), showing again that *tps1*$^+$ is involved in trehalose synthesis in *S. pombe*. Moreover transformation of *S. pombe tps1* mutants with a plasmid containing *TPS1* from *S. cerevisiae* expressed from its own promoter restored the ability to synthesize trehalose (Table 1). During heat shock treatment of a culture of *S. pombe*, trehalose accumulated almost linearly and reached a plateau between 30 and 60 min after initiation of the treatment (Fig. 5a). Northern blot analysis (Fig. 5b) showed that a 1.8-kb RNA species hybridizing to the *tps1*$^+$ probe increased during the first minutes of treatment, reaching a peak at about 20 min and decreasing again to initial levels after 60 min.

DISCUSSION

We have isolated from *S. pombe* the *tps1*$^+$ gene, whose functionality is necessary for the germination of spores. This gene encodes trehalose-6-P synthase, as evidenced by the following facts; (i) the gene has 65% sequence identity at the amino acid level and 60% identity at the nucleotide level with the *TPS1* gene from *S. cerevisiae* (2, 19, 47); (ii) an *S. pombe tps1* mutant is defective in trehalose synthesis; (iii) the *S. pombe* gene complemented the growth defect of an *S. cerevisiae tps1* mutant and increased its trehalose content; (iv) the *S. cerevisiae TPS1* gene complemented the lack of trehalose synthesis in an *S. pombe tps1* mutant; and (v) trehalose-6-P synthase activity was partially restored to a *S. cerevisiae tps1* mutant by the *S. pombe* gene.

The phenotype produced by disruption of the *tps1*$^+$ gene in *S. pombe* is quite different from that produced by disruption of the *TPS1* gene in *S. cerevisiae* or the homologous *GGS1* gene in *K. lactis*. While a disruption of these genes causes inability to grow on glucose in *S. cerevisiae* (19) and in *K. lactis* (30), the disruption of *tps1*$^+$ in *S. pombe* does not influence its capacity to grow on this sugar. This difference may be explained by the different behaviors of the hexokinases of *S. pombe* and the other yeasts toward trehalose-6-P. *S. pombe* hexokinase is not inhibited either by glucose-6-P (3a) or, as shown here, by trehalose-6-P, while it inhibits the hexokinases from *S. cerevisiae* and *K. lactis* (5). Lack of trehalose-6-P causes in *S. cerevisiae* (5), and likely in *K. lactis*, an uncontrolled flux through hexokinase that cannot be matched by the energy-producing glycolytic reactions, thus resulting in loss of ATP and absence of growth. Apparently, *S. pombe* controls the initial steps of glycolysis in a different way.

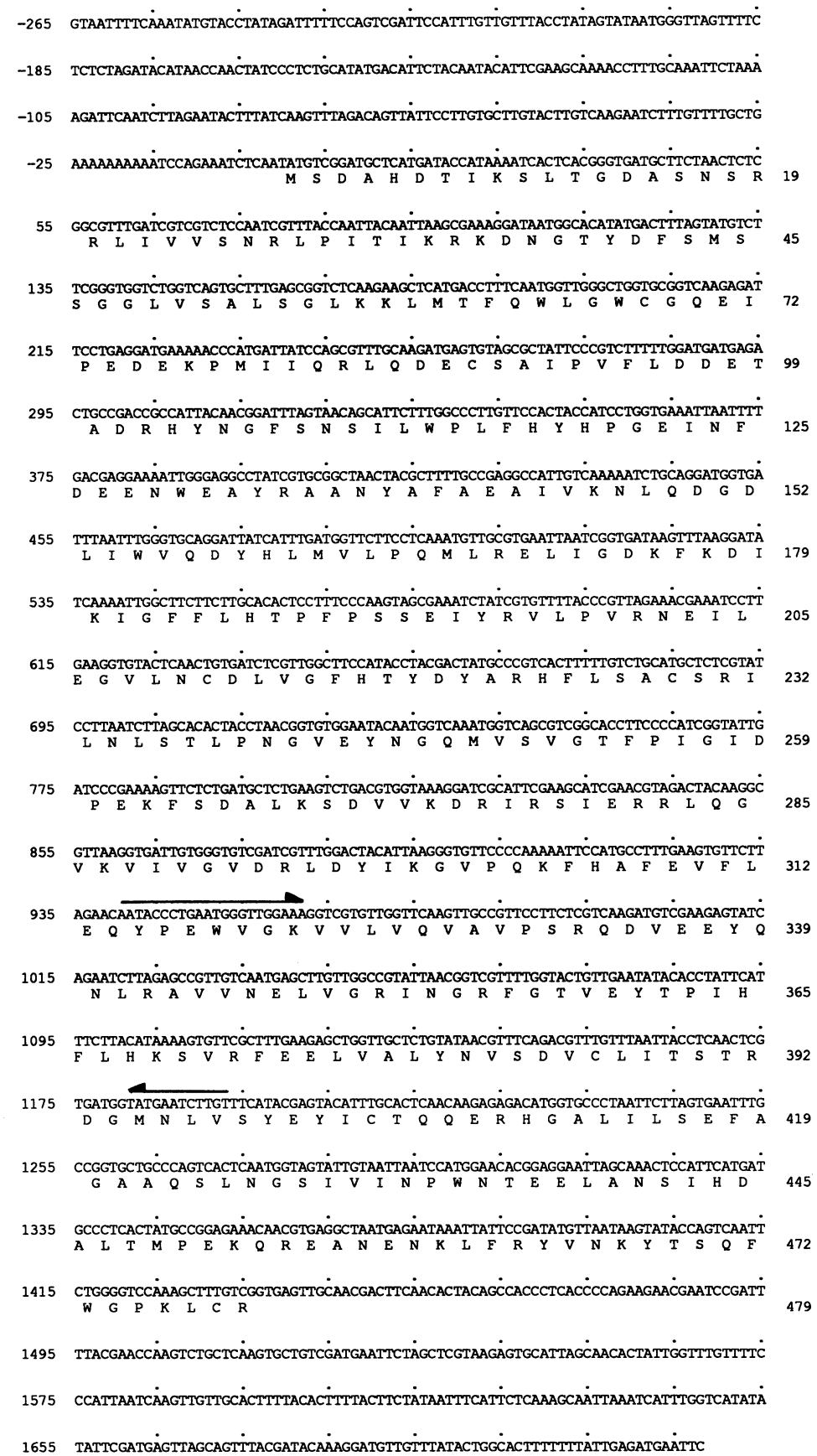

FIG. 3. Nucleotide sequence of the *S. pombe tps1*$^+$ gene and deduced amino acid sequence. Arrows indicate positions of the primers used for PCR. The 250-bp region isolated and used in the screening lies between the arrows.

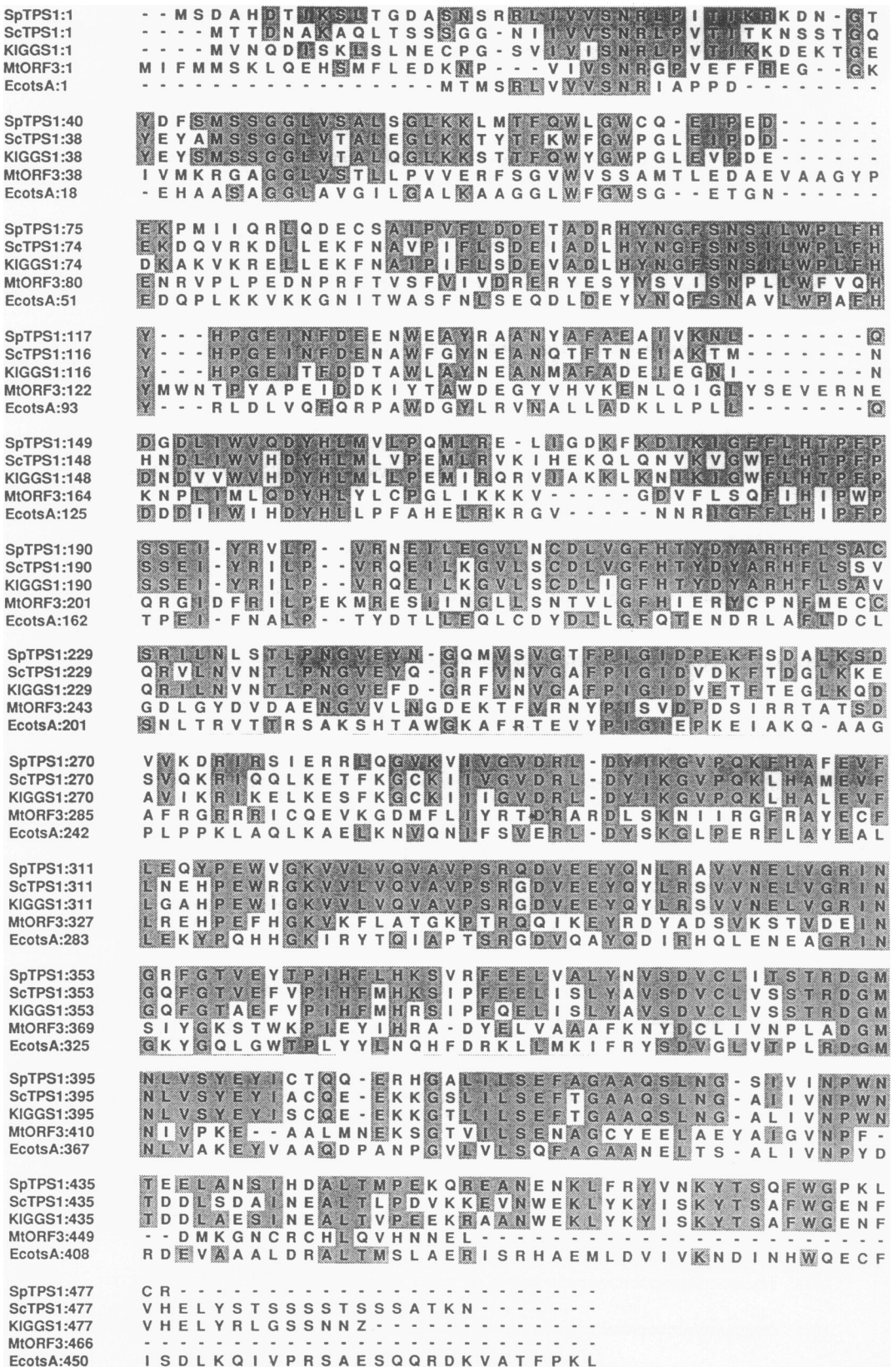

FIG. 4. Comparison of the deduced amino acid sequences of the proteins encoded by, from top to bottom, *S. pombe tps1*$^+$, *S. cerevisiae TPS1*, *K. lactis GGS1*, *M. thermoautotrophicum* open reading frame 3, and *E. coli otsA*. Amino acids identical to the sequence encoded by *tps1*$^+$ are shaded.

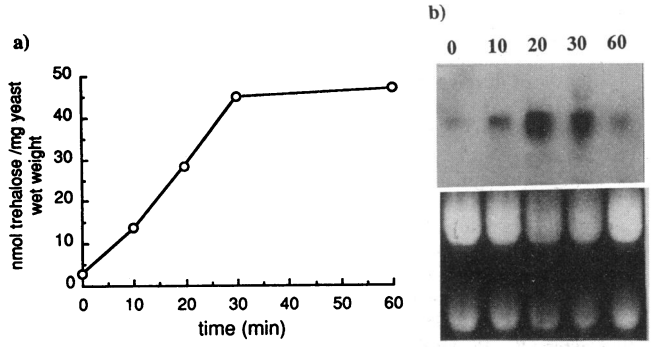

FIG. 5. Expression of *tps1*$^+$ from *S. pombe* during heat shock. The cells were grown in glucose medium and heat shocked for the times indicated. RNA was extracted and trehalose was assayed as described in Materials and Methods. (a) Trehalose content; (b) Northern (RNA) blot analysis of the different samples. Fifty micrograms of RNA was applied to each lane. The probe used was the 250-bp fragment isolated in the initial PCR (see Fig. 4). The rRNAs corresponding to each lane are shown at the bottom.

Trehalose is often found in fungal spores, and in *S. cerevisiae* it has been thought to serve as an energy source during germination. However, Donini et al. (14) showed that germination was possible in this yeast in the absence of trehalose mobilization, and Campbell-Burk and Shulman (7) concluded that the amount of energy produced during trehalose breakdown in *S. cerevisiae* was small compared with that derived from the sugar in the medium. Consistent with this finding, disruption of the two copies of the *TPS1* gene in an *S. cerevisiae* diploid has no effect either on sporulation (8) or on germination (4a). In *S. pombe*, disruption of *tps1*$^+$ prevents the germination of spores. Synthesis of trehalose in a sporulating culture of *S. pombe* is observed only after the appearance of asci (24), while in *S. cerevisiae*, trehalose appears well before the asci are observable (26, 39). It may be thought that each spore synthesizes its own trehalose; accordingly, spores with an interruption of *tps1*$^+$ would be unable to synthesize it. This idea would provide a plausible explanation for the effect of the disruption of *tps1*$^+$ on germination if trehalose were necessary for maintenance of spore viability or for germination itself. It is worth noting that *S. pombe* spores have a higher trehalose content than those of *S. cerevisiae*, 2.3 versus 0.4 $\mu g/10^6$ cells (46). However, the mode of action of *tps1*$^+$ in germination cannot be yet accurately defined, and it may act in another, unknown way.

The enzymes synthesizing trehalose in *S. cerevisiae* seem to form a complex of at least three proteins (12, 47): trehalose-6-P synthase (TPS1), trehalose-6-P phosphatase (TPS2), and a third protein, TSL1, that could play a regulatory role. The complementation of the *tps1* mutation of *S. cerevisiae* by the *S. pombe tps1*$^+$ gene implies that the protein encoded by this gene is able either to interact productively with the other components of the *S. cerevisiae* complex or to act by itself as trehalose-6-P synthase. The facts that the product of the gene *TPS1* from *S. cerevisiae* can complement a defective *otsA* gene in *E. coli* (32) and that *S. cerevisiae* mutants lacking trehalose-6-P phosphatase activity are able to synthesize trehalose-6-P (12) suggest that the product of *tps1*$^+$ could be active without being part of the complex. However, the high levels of trehalose-6-P in *S. cerevisiae* CJM221/pMR85 and CJM221/pMR82 after heat shock could suggest some defect in the coupling between the reactions catalyzed by the products of *tps1*$^+$ and *TPS2*. In this regard, it is noteworthy that the *TPS1* gene from *S. cerevisiae* has a C terminus rich in serine and threonine residues which is missing in the *S. pombe* gene (Fig. 4). The promoters of *tps1*$^+$ and *TPS1* were functional in both yeast species, but the levels of trehalose were always lower when the genes were expressed from the heterologous promoter. Also, the activity of trehalose-6-P synthase in vitro was lower in this case. This could be due to in vitro instability of the complex between trehalose-6-P synthase and the other proteins involved in trehalose synthesis. It has been reported that the activity of trehalose-6-P phosphatase is not measurable in *S. cerevisiae tps1* mutants, probably as a result of the absence of complex formation (47). Differences between in vivo and in vitro activities due to protein-protein interactions are also well documented in yeast strains (9).

Trehalose increases in stationary phase and upon heat shock in yeasts (38, 46, 48). We have observed that heat shock caused an increase in an RNA that hybridized with a probe specific for the *tps1*$^+$ gene. This RNA reached its peak level after 20 min of heat shock and decreased thereafter, while trehalose continued to increase. A similar behavior has been described for *S. cerevisiae TPS2* gene expression (12). These results suggest that trehalose accumulation is a consequence of the synthesis of trehalose-6-P synthase after heat shock. In fact, the increase in trehalose after heat shock in *S. cerevisiae* is blocked by cycloheximide (37). However, De Virgilio et al. (13) reported that trehalose synthesis in *S. pombe* could proceed after heat shock even in the absence of protein synthesis and concluded that the enzymes of trehalose metabolism were regulated by some kind of posttranslational modification. Our results indicate that the picture could be more complex and that several factors could contribute to the increase in trehalose content after heat shock.

The results presented show that although the genes encoding trehalose-6-P synthase are very similar in the different yeast species studied, there are important differences in the physiological utilization of the trehalose-synthesizing pathway.

ACKNOWLEDGMENTS

We thank S. Moreno (Instituto de Microbiología Bioquímica, CSIC, Salamanca, Spain) for the gift of plasmids and the library of *S. pombe*, Christa Schwarzlose for technical assistance, Juana M. Gancedo for discussions, and T. G. Cooper, M. J. Mazón, and F. J. Gamo for critical reading of the manuscript.

M. A. Blázquez was supported by a fellowship of the Comunidad Autónoma de Madrid. In the initial steps of this work, M.A.B. benefited from a FEBS Summer Fellowship. This work was supported by grants PB91-0056 from the Spanish CICYT and Fonds der Chemischen Industrie and Deutsche Forschungsgemeinschaft (SFB 190) München.

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