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# The role of the trehalose transporter during germination

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**Abstract**

Previous studies on the resistance of yeast cells to dehydration pointed towards the protective role of trehalose and the importance of the specific trehalose transporter in guaranteeing survival. The present report demonstrates that the trehalose transporter is essential during the germination process in order to translocate trehalose from the cytosol to the external environment. Diploids that lack the trehalose transporter germinate poorly and do not form 4 spore tetrads although they accumulate trehalose and show trehalase activity. Furthermore, addition of exogenous trehalose to the germination medium enhances germination and normal segregation. The ability to transport trehalose is dominant and seems to be related to a single gene. © 1997 Elsevier Science B.V.

*Keywords:* Trehalose transporter; Germination; Stress; (*Saccharomyces cerevisiae*)

## 1. Introduction

Transporters mediate two types of transport processes in yeast cells: facilitated diffusion and active transport. In the first case, solutes are transported down a concentration gradient without energy requirement. In contrast, active transport systems accumulate solutes in the cells against a concentration gradient and require energy to do so. The yeast *Saccharomyces cerevisiae* consumes mono- and disaccharides preferentially to any other carbon source (For a review see [1,2]).

In the case of trehalose which is not normally found in nature as a carbon source for microorganisms, a special role has been assigned for the transporter. Trehalose accumulates inside the yeast cell as a response to an environmental stress [3–5]. Studies with unilamellar liposomes indicate that for maximal retention of entrapped solutes, during dehydration, it is necessary to have trehalose on both sides of the membrane [6]. We have demonstrated, in our previous studies, that it was necessary to transport trehalose from the cytosol to the outer side of the membrane to enable the sugar to protect the yeast cell during dehydration. *S. cerevisiae* mutant strains unable to transport trehalose do not survive when dried although they accumulate endogenous trehalose. Furthermore, when mutants defectives in transport are dehydrated in the presence of exogenous trehalose, the cells become more resistant showing increased survival [7]. Recently, it was reported that the maltose transporter is fully reversible and capable of catalyzing influx as well efflux transport [8].

The first evidence that a trehalose transporter exists in yeast cells was presented in 1979 [9]. When it became apparent that trehalose plays a role in stabilizing cellular constituents, the interest in the mecha-

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nism of trehalose transport was renewed. It was shown that glucose both represses and inactivates trehalose transport and that external trehalose induces its synthesis [10]. Trehalose transport in *S. cerevisiae* occurs against a concentration gradient and is characterized as a high affinity proton symporter [11].

Formation of spores is a response to the stress of starvation and accumulation of trehalose under conditions leading to sporulation has been reported by Roth [12] and later on by Ferreira and Panek [13]. It has been pointed out that trehalose is needed to carry out the budding process at a constant time [14]. Should we interpret this fact exclusively as a result of an activation of trehalase [15] or also as a consequence of modifications in the membrane due to the stress of germination which would require protection by trehalose?

In this report, we wish to address the question of the requirement of trehalose transport from the inside to the outside of the cell membrane during germination. We have previously shown that in the absence of accumulated trehalose (in diploids homozygous for a defect in the trehalose synthesizing system) sporulation is poor and no 4-spore tetrads are observed upon germination [16].

## 2. Materials and methods

The yeast strains used in this work are listed in Table 1.

### 2.1. Growth conditions

Cells were routinely grown by shaking at 160 rpm at 28°C in 100 ml medium, containing 2% of carbon source (glucose or trehalose), 1% yeast extract and 2% peptone pH 5.2 or 2% of glucose, 1.3% yeast extract, supplemented with 0.2% $(NH_4)_2SO_4$ and 0.2% $KH_2PO_4$, pH 5.2. The ability of strains to grow on trehalose was tested on minimal medium (2.0% trehalose, 0.67% Yeast Nitrogen Base without aminoacids, 0.01% aminoacid requirements and 2.0% agar).

### 2.2. Genetic analysis

Isolations of diploids, induction of sporulation and dissections were performed as previously described [17,18].

### 2.3. Measurement of [$^{14}$C]trehalose transport

The protocol used was based on the one previously described [19]. 150 μl of a cell suspension (20 mg cell/ml) were added to 150 μl of 100 mM succinate–Tris buffer pH 5.0. After 4 min at room temperature, 17 μl of a 100 mM [$^{14}$C]trehalose solution containing 35 mCi.nmol$^{-1}$ was added. At timed intervals (0.5, 1.5 and 3.0 min), 100 μl aliquots were removed and cells were rapidly filtered on Millipore filters and rinsed with 5 ml of ice-cold distilled water. The filters were placed into scintillation vials containing 5 ml scintillation cocktail for intracellular radioactivity measurements. Controls using previously boiled cells were subtracted from all data.

### 2.4. Trehalose determination

Samples of 12 mg cells (dry weight) were extracted with 0.5 M trichloroacetic acid and determined by the anthrone method, as previously de-

Table 1
Yeast strains employed: genotypes and sources

| Strains | Genotype and phenotype [a] | Source |
|---|---|---|
| 582-6-1 | *MAT a / α*, homothalic, monosporic, TRE | 1 |
| WH92-7A | *MAT a*, *gal4*, tre | 1 |
| 2592-1B-1A | *MAT a*, tre | 1 |
| AP1 | [1403-7A (*a MAL4 trp1 ura3 lys2*, TRE) × D213-1B (*α ade1 trp1 mal*, tre)], TRE | 2 |
| AP3 | [AP1-5C (*a MAL4 trp1 ura3*, TRE) × D213-1B], TRE | 2 |

[a] TRE represents growth on trehalose.
1: Naumov, Moscow University, Russia.
2: Panek, University of Rio de Janeiro, Brazil.

scribed [20]. The presence of trehalose was confirmed using immobilized trehalase [21].

### 2.5. Heat shock

Cells were harvested in stationary phase and immediately exposed to 50.5°C for 8 min. Samples of 50 mg cells (dry weight) were collected by centrifugation and treated as described below.

### 2.6. Trehalase assay

Harvested cells (50 mg dry weight) were washed twice with cold distilled water and cell free extracts prepared as described by Panek et al. [22]. Protein was determined according to Stickland [23] using bovine serum albumin as standard. Neutral and acid trehalase activities were determined according to Dellamora-Ortiz et al. [24] and Mittenbuller and Holzer [25], respectively.

### 2.7. Special chemicals

[$^{14}$C]Trehalose was a kind gift from Dr. Soares de Araujo (Institute of Chemistry, University of São Paulo, Brazil) and was prepared according to Stambuck et al. [19].

## 3. Results and discussion

Two strains of *S. cerevisiae*, isolated from nature, unable to transport trehalose, were investigated in an attempt to further characterize the trehalose transporter as well as its role in the physiology of yeast cells. These strains, possessing a mutation selected by the environment, were collected in fruits from the Botanical Garden, Rio de Janeiro, Brazil. Our previous studies on the role of the transporter during dehydration were carried out with mutants obtained in our laboratory by mutagenesis using ethyl methane sulfonate [7].

### 3.1. Trehalose transport and trehalase activity

Cells were grown on 2% glucose up to stationary phase and transport was measured as described in Section 2 (Fig. 1A).

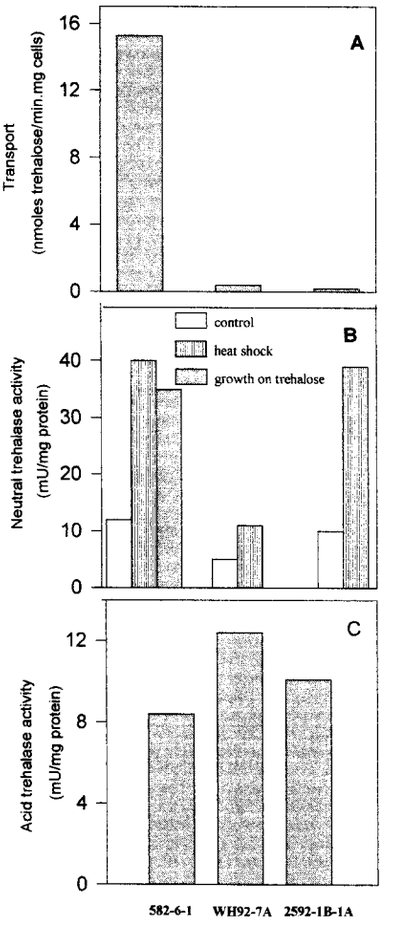


Fig. 1. Trehalose transport (A) and trehalase activities (B, C) were determined as described in Section 2. Cells were grown on glucose and collected in stationary phase. To analyze the effect of heat shock on neutral trehalase activity, cells were treated at 50.5°C/8 min. Neutral trehalase activity was also determined in the control strain (582-6-1) grown on trehalose as carbon source.

The lack of trehalose transport could be due to the absence of trehalase activity. Therefore, cell free extracts from cells grown under the same conditions were prepared and trehalase activity determined as described in Section 2. Both strains unable to transport trehalose showed the capacity of breaking down the sugar in vitro (Fig. 1B). The effect of a heat shock that normally activates neutral trehalase 4- to 8-fold [26] is less effective in the case of the mutant strain WH92-7A. This result could suggest that the mutant might also lack the $Ca^{2+}$/CaM trehalase inhibitor, which is normally removed by heat shock [27], since the levels of trehalase activity before and after heat stress were almost the same.

However, the levels of neutral trehalase activity present in the mutants in stationary phase were very similar to the control strain, indicating that the capacity of transporting trehalose does not depend upon high trehalase activity. Noteworthy is the fact that when the control strain (582-6-1) was grown on 2% trehalose as the carbon source, trehalase activity increased 3-fold (Fig. 1B). This result might suggest that growth on trehalose would induce the transcription, by the substrate, of the *NTH1* gene, which codes for neutral trehalase [28]. Alternatively, it could be interpreted as a phenomenon of derepression in the absence of glucose [29], without the need of induction.

Besides neutral trehalase, *S. cerevisiae* contains an acid trehalase, encoded by the gene *ATH1*, which is located in the vacuole [30]. In contrast to the cytosolic neutral trehalase, the role of the vacuolar acid trehalase is not clear yet. In a recent report only the vacuolar trehalase is indicated as responsible for hydrolysing external trehalose and the authors suggest that the enzyme would gain access to the substrate by endocytosis [31]. Contradicting this result, another recent report states that acid trehalase would be involved in the hydrolysis of endogenous trehalose [32]. Irrespectively of the exact role of the vacuolar isoform, the mutants showed similar levels of acid trehalase activity to the control strain (Fig. 1C), indicating, once more, that the impaired utilization of external trehalose by the mutants was due to the absence of trehalose transport activity.

In conclusion, trehalose transport activity does not seem to be related to high total trehalase activity albeit growth of cells on trehalose as a carbon source will depend upon the capacity of the cell of hydrolysing it.

### 3.2. Genetic analysis of trehalose transport

The diploid strains AP1 and AP3 were constructed from one haploid strain with the capacity and one without the capacity of growing on trehalose (Table 1). In both diploids, we found increased trehalase and trehalose transport activities and normal levels of accumulated trehalose (results not shown).

For genetic analysis of trehalose transport, the diploids AP1 and AP3 were sporulated, dissected and analyzed for their ability to transport trehalose and to grow on trehalose. The results indicated that both trehalose transport and growth on trehalose segregated 2:2 in the eight tetrads analyzed.

In order to learn more about the characteristics of trehalose transport two segregants (AP1-11D and AP1-12A) positive for trehalose transport and growth on trehalose were picked and crossed with two negative mutants (WH92-7A and 2592-1B-1A). All four resulting diploids were capable of growing on trehalose, indicating that trehalose transport is a dominant characteristic.

The data obtained suggest that the trehalose transporter is coded by a single gene albeit a regulator might be involved since growth on maltose and α-methylglucoside increased in activity (Stambuk, unpublished results).

### 3.3. Trehalose transport during germination

For an organism to survive successfully, a tight control between growth and response to stress must be accomplished. Initiation of growth in media lacking essential nutrients is lethal [33]. The interplay between growth control and stress tolerance is not surprising if one considers that the main goal of a stress response is adaptation to and growth under different environmental conditions. STRE-binding factors which are activated by a large spectrum of stresses seem to integrate the effects of different stress signals and trigger an optimal transcriptional

Table 2
Trehalose transport and accumulation

| Strains | Trehalose transport (nmol/min.mg cell) | Accumulated trehalose (mg/g dry weight cells) |
|---|---|---|
| AP1-10C | 0.71 | 40.4 |
| AP1-12C | 0.62 | 28.0 |
| AP1-13A | 7.19 | 42.0 |
| AP1-14A | 0.40 | 35.4 |
| AP1-14B | 1.63 | 36.0 |
| AP1-14C | 0.47 | 24.0 |
| AP3-10B | 0.39 | 37.5 |
| AP3-12A | 0.58 | 38.0 |

The strains employed are segregants obtained from the sporulation of diploids AP1 and AP3. Cells were grown on glucose as carbon source and collected in stationary phase. Trehalose transport and trehalose determination were carried out as described in Section 2.

Table 3
Role of the trehalose transporter during germination. Germination occurred in the presence (+T) or absence (−T) of 250 mM trehalose

| Crosses | Dissected asci | Germination (%) | | 4 spore tetrads (%) | |
|---|---|---|---|---|---|
| | | −T | +T | −T | +T |
| AP1-12C × AP3-10B | 10 | 30.0 | 85.0 | 0 | 90.0 |
| AP1-14A × AP3-10B | 10 | 28.0 | 85.0 | 0 | 70.0 |
| AP1-10C × AP3-12A | 10 | 32.5 | 70.0 | 10.0 | 75.0 |
| AP1-14A × AP1-14C | 8 | 43.7 | 67.0 | 12.5 | 65.0 |
| AP1-13A × AP1-14B | 10 | 88.0 | nd [a] | 60.0 | nd [a] |

[a] Not determined.

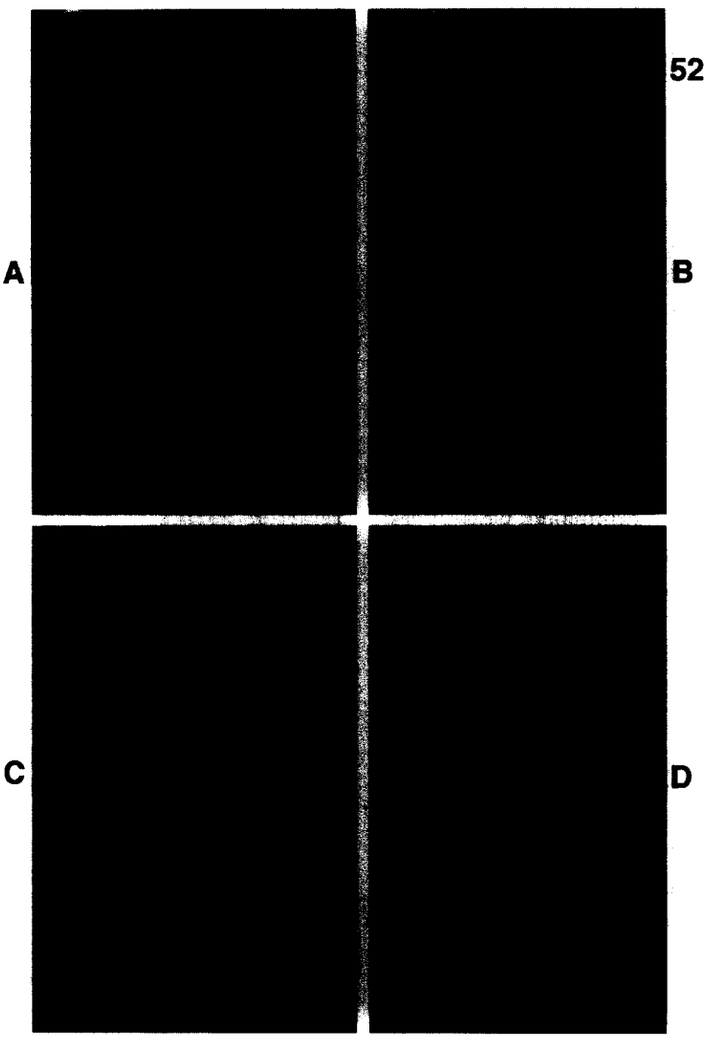


Fig. 2. Germination of spores in the presence and in the absence of external trehalose. (A) Diploid AP1-13A × AP1-14B (control). (B) Diploid AP1-12C × AP3-10B homozygous for the lack of trehalose transport. (C) Diploid AP1-12C × AP3-10B in the presence of 250 mM external trehalose. (D) Diploid AP1-14A × AP3-10B homozygous for the absence of trehalose transport in the presence of 250 mM exogenous trehalose.

output resulting to adaptation to adverse conditions [34].

During their life cycle cells of *S. cerevisiae* accumulate trehalose during sporulation as a response to the stress caused by nutrient deprivation [13]. On the other hand, diploids unable to accumulate trehalose sporulate poorly and are unable to germinate normally [16]. Germination is a response to the abrupt addition of nutrients which could cause an osmotic stress against which cells generally protect themselves by accumulating trehalose [35]. Furthermore, this process involves morphological alterations in the membrane caused by budding. Would trehalose be required, during the germination process, on both sides of the bilayer in order to protect the plasma membrane?

In order to address this intriguing question, we made crosses between haploid strains unable to transport trehalose, however, capable of accumulating endogenous trehalose (Table 2).

The diploids were sporulated and the percentage of fully germinated tetrads analyzed. As can be seen in Tables 2 and 3 although all strains were capable of accumulating trehalose, germination was poor in those diploids in which trehalose transport activity was low. Moreover, formation of complete tetrads was strongly impaired.

When trehalose was added (250 mM) to the germination medium all diploids germinated well and 4 spore tetrad formation was above 60% (Table 3). In Fig. 2, we can observe germination of the control diploid (AP1-13A × AP1-14B) and of two diploids homozygous for the absence of the transporter (AP1-12C × AP3-10B and AP1-14A × AP3-10B).

These results suggested that trehalose must be present on both sides of the membrane during germination. Most probably, a trehalose permease carries part of the endogenously accumulated trehalose to the outer side of the bilayer thus protecting the membrane during the stress caused by the germination process. Cloning of the gene responsible for coding of the transporter will confirm this hypothesis.

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