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Correlation of trenalose content and heat resistance in yeast mutants altered in the RAS/adenylate cyclase pathway: is trehalose a thermoprotectant?

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Trehalose content and thermotolerance were closely correlated in wild type yeast (*Saccharomyces cerevisiae*) and in *cyr1-2* and *bcy1-1* mutants both during exponential growth at 27°C and during heat shock at 40°C. Trehalose levels were high when heat shock proteins (hsps) were expected to be induced and low when hsps were presumably absent. It was tried to uncouple trehalose biosynthesis and hsp-induction. Various non-heat stresses affected trehalose levels of wild type cells in a similar way as they would have affected hsps. However, no trehalose was accumulated when cells were treated with canavanine, a well-known inducer of hsps but not of the thermotolerant state.

Trehalose; Heat shock; Thermotolerance; RAS; Adenylate cyclase; cyclic AMP

1. INTRODUCTION

In baker's yeast (*Saccharomyces cerevisiae*) the non-reducing disaccharide, trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is widely believed to fulfil a storage function [1]. Trehalose levels hare high in resting cells (e.g., stationary phase cells and ascospores) and extremely low during rapid growth [1]. Interestingly, trehalose metabolism is dramatically influenced by heat stress: large amounts of the disaccharide are synthesized when log-phase cultures of *S. cerevisiae* are heated to 40°C [2–4]. Transfer of such cultures back to normal growth temperature results in rapid degradation of accumulated trehalose [2–4]. It was found that trehalose content and thermotolerance of cells were closely correlated both during heat shock and during recovery [3], suggesting that trehalose might act as a thermoprotectant. In view of this hypothesis, it was of interest to compare trehalose levels and heat resistance in mutants altered in the activity of the RAS/adenylate cyclase pathway. Earlier studies have shown that cells mutated in the regulatory subunit of cAMP-dependent protein kinase, *bcy1-1*, are unable to acquire thermotolerance and to synthesize hsps upon heat shock [5]. On the other hand, cells harboring *cyr1-2*, a mutation in the adenylate cyclase structural gene, are thermotolerant without a conditioning heat treatment and constitutively synthesize some hsps [5].

In this report, we show that trehalose levels and thermotolerance are closely correlated in wild type yeast as well as in *cyr1-2* and *bcy1-1* mutants. In all 3 strains, trehalose levels are equally indicative of thermotolerance as are hsp levels. In order to differentiate between possible contributions of trehalose and hsps to thermotolerance, we examined a number of stimuli whose effects on hsp levels are known. A difference between the behaviour of trehalose and hsps was found in only 1 case: the amino acid analogue, canavanine, did not induce trehalose accumulation although it is known to stimulate hsp production whilst failing to render cells thermotolerant [6].

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2. EXPERIMENTAL

2.1. *Yeast strains and culture conditions*

The following strains of *S. cerevisiae* were kindly provided by Dr Tatsuo Ishikawa: P28-24C (MATa pho3-1, wild type strain), AM110 (MATa pho3-1 cyr1-2 leu1) and M203-1A (MATα pho3-1 bcy1-1 his7 gal7). Strain C276 a/α was a gift from Dr J.R. Pringle. Cultures were grown to mid log-phase (about 10^7 cells/ml) on synthetic medium (see [4]) supplemented with leucine and histidine (1 mM each). Glucose (2%, w/v) served as the carbon source. The cultivation temperature was 27°C.

2.2. *Heat shock treatment and determination of thermotolerance*

Heat shock treatment was carried out by transferring cultures growing at 27°C to a water bath maintained at 40°C. For the determination of thermotolerance, 1-ml samples were briefly sonicated (25 W, 30 s), heated to 52°C for 5 min and further treated as in [6].

2.3. *Determination of trehalose and protein*

Trehalose was determined by quantitative thin-layer chromatography as described [2]. Protein was measured according to Peterson [7], using bovine serum albumin as the standard.

2.4. *Chemicals and enzymes*

α-Factor was a gift from Dr H. Riezman (Biocenter, Basel). All other chemicals were bought from Fluka AG (Buchs, Switzerland) and were of the highest purity available.

3. RESULTS

The trehalose content of strains P28-24C (wild type), AM110 (*bcy1-1*) and M203-1A (*cyr1-2*) was compared during exponential growth at 27°C and during heat shock at 40°C (fig.1A). In the wild type strain, only minimal amounts (about 0.02 g/g protein) of trehalose could be detected at 27°C. However, as much as 0.3 g trehalose per g protein was accumulated during incubation at 40°C (fig.1A). In contrast to the wild type, the *cyr1-2* strain contained large quantities of trehalose at 27°C. Since *cyr1-2* was reported to be a temperature-sensitive mutant [8] this result may seem surprising. However, the *cyr1-2* mutation apparently also lowers adenylate cyclase activity at permissive temperature [9]. Upon a shift to 40°C, *cyr1-2* cultures accumulated additional trehalose up to a final concentration of about 1 g/g protein. In the *bcy1-1* strain, no trehalose was found during growth at 27°C. Upon a heat shock, a small but distinct amount of trehalose was produced. Since it has been reported [10] that, during stationary phase, *bcy1-1* strains do not contain any trehalose at all, we checked whether trehalose formation by our strain could be ascribed to reversion of the *bcy1-1* allele in part of the cells. This proved not to be the case as judged by staining for glycogen (see [10]). Hence, the *bcy1-1* mutation strongly diminished but not completely abolished trehalose accumulation at 40°C. Similar results as with the *bcy1-1* mutant were obtained with a $RAS2^{val19}$ strain.

In order to compare the trehalose content of cells and their heat resistance, exponentially growing cultures of strains P28-24C, AM110 and M203-1A were either heated to 40°C for 90 min or maintained at 27°C for the same time. Thereafter, samples were withdrawn, heat-stressed at 52°C for 5 min and plated onto YPD-agar in order to determine cell survival. The same cultures were used as in the experiment shown in fig.1A. Trehalose was found to be an excellent marker for the thermotolerant state (fig.1A and B): cells containing low amounts of the disaccharide (wild type cells at 27°C, *bcy1-1* cells at 27°C or 40°C (fig.1), $RAS2^{val19}$ cells (not shown)) were heat sensitive, whereas cultures exhibiting high trehalose levels were heat resistant. Most notably, the thermotolerance of wild type cells heat-shocked at 40°C and of *cyr1-2* cells maintained at 27°C was almost equal, as were trehalose levels under these conditions (fig.1A and B). Note also that the heat resistance of the *cyr1-2* mutant increased by a factor of about 2 (fig.1A) upon incubation at 40°C and that this was paralleled by about a 3-fold increase in cellular trehalose (fig.1B). These data show that trehalose content and thermotolerance of yeast cells are closely correlated and coordinately regulated by the activity of the RAS/adenylate cyclase pathway.

Under the conditions used in our experiments, heat shock proteins would have behaved in parallel with trehalose both in the wild type and in the mutant strains [5]. It was impossible, therefore, to distinguish between possible contributions of trehalose and heat shock proteins to the thermal stability of yeast cells. Consequently, we tried to uncouple trehalose production from hsp synthesis by exposing cells to a varity of stressful treatments with known effects on hsp biosynthesis. The results are given in table 1.

Since trehalose is accumulated under conditions leading to growth retardation or even growth stop (e.g., heat or stationary phase, see table 1) it was tested whether trehalose biosynthesis could be

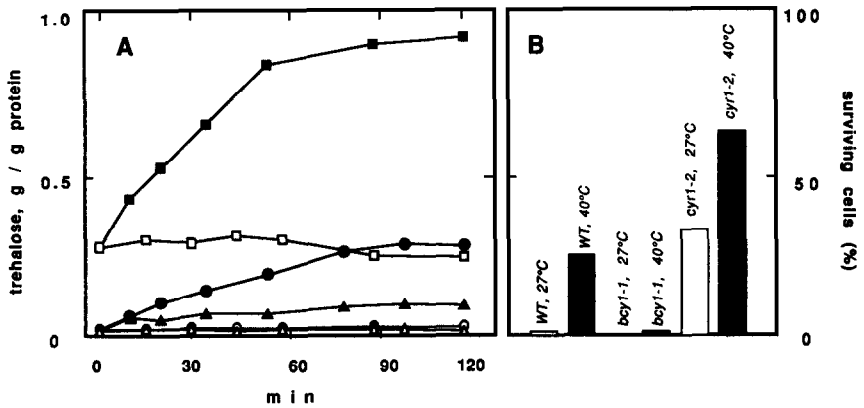

Fig.1. (A) Effect of mutations in the RAS/adenylate cyclase pathway of trehalose levels of *S. cerevisiae*. Cells of strains P28-24C (wild type, circles), AM110 (*cyr1-2*, squares) and M203-1A (*bcy1-1*, triangles) were grown to mid log-phase at 27°C and transferred to a water bath at either 40°C or 27°C at time 0. At intervals, samples were withdrawn for the determination of trehalose and protein. Open symbols: cultures at 27°C. Closed symbols: cultures at 40°C. (B) Thermotolerance of the cultures from (A) after 90 min at 27°C (open columns) and 40°C (filled columns), respectively.

stimulated by *o*-phenanthroline or α-factor, 2 agents known to arrest cell division without inducing hsps [11]. Phenanthroline (1 mM) rapidly stopped growth of wild type cultures but did not stimulate trehalose production (table 1). Note that the chelator did not interfere with trehalose-6P-synthase activity in vitro (not shown). Treatment with α-factor stopped cell division and induced shmoo-formation within 4 h. α-Factor treated cells did not, however, accumulate trehalose (table 1). Heavy metals such as cadmium and copper are well known non-heat inducers of heat shock proteins [12]. High concentrations of both metals stimulated trehalose production, although to a lesser extent than did heat stress (table 1). Osmotic stress (1.2 M sorbitol) which is known to be ineffective in inducing hsps [13] had no influence on trehalose levels (table 1). Thus, in all the cases described above, hsp induction and trehalose accumulation proved to be coupled. It was surprising, therefore, that the amino acid analogue, canavanine, a potent inducer of hsps which nevertheless fails to render cells thermotolerant [6], did not stimulate trehalose production (table 1). Note that for the canavanine

Table 1

Effect of various stimuli on trehalose production and hsp synthesis of *S. cerevisiae* strain P28-24C

Stimulus	Trehalose (g/g protein)		Induction factor (test/control)
	Test	Control	
Heat (40°C, 90 min)	0.295	0.015	19.7
Stationary phase	0.357	0.015	23.8
$CdCl_2$ (250 μM, 2 h)	0.073	0.021	3.5
$CdCl_2$ (1 mM, 2 h)	0.094	0.021	4.5
$CuSO_4$ (5 mM, 1 h)	0.054	0.021	3.0
o-Phenanthroline (1 mM, 1 h)	0.030	0.026	1.15
α-Factor (10^{-6} M, 2 h)	0.038	0.040	0.95
α-Factor (10^{-6} M, 4 h)	0.034	0.050	0.68
Sorbitol (1.2 M, 1 h)	0.021	0.019	1.10
Canavanine (83 μM, 1 h)	0.019	0.022	0.86
Canavanine (250 μM, 1 h)	0.025	0.024	1.05

experiment histidine has been omitted from the medium in order to prevent competition between canavanine and histidine. The effect of canavanine on a prototrophic strain, C276, was also tested, and consistent results were obtained (not shown).

4. DISCUSSION

In yeast, as in many other organisms, a mild heat shock causes an increase in thermotolerance (review in [12]). Heat-induced production of stress proteins is commonly thought to be responsible for this phenomenon [12] although evidence is contradictory [6,12,14].

In earlier work, we have shown that trehalose accumulates in yeast cells upon exposure to heat shock [2,3]. The well-established potential of trehalose to preserve membranes and proteins during dehydration [15], and the fact that polyhydroxy compounds of the type of trehalose stabilize enzymes against thermal damage ([16]; our own unpublished results) lead us to speculate that trehalose might act as a thermoprotectant. The results presented in this paper are compatible with this hypothesis: in wild type cells as well as in *bcy1-1* and *cyr1-2* mutants of *S. cerevisiae*, trehalose and thermotolerance were closely correlated. The behaviour of the trehalose pool mimicked that expected for heat shock proteins: in the *bcy1-1* mutant, which is unable to mount a heat shock response [5], little trehalose was accumulated at 40°C (fig.1A). Conversely, *cyr1-2* cells, which are known to constitutively synthesize some hsps [5], contained high amounts of trehalose even at 27°C. We tried to uncouple trehalose accumulation and hsp synthesis by exposing wild type yeast to a variety of stresses. This proved to be impossible in mose cases (see also [4]). The coordinate induction of trehalose and hsps renders it difficult to differentiate between their possible contributions to thermotolerance. In this context, it is of interest that no trehalose is accumulated in the presence of canavanine. This amino acid analogue, a powerful inducer of hsps [6,12], fails to render cells thermotolerant [6]. Thus, in the case of canavanine-stressed cells, trehalose is a better marker for thermotolerance than are hsps. The effect of canavanine on the formation of trehalose and hsps therefore deserves further study.

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