Activation of neutral trehalase by glucose and nitrogen source in *Schizosaccharomyces pombe* strains deficient in cAMP-dependent protein kinase activity

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Abstract ***Schizosaccharomyces pombe*** **cells carrying a disruption in the *PKA1* gene, that encodes the catalytic subunit of cAMP-dependent protein kinase (PKA), lacked the glucose- and nitrogen-source-induced activation of trehalase at stationary-phase but rised trehalase activity in response to these compounds during the exponential phase of growth. Treatment by phosphatase of either glucose- or nitrogen-source-activated trehalase resulted in trehalase deactivation suggesting that phosphorylation of the enzyme protein occurs during activation. These data indicate that in growing cells of this yeast the mechanism responsible for the activation of trehalase can be independent of interactions with free catalytic subunits of PKA and related to a signaling pathway involving a type of protein kinase different from PKA.**

Key words: Trehalase activation; Protein kinase A; Signal transduction; Enzyme phosphorylation; *Schizosaccharomyces pombe*

1. Introduction

In *Saccharomyces cerevisiae* the addition of nitrogen sources causes activation of trehalase in the presence of glucose [1]. This stimulation of trehalase appears to be due to phosphorylation of the enzyme protein but, contrariwise to the glucose-induced activation of trehalase, is not associated with changes in the cAMP level [2]. A model has been suggested for this yeast in which nitrogen sources activate trehalase by means of a novel signaling pathway which includes direct stimulation of free catalytic subunits of cAMP-dependent protein kinase (cAPK, protein kinase A or PKA) without any role of cAMP as second messenger [3].

Glucose and nitrogen sources are also able to trigger activation of trehalase in the fission yeast *Schizosaccharomyces pombe* [4], an organism which has diverged markedly from the budding yeast *S. cerevisiae* [5]. *Schiz. pombe* has only one gene (*PKA1*) coding for the catalytic subunit of PKA [6,7] whereas the homologous subunits in the budding yeast are encoded by each of the *TPK1*, *TPK2* and *TPK3* genes [8]. In this paper we have analysed the activation of trehalase by glucose and nitrogen sources in normal and *PKA1*-disrupted cells of the fission yeast and found that disruption of this single gene has no negative effect on the activation response of growing cells. These results suggest that in *Schiz. pombe* a model different to that proposed for *S. cerevisiae* has to be considered to explain the transduction of the glucose- and nitrogen-source-induced signal(s) and that a kinase distinct from the *PKA1* gene product may be involved in the in vivo activation of trehalase by these and other various inducers.

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2. Materials and methods

2.1. Strains, media and growth conditions

Yeast strains used in this study included wild type L975 h^+ and L968 h^{90} and their corresponding derivatives JZ636 (h^+ *ade6-M210 leu1 ura4-D18* Δpka1::URA4) and JZ633 (h^{90} *ade6-M216 leu1 ura4-D18* Δpka1::URA4). The *PKA1*-disruptants were kindly supplied by M. Yamamoto (Tokyo) and have been previously described 6. Strain RP91 (h^+ Δras1::LEU2$^+$ leu1) [9] and its counterpart strain Eg545 (h^+ Δmat2,3::LEU2$^+$ leu1$^+$) [10] were received from O. Nielsen (Copenhagen). Complete media contained per liter 20 g of glucose or glycerol and 6 g of yeast extract. Cultures were maintained at 28°C with shaking. Repressed or derepressed growing cells were obtained from mid-exponential phase cultures containing either glucose or glycerol, respectively. Resting cells were harvested from glucose-grown stationary-phase cultures after glucose exhaustion.

2.2. Induction of trehalase activation and enzyme assays

Cells were washed with cold distilled water and resuspended in 10 mM MES/KOH buffer pH 6 (suspension buffer) at a concentration of 50 mg wet weight ml^{-1} (approximately 8×10^8 cells ml^{-1}). The cell suspensions were incubated in a shaking water bath and allowed to equilibrate at 30°C for 5 min. Before and after addition of glucose or nitrogen-source as inducers of trehalase activation samples were removed at timed intervals and the cells washed as above in the cold. Cell-free extracts were obtained, centrifuged and analysed for trehalase and protein as previously described [11]. Specific activity of trehalase is expressed as nmol glucose liberated min^{-1} mg protein^{-1}. Fructose-1,6-bisphosphatase activity was assayed spectrophotometrically as indicated in [12] except that imidazole buffer was replaced by 10 mM MES/KOH at pH 7. cAMP was determined as previously indicated [11].

2.3. Deactivation of trehalase by phosphatase

After activation in vivo, enzyme samples (0.4 ml) were added to the same volume of 100 mM Tris-HCl pH 8 without or with 100 units alkaline phosphatase per mg protein (Boehringer Mannheim, Grade I). The mixture was incubated for 10 min at 37°C and aliquots were removed to determine the remaining activity in standard trehalase assays. The phosphatase was free of proteinase contamination when tested on 1% (w/v) casein. To assess the specificity of deactivation, we assayed the α-glucosidase present in cell extracts as an internal control [13]. No difference in activity between samples maintained in the absence or presence of phosphatase was found.

3. Results and discussion

When glucose-derepressed cells of the wild type strain L975 of *Schiz. pombe* were resuspended in buffer and given 100 mM glucose, a transient activation of trehalase was observed both in growing and resting cells (Fig. 1A,B). Confirming previous results [4] this type of response was not seen in glucose-

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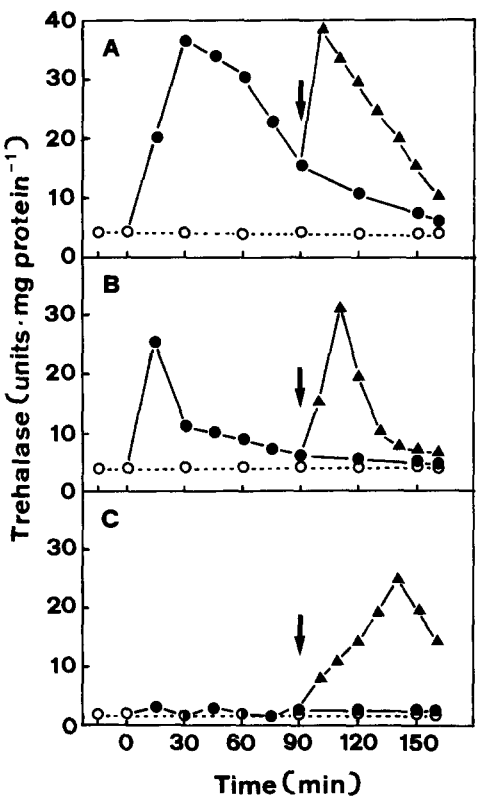

Fig. 1. Trehalase activity after addition of glucose (100 mM, zero time) to cells of the wild type strain L975 of *Schiz. pombe* in suspension buffer followed by addition of a nitrogen-source (10 mM asparagine, arrow). (A) Derepressed growing cells harvested from mid-exponential cultures in glycerol-containing medium. (B) Derepressed resting cells from glucose-grown stationary-phase cultures after sugar exhaustion. (C) Repressed growing cells from mid-exponential cultures in glucose-containing medium. Glucose remaining in the buffer at the time of the addition of asparagine was 35, 32 and 20 mM in panel A, B and C, respectively. Symbols: ●, glucose-induced activation of trehalase; △, nitrogen-source-induced reactivation of trehalase; ○, basal levels of trehalase without additions.

repressed cells (Fig. 1C). Since nitrogen sources alone fail to induce trehalase activation in *Schiz. pombe*, but they increase the activity of the enzyme in the presence of glucose [4], we analysed the effect of nitrogen source by adding 10 mM asparagine to cells suspended in buffer after a previous activation by glucose in the presence of remaining sugar (20–35 mM). A clear nitrogen-source-induced activation of trehalase was thus found in both growing and resting derepressed cells (Fig. 1A,B) and also, in contrast to the activation triggered by glucose, in repressed cells (Fig. 1C). Similar data to those illustrated for strain L975 were found by using the homothallic wild type strain L968 (results not shown). Essentially the same type of results was also observed when cells from strains RP91 (*Δras1*) or Eg545 (*RAS1*) were examined as to their ability to respond to the addition of glucose or asparagine. Hence, neither the glucose-induced nor the nitrogen-source-induced signaling pathways causing activation of trehalase in the fission yeast are RAS-dependent. By contrast, RAS function is required in *S. cerevisiae* for glucose-induced activation of trehalase, although not for stimulation of the enzyme by nitrogen sources [3].

The addition of exogenous cAMP to *Schiz. pombe* cells containing the wild-type *PKA1* gene can activate trehalase [4]. Because this effect was not seen in *PKA1*-disrupted cells from strains JZ636 or JZ633 we anticipated that neither the glucose-induced signal nor the nitrogen-source-induced signal should be functional in *pka1* cells if a cAMP-PKA phosphorylation pathway was the only mechanism accounting for trehalase activation. However, when experiments similar to those described above were performed with *pka1* cells from strain JZ636 the results revealed both glucose- and nitrogen-source-induced activation of trehalase in disruptants harvested from growing cultures (Fig. 2A,C) and lack of activation response to either of these nutritional signals in disruptants harvested from resting cultures (Fig. 2B). Identical conclusions were reached when *pka1* cells from the homothallic strain JZ633 were used, i.e. trehalase activity increased in the cells devoid of *PKA1* gene product in response to the inducers, irrespective of whether obtained from derepressed or repressed cultures, provided they were growing (results not shown).

For unknown reasons, earlier attempts to obtain significant deactivation of glucose-activated trehalase from *Schiz. pombe* by phosphatase were unsuccesful when performed according to the dephosphorylation procedure referred in [14] (reported as unpublished results in [11]). More recently, however, we have been able to accomplish this by using the treatment indicated in Materials and methods. Accordingly, we examined the effect of phosphatase on trehalase extracted from wild type cells of strain L975 following activation by 10 mM asparagine after a previous pulse with glucose. As shown in Table 1, a marked deactivation was obtained. Trehalase reactivated in *pka1* cells from strain JZ636 exposed to a nitrogen-source after a previous activation–deactivation cycle induced by glucose was similarly sensitive to phosphatase treatment (Table 1). Taken together, these results can be interpreted to indicate that trehalase stimulation is due to phosphorylation of the enzyme protein and that PKA is not the only kinase able to activate trehalase.

Although the activation of trehalase by glucose is a glucose-repressible event in control cells we found this type of stimulation in glucose-growing *pka1* cells (see Figs. 1C and 2C). This might be related to the fact that disruption of the *PKA1* gene supress some phenotypic characteristics typical of glucose-repressed cells. For intance, transcription in *Schiz. pombe* of the *FBP1* gene, encoding the gluconeogenic enzyme fructose-1,6-bisphosphatase, is subject to glucose repression in wild type cells but constitutively derepressed in *pka1* mutants [15–17, and our own observations]. Similarly, *pka1* cells might show derepression for some component of the glucose-induced pathway(s) needed to transduce the glucose signal, that is normally

Table 1
Deactivation of nitrogen-source-activated trehalase by phosphatase treatment

Strain	Trehalase (specific activity)		
	Control	Alkaline phosphatase	Deactivation (%)
L975	12.7	2.6	79.6
JZ636	13.8	4.1	71.1

Cells harvested from mid-exponential cultures growing on glucose were suspended in buffer and supplemented with 100 mM glucose for 90 (L975 strain) or 120 min (JZ636 strain) as indicated in Fig. 1C and Fig. 2C, respectively. The cells were pulsed then for 40 min with 10 mM asparagine and cell extracts obtained. Enzyme samples were treated as described in section 2.

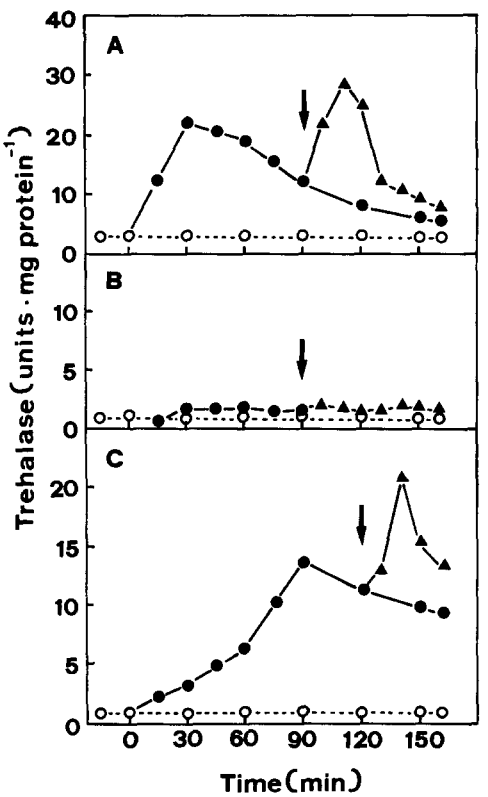

Fig. 2. Trehalase activity upon addition of glucose (100 mM, zero time) and a nitrogen-source (10 mM asparagine, arrow) to cells of the JZ636 strain in suspension buffer. (A) derepressed growing cells; (B) derepressed resting cells; (C) repressed growing cells. Glucose remaining in the buffer at the time of the addition of asparagine was 32, 35 and 20 mM in panel A, B and C, respectively. Symbols are as in Fig. 1.

repressed in glucose-grown *PKA1* cells [4,11]. Such explanation is consistent with the notion that the expression of a whole set of genes, whose transcription is under regulatory control by the cAMP-PKA pathway, is allowed in the absence of PKA function. Among other genes, *FBP1* and *MEI2* in *Schiz. pombe* [17,18] and *UBI4*, *CTT1*, *ADH2*, *SSA3*, *HSP12* and *CYC7* in *S. cerevisiae* are controlled in this way [19–26].

The activation of trehalase by glucose and nitrogen source in cells of *Schiz. pombe* devoid of PKA indicates that the mechanisms underlying these stimulations do not fit entirely the models previously propossed for the increase in trehalase induced by the same sources in *S. cerevisiae* [1–3]. Although the action of a kinase different to PKA has to be assumed to account for the induced activations in *PKA1*-disrupted cells, this observation does not invalidate previous findings on the existence of a glucose-induced cAMP-PKA-mediated phosphorylation pathway causing trehalase activation in this yeast [4,11]. Rather, these and other results (T. Soto et al., manuscript in preparation) sustain that trehalase in the fission yeast may be a target common to various signaling pathways.

Data presented in this work (Fig. 2B) suggest that only in resting cells is PKA conclusively involved in the rise of trehalase activity ensuing the addition of glucose or a nitrogen source. Moreover, the same results point out that trehalase is a natural substrate for PKA in *Schiz. pombe*. Congruent with the presumed function of PKA in resting cells, and lending support to a possible role of cAMP as second messenger in the nitrogen-source-induced signaling pathway for trehalase activation, the addition of asparagine to these cells causes a transient cAMP signal that is even more pronounced than that triggered by glucose (3-fold vs. 12-fold increase above a basal level of 250 pmol cAMP·g wet weight^{-1}). A similar nitrogen-induced cAMP signal has never been seen in *S. cerevisiae* [2]. Therefore, in resting *PKA1* cells of the fission yeast, the mechanism causing trehalase activation by nitrogen sources could be somewhat similar to that occuring after glucose addition, i.e. via cAMP-PKA [11]. However, in growing cells, trehalase activation can be mediated by the alternative kinase enzyme instead of, or in addition to PKA. Most likely the glucose- and the nitrogen-source-induced pathways leading to trehalase activation share a part of the activation machinery. Nevertheless, the transduction of the initial input signal triggered by the nitrogen source occurs in both repressed and derepressed cells whereas that of the glucose signal is only operative in derepression. This particular aspect appears to be conserved in both *Schiz. pombe* and *S. cerevisiae* [3].

Up to now, no other mechanism for regulation of trehalase activity in yeast cells has been identified besides phosphorylation by PKA. Our results, however, demonstrate the presence in the fission yeast of another protein kinase able to phosporylate trehalase in addition to the activating enzyme PKA. Further characterization of this alternative, PKA-independent signaling pathway for trehalase activation will help to elucidate its physiological meaning and to promote the understanding of key cellular processes in *Schiz. pombe*.

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