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Rapid Report

cAMP-dependent protein kinase is not involved in catabolite inactivation of the transport of sugars in *Saccharomyces cerevisiae*

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Abstract

It has been reported that catabolite inactivation of sugar transport systems in *Saccharomyces cerevisiae* requires cAMP-dependent protein kinase activity (cAPK) and that the levels of these transport systems are decreased in the absence of a functional cAPK regulatory subunit. We have re-examined these possibilities and have found that catabolite inactivation does not require cAPK activity and that normal levels of the transports occur independently from the presence of the regulatory subunit. With the available information, it is difficult to ascertain the reasons for the discrepancy between our results and the ones previously reported. The inadequacy of the method used to measure the sugar transport activities might contribute to this discrepancy.

Key words: Sugar transport; Catabolite inactivation; cAMP-dependent protein kinase; Protein kinase; (*S. cerevisiae*)

Sugar transport systems are inactivated in *Saccharomyces cerevisiae* when protein synthesis is impaired [1–5]. This inactivation, known as catabolite inactivation [6], is dependent on energy, stimulated by fermentable substrates [2–4] and due to proteolysis [7]. It has been reported that catabolite inactivation of the glucose and galactose transporters requires cAMP-dependent protein kinase activity (cAPK) [8]. This conclusion was based on the observation that mutants with markedly reduced cAPK activity did not inactivate these transporters [8]. In addition, it has been claimed that in mutants with unbridled cAPK activity, due to the presence of a non-functional regulatory subunit, the transport systems were absent or present at very low levels [8]. We initiated this work to elucidate whether or not the levels of maltose transport and its catabolite inactivation are similarly affected by mutations in the cAPK components. We found that in the case of this transport both phenomena were independent of cAPK activity. This prompted us to re-examine the reported results on the glucose and galactose transport systems. We present evidence showing that cAPK activity is not implicated either in the inactivation of these systems or in the occurrence of normal levels.

Characteristics of the strains and plasmid used are described in Table 1. Cells were grown aerobically at 30°C until late exponential growth in YP (1% yeast extract and 2% peptone (w/v)) in the presence of 3 ppm antimycin A and 2% of the sugars indicated in each case. At this stage of growth the high-affinity glucose transport is present in the cells [12,13]. Cell growth was monitored by measuring optical densities at 640 nm. Inactivation of the sugar transports was routinely achieved by suspending growing cells in an ammonium-free medium as described in Ref. [3] in the presence of 2% glucose and 250 μg/ml tetracycline chlorhydrate, to avoid bacterial growth, at a cellular density of 0.5 mg/ml (wet wt). Only when indicated in the text the inactivation conditions described in Ref. [8] were used. In this case the cells were suspended in yeast nitrogen base in the presence of 2% glucose at 2.5 mg/ml (wet wt). In both cases incubation was performed at 30°C in a rotatory shaker (200 rpm). Glucose transport was measured as described in Ref. [14] using as substrate 170 mM xylose, a non-metabolizable analogue of glucose, and incubating 30 s at 30°C. Galactose transport was measured as described in Ref. [4] using 5 mM galactose and incubating 15 s at 20°C. Maltose transport was measured as described in Ref. [15] using 4 mM maltose and incubating 15 s at 20°C. At these sugar concentrations the activities of the

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Table 1
cAMP-dependent protein kinase activity in the strains used in this work

Strain	Genotype	Activity (nmol P_i/min per mg protein)	
		+ cAMP	− cAMP
W303-1A-pRM1-1 [a]	{*MAT* a *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*} *TPK1 TPK2 TPK3 BCY1*	3.5	0.34
MB23-pRM1-1 [a]	{Isogenic with W303-1A} *TPK1 tpk2::HIS3 tpk3::URA3 BCY1*	1.2	0.25
MB13-pRM1-1 [a]	{Isogenic with W303-1A} *tpk1::LEU2 TPK2 tpk3::URA3 BCY1*	1.2	0.14
MB12-pRM1-1 [a]	{Isogenic with W303-1A) *tpk1::LEU2 tpk2::HIS3 TPK3 BCY1*	0.14	0.07
238-pRM1-1 [b]	{*MAT* a *ade leu2 ura3-52 his3 trp1 CTT1::LacZ*} *TPK1 TPK2 TPK3 BCY1*	2.0	0.29
242-pRM1-1 [b]	{Isogenic with 238} *TPK1 tpk2::HIS3 tpk3::TRP1 bcy1::LEU2*	1.3	1.2
243-pRM1-1 [b]	{Isogenic with 238} *tpk1^w tpk2::HIS3 tpk3::TRP1 bcy1::LEU2*	0.14	0.12

[a] Strains are described in Ref. [9]. [b] Strains were obtained from K. Tatchell. All these strains were unable to grow on maltose and were transformed with the multicopy plasmid pRM1-1 that carries the MAL1 locus [10]. cAPK activity was measured in extracts from cells exponentially growing on YP 2% maltose as described in Ref. [11].

high-affinity components of the sugar transport systems are determined [3,4,15]. We have focused our attention exclusively on these components because the occurrence of the low-affinity ones has been questioned [16,17,25]. Immunodetection of the maltose transporter was performed in crude extracts using polyclonal antibodies as previously described [7]. cAMP-dependent protein kinase activity (cAPK) was measured using labeled [γ-^{32}P]ATP and the synthetic peptide kemptide as described in Ref. [11]. Protein content was determined after precipitation with trichloroacetic acid using the method of Lowry et al. [18].

Two sets of cAPK deficient mutants have been used in this work. The first set carries a wild-type *BCY1*

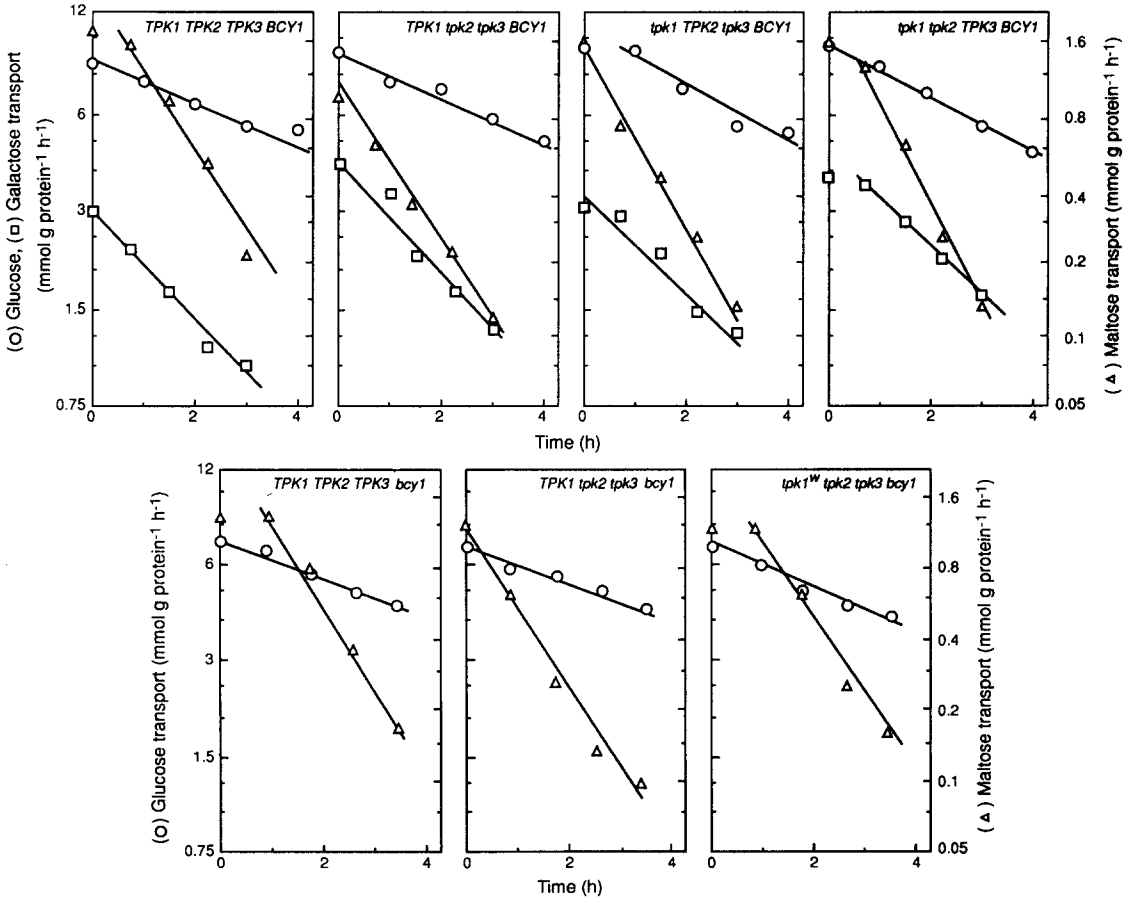

Fig. 1. Inactivation rate of sugar transports in cAPK deficient mutants. Cells were harvested at the exponential phase of growth on glucose (○), galactose (□), or maltose (△), washed and suspended in 3-times the initial volume of the inactivating medium. After incubation at 30°C for the indicated times cells were harvested, washed and assayed for sugar transport activities.

gene in the presence of only one of the three *TPK* genes, i.e., *TPK1* or *TPK2* or *TPK3*, respectively. The second set carries a *BCY1* gene disruption in the presence of the *TPK1* gene or a *tpk1*^{w} allele with markedly reduced phosphorylating activity (Table 1). Due to the mutations in the different catalytic subunits, cAPK activity in these two sets of mutants ranged between 5 and 100% of the normal value (Table 1). As expected cAPK activity was independent on the presence of cAMP in the mutants that carry the *BCY1* disrupted gene (Table 1).

In all these strains we measured sugar transport activities. As shown in Fig. 1 we found similar levels of sugar transport activities as well as similar inactivation rates. In all strains half-life values during the inactivation process of about 5, 2 and 1 h for the glucose, galactose, and maltose transport systems could be, respectively, calculated (from data of Fig. 1). Control experiments run in parallel demonstrated that cAPK activity remained constant during the inactivation period (results not shown). These results strongly indicate that levels and inactivation of sugar transports in *S. cerevisiae* occur independently on cAPK activity. This conclusion is supported by other experiments performed with the maltose transport system in which content of the transporter instead of transport activity

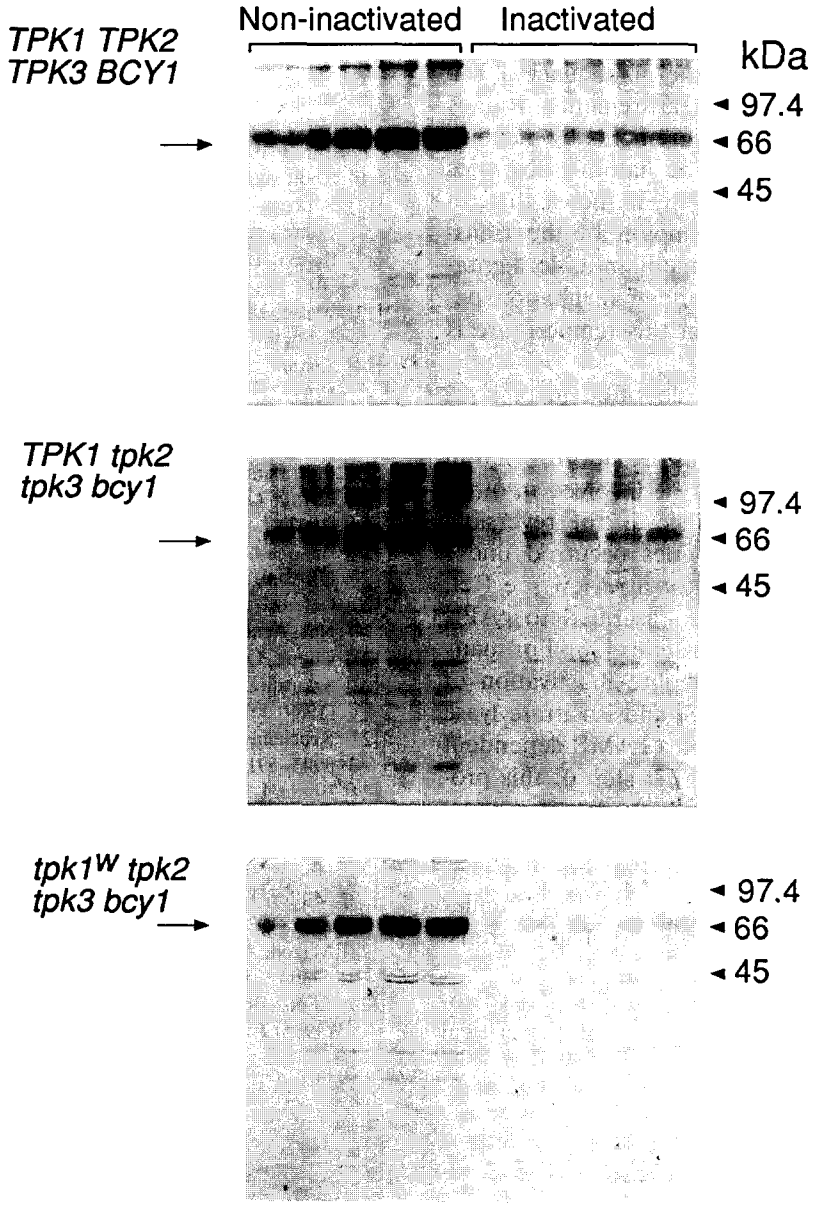

Fig. 2. Content and decrease of the maltose transporter in cAPK deficient mutants during inactivation. Cells were harvested at the exponential phase of growth, washed, and suspended as described in the text Crude extracts were obtained immediately (non-inactivated cells) or after 3 h of incubation at 30°C (inactivated cells). Samples containing 5, 10, 15, 20, and 25 μg protein, respectively, were analyzed using the polyclonal antibodies as described in Ref. [7]. The band corresponding to the maltose transporter is indicated by the arrow.

was measured. As shown in Fig. 2, maltose transporter showed similar levels whether a functional cAPK regulatory subunit was present or not. In addition, and as expected from the calculated half-life of this transporter, its content decreased by about 80% in 3 h of inactivation independently on the cAPK activity of the cells (Fig. 2).

Ramos and Cirillo observed no inactivation of sugar transports in mutants similar to those used in this work [8]. The reasons for the discrepancy between the results of these authors and our results are difficult to asses. This discrepancy cannot be ascribed to differences in the inactivating conditions used since experiments performed in the conditions described by these authors (see above) gave similar results to those shown in Fig. 1 (results not shown). One reason that might contribute to this discrepancy is the inadequacy of the procedures used in transport measurements. Ramos and Cirillo performed their measurements by incubating the cells in the presence of the natural substrates for 1 min at 30°C [8] and it has been shown that, in these conditions, a substantial amount of the transported sugar may be lost in the medium as fermentation products [15]. The recommended incubation period with the natural substrates at this temperature is only 5 s [17,19].

All sugar transporters so far identified in *S. cerevisiae* contain cAPK substrate sites [20–24]. Although our results indicate that these sites are not involved in catabolite inactivation they might be involved in other regulatory mechanisms, for instance, in the reversible changes of sugar transport activities occurred under certain physiological conditions (for a review see Ref. [25]). This kind of role has been ascribed to cAPK substrate sites present in other proteins [26]. For many years it was supposed that catabolite inactivation of fructose-1,6-bisphosphatase [27,28] and isocitrate lyase [29] in *S. cerevisiae* is triggered by a cAMP-dependent phosphorylation occurred at the first step of this process [26]. However, the present view is that this phosphorylation serves instead to increase susceptibility of these enzymes to their allosteric regulatory mechanisms [26]. It might be interesting to check if a similar mechanism takes place in the regulation of sugar transporters.

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