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The high-affinity glucose uptake system is not required for induction of the RAS-mediated cAMP signal by glucose in cells of the yeast *Saccharomyces cerevisiae*

Kaishusha Mbonyi and Johan M. Thevelein

Laboratorium voor Cellulaire Biochemie, Katholieke Universiteit te Leuven, Leuven (Belgium)

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Addition of glucose or related fermentable sugars to yeast cells grown on non-fermentable carbon sources, triggers a RAS-protein mediated cAMP signal which induces a protein phosphorylation cascade. The high-affinity glucose uptake system in yeast cells is known to be glucose-repressible and only functional in strains containing at least one active kinase. In strains containing point or disruption mutations in the *SNF3* gene, which codes for the high-affinity glucose carrier, the glucose-induced cAMP signal is still present. This indicates that the previously demonstrated requirement of a functional kinase for the induction of the cAMP signal, does not reflect requirement of high-affinity sugar transport. It also indicates that the unknown glucose-repressible protein in the induction sequence of the RAS-mediated cAMP signal is not the high-affinity sugar carrier.

Addition of glucose or related fermentable sugars to yeast cells grown on non-fermentable carbon sources, stationary-phase yeast cells or yeast ascospores, induces a cAMP signal which triggers a protein phosphorylation cascade. This cascade is responsible for mobilization of the storage sugar trehalose through activation of trehalase, stimulation of glycolysis through activation of phosphofructokinase 2 and inhibition of gluconeogenesis through inactivation (directly by phosphorylation and/or allosterically by fructose 2,6-bisphosphate) of fructose-1,6-bisphosphatase (for a recent review, see Ref. 1).

The mechanism by which glucose induces the cAMP signal is not well understood. Recently, we showed that its induction requires the presence of one functional *RAS* gene and that the product of a yeast equivalent, *RAS2*^{val19}, of the human oncogene H-*ras*^{val12} is completely deficient in transmission of the cAMP signal [2]. Yeast strains lacking functional CDC25 proteins, which probably serve as specific stimulators of GDP/GTP exchange on the RAS proteins [3–6] are also deficient in induction of the cAMP signal (Van Aelst, L., unpublished results). In addition, we have shown that the presence of one of the three glucose phosphorylating enzymes (hexokinase 1, hexokinase 2 or glucokinase) is needed for induction of the cAMP signal by glucose and one of the two hexokinases for induction by fructose [7]. This could indicate the importance of sugar phosphorylation for induction of the cAMP signal. However, the presence of an active kinase is also necessary

Abbreviation: Mes, morpholineethanesulfonic acid.

Correspondence: J.M. Thevelein, Laboratorium voor Cellulaire Biochemie, Katholieke Universiteit te Leuven, Kardinaal Mercierlaan 92, B-3030 Leuven-Heverlee, Belgium.

for high-affinity glucose (and fructose) transport in yeast [8]. Hence, requirement for an active kinase could reflect requirement for high-affinity sugar transport. In addition, high-affinity transport is glucose-repressible [9], which is also the case for the cAMP signal [7].

Recently, sucrose non-fermenting mutants containing point mutations in the *SNF3* gene were shown to be deficient in high-affinity glucose transport [10]. The *SNF3* gene, which appears to code for the high-affinity glucose carrier, has been cloned and strains containing a disrupted *SNF3* gene have been constructed [11]. We have used the point mutant *snf3*-217, the disruption mutant *snf3*-Δ4::*HIS3* and the corresponding wild type *SNF3* to investigate the requirement of high-affinity glucose transport for induction of the cAMP signal by glucose. Similar experiments were carried out with fructose. Glucose and fructose use the same transport system [8]. In addition, we also measured the cAMP increase caused by the addition of dinitrophenol. This compound stimulates cAMP synthesis because it lowers the intracellular pH [12–15]. The mechanism involved is unclear. The effect, however, is (for the greatest part) not due to direct activation of adenyl cyclase, as was previously thought. The action point of lowered intracellular pH must be located somewhere after sugar phosphorylation and at or before the CDC25 protein in the activation pathway of adenyl cyclase (Ref. 2 and unpublished results).

The following yeast strains were provided by M. Carlson (Columbia University, New York): MCY 1093: wild-type *SNF3* strain (genotype: *Mata SNF3 his*4-539 *lys*2-801 *ura*3-52 *SUC*2$^+$ *GAL*$^+$) MCY 1409: *snf3* disruption mutant (genotype: *Matα snf3*-Δ4::*HIS3 lys*2-801 *ura*3-52 *SUC*2$^+$ *GAL*$^+$ *his*3Δ) MCY 1609: *snf3* point mutant (genotype: *Matα snf3*-217 *ade*2-101 *SUC*2 *gal*2).

The cells were grown on rich glycerol medium as described previously [2]. The sugar- and dinitrophenol-induced cAMP increases were measured at 25 °C in Mes buffer (pH 6) as described previously [2]. The sugars were added at a concentration of 100 mM, dinitrophenol was added as a solution in ethanol to give a final concentration of 2 mM (and 2.5% ethanol). Determination of cAMP levels was performed as described previously [15]. All experiments were repeated at least twice with consistent results. Representative results are shown.

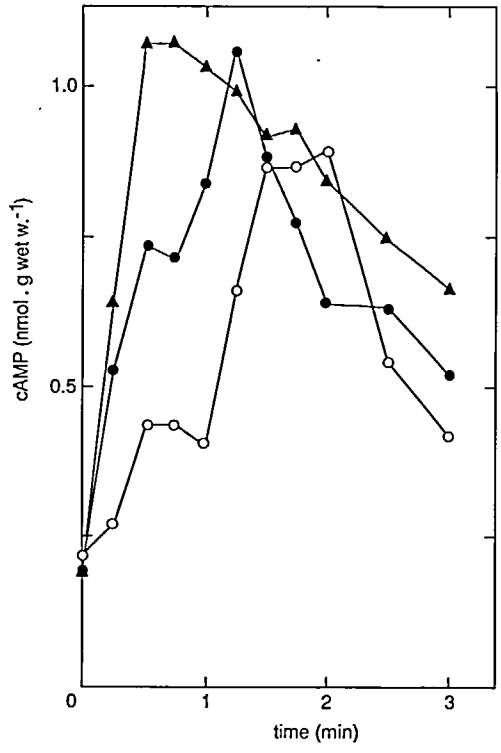

Fig. 1. Glucose-induced cAMP signal in the *SNF3* wild-type strain (●), the *snf3*-Δ4::*HIS3* disruption mutant (○) and the *snf3*-217 point mutant (▲). The cells were incubated in Mes buffer and glucose was added at time zero.

Addition of glucose to derepressed cells of the wild-type strain or mutant strains deficient in high-affinity sugar transport induced similar cAMP signals (Fig. 1). The cAMP signal in the *snf3* disruption mutant was somewhat retarded compared to the control (Fig. 1). However, this was only true at 25 °C. At 32 °C and 37 °C, induction of the cAMP signal in wild-type and mutant strains was equally rapid (results not shown). Induction of the cAMP signal by fructose was also not significantly affected by the absence of the high-affinity sugar carrier (Fig. 2). Addition of dinitrophenol caused similar large increases in the cAMP level in both the wild-type strain and the mutants lacking high-affinity transport (Fig. 3).

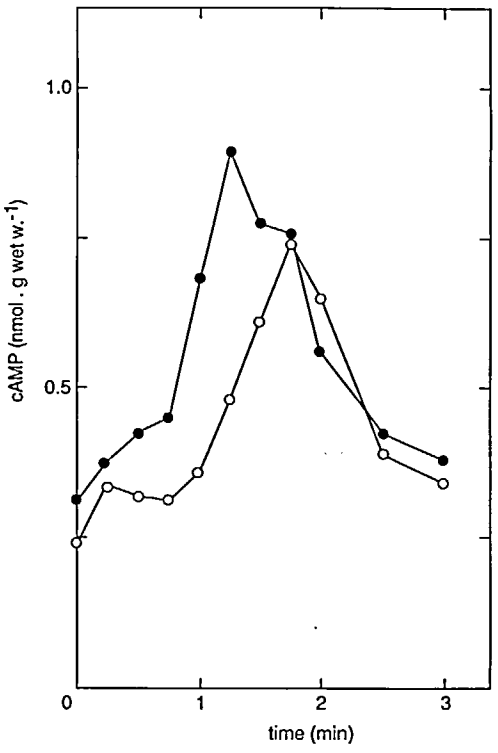

Fig. 2. Fructose-induced cAMP signal in the *SNF3* wild-type strain (●) and the *snf3*-Δ4::*HIS3* disruption mutant (○). The cells were incubated in Mes buffer and fructose was added at time zero.

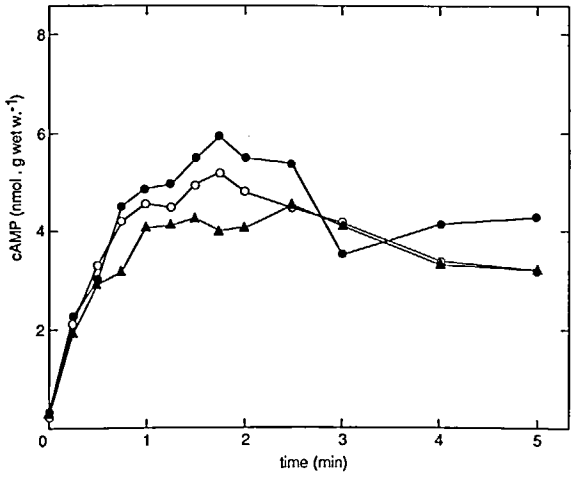

Fig. 3. Dinitrophenol-induced cAMP increase in the *SNF3* wild-type strain (●), the *snf3*-Δ4::*HIS3* disruption mutant (○) and the *snf3*-217 point mutant (▲). The cells were incubated in Mes buffer and dinitrophenol added at time zero.

The present results clearly indicate that high-affinity sugar transport is not needed for induction of the RAS-mediated cAMP signal by glucose or fructose in derepressed yeast cells. Hence, the previously demonstrated requirement of a functional kinase for the induction of the cAMP signal [7] does not reflect a requirement of high-affinity sugar transport or for the mere presence of this transport system. It also indicates that the unknown glucose-repressible protein in the induction sequence is not the (glucose-repressible) high-affinity sugar carrier. Nor do mutations in the high-affinity uptake system affect the dinitrophenol-induced cAMP increase, which is also consistent with the idea that this sugar uptake system is not involved in the regulation of cAMP metabolism.

The conclusions above support our previous suggestion that the low-affinity glucose carrier acts as the receptor for glucose in the induction of the RAS-mediated cAMP signal [7]. This suggestion was based on the fact that the K_m of this carrier and that for induction of the cAMP signal by glucose are both about 20 mM, which is at least one order of magnitude higher than the K_m values of the high-affinity sugar carrier and the kinases. Since requirement of an active kinase for the induction of the cAMP signal does not reflect the need for high-affinity sugar transport, it apparently reflects the need for phosphorylation of the sugar. Previously we have presented evidence that the sugar phosphates do not appear to act as the triggers for the cAMP signal [7]. At this point it is important to mention that evidence exists of coupling between sugar transport and sugar phosphorylation in yeast [16–18]. The initial trigger of the glucose-induced RAS-mediated cAMP signal might therefore be localized at the point of transport-associated phosphorylation of the sugar. Recent experiments in our laboratory, however, appear to indicate that sugar transport by the low-affinity sugar carrier in yeast does not involve transport-associated phosphorylation (Beullens, M., unpublished results).

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