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The activity of the gluconate-H^+ symporter of *Schizosaccharomyces pombe* cells is down-regulated by D-glucose and exogenous cAMP

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Abstract ***Schizosaccharomyces pombe*** **cells take up D-gluconate, as an alternative carbon source for growth, during glucose starvation or when cultured on glycerol-containing medium. Gluconate uptake is not detectable while cells are growing logarithmically on glucose. The addition of D-glucose as well as its non-metabolizable analogues to glycerol-grown cells causes an immediate loss of gluconate transport within 1 min. The reversible down-regulation of the gluconate carrier occurs after glucose has been internalized. This regulation is triggered not only by D-glucose but also by extracellular cAMP even in the absence of the cAMP-dependent protein kinase (PKA1).**

Key words: Gluconate transport; cAMP; Carrier regulation; *Schizosaccharomyces pombe*

1. Introduction

Cells use inducible transport proteins to tap additional substrates. Addition of the preferred carbon source results in the inactivation (catabolite inactivation) of these transport proteins and/or in the transcriptional repression of the encoding genes (catabolite repression). In enteric eubacteria, like *Escherichia coli*, catabolite inactivation is mediated by a soluble subunit of the phosphotransferase system (PTS), referred to as enzyme III^{GLU} [1]. For example, lactose transport is inhibited by the interaction of the lactose-H^+ symport protein (LacY) with the dephosphorylated enzyme III^{GLU}. This occurs upon the addition of a sugar substrate for the PTS system [2]. Catabolite inactivation in eucaryotic organisms has been studied extensively in *Saccharomyces cerevisiae*. It has been shown that glucose down-regulates irreversibly the galactose uniporter [3,4] as well as the maltose-H^+ symporter [5–7]. The latter protein is inactivated, endocytosed and degraded inside the vacuole [6,7]. Although changes in carrier phosphorylation have been suggested to be responsible for the observed regulation [7], the glucose-sensing mechanism [8] as well as the pathways causing the inactivation of the carrier protein remain unknown [9].

For the fission yeast *Schizosaccharomyces pombe*, down-regulation of transport proteins has not yet been reported. This work presents the observation that the uptake of D-gluconate, which can be used by fission yeast cells as an alternative carbon and energy source [10], is rapidly inhibited by the addition of D-glucose as well as by exogenous cAMP.

There are several regulatory phenomena in *S. pombe* which are triggered by glucose. Glucose prevents the onset of mating and meiosis of starved homothallic cells [11,12] and regulates the expression [13,14] as well as the activity [15] of some enzymes. Several studies support the hypothesis that cAMP [11,12,15] and the cAMP-activated protein kinase (PKA1) [14,16,17], but not the Ras-protein [18], are involved in regulation caused by glucose.

2. Materials and methods

2.1. Strains, media and growth conditions

The following fission yeast strains were used in this study: wild-type strain $972h^-$ [19], mutant strain JZ633 (h^{90} ade6-M216, leu1, ura4-D18, Δpka::ura4$^+$) lacking cAMP-dependent protein kinase (PKA1) [16], glucose-transport deficient strain YGS-B25 (h^-, leu1-32) [20], and strain YGS-B25 expressing the *Chlorella kessleri* glucose-H^+ symporter (TCY96) [21]. Cells were grown at 29°C in rich medium containing 0.5% yeast extract and 3% D-glucose. For derepression of gluconate uptake, cells were cultivated on 3% glycerol. The transgenic strain TCY96 was grown on 0.67% yeast nitrogen base and 3% potassium gluconate.

2.2. Uptake measurements

To measure uptake of labeled gluconate, the following procedure was used. Cells were grown to an OD_{578} of 1–2 (1 g fresh weight of cells corresponds to 1560 OD_{578} units of cells). For each test, 5 OD_{578} units of cells were pelleted, washed twice with 100 mM Na-citrate buffer (pH 4.3), and resuspended in this buffer to a final volume of 1 ml. Ethanol was added to 120 mM to achieve energization during uptake [22]. Cells were agitated in a rotary shaker at 32°C and the test was started by adding D-[U-^{14}C]gluconate (1.8 μCi/μmol, Amersham Buchler Braunschweig, Germany) to a final concentration of 100 μM. Aliquots were taken at given intervals, filtered through nitrocellulose filters (0.8 μm pore size), and washed thoroughly with water. Incorporated radioactivity was determined by scintillation counting.

3. Results and discussion

3.1. Glucose depletion triggers gluconate uptake

Fission yeast wild-type cells start to transport gluconate when they suffer from glucose starvation during exponential growth or when cells have reached their stationary growth phase. However, when cells are growing logarithmically in rich medium supplemented with 3% glucose, gluconate uptake is not detectable (Fig. 1). These observations indicate that the decrease in extracellular glucose concentration is the signal for the appearance of the gluconate-H^+ symport activity, which enables the cells to grow on this alternative carbon source [10]. In addition, gluconate uptake was also observed when fission yeast cells were grown on glycerol. Glycerol, as a carbon source, was chosen to cultivate the different strains used in this study due to the inability of the mutant strain JZ633 to

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Dedicated to Prof. Dr. Widmar Tanner on the occasion of the 25th anniversary of his department.

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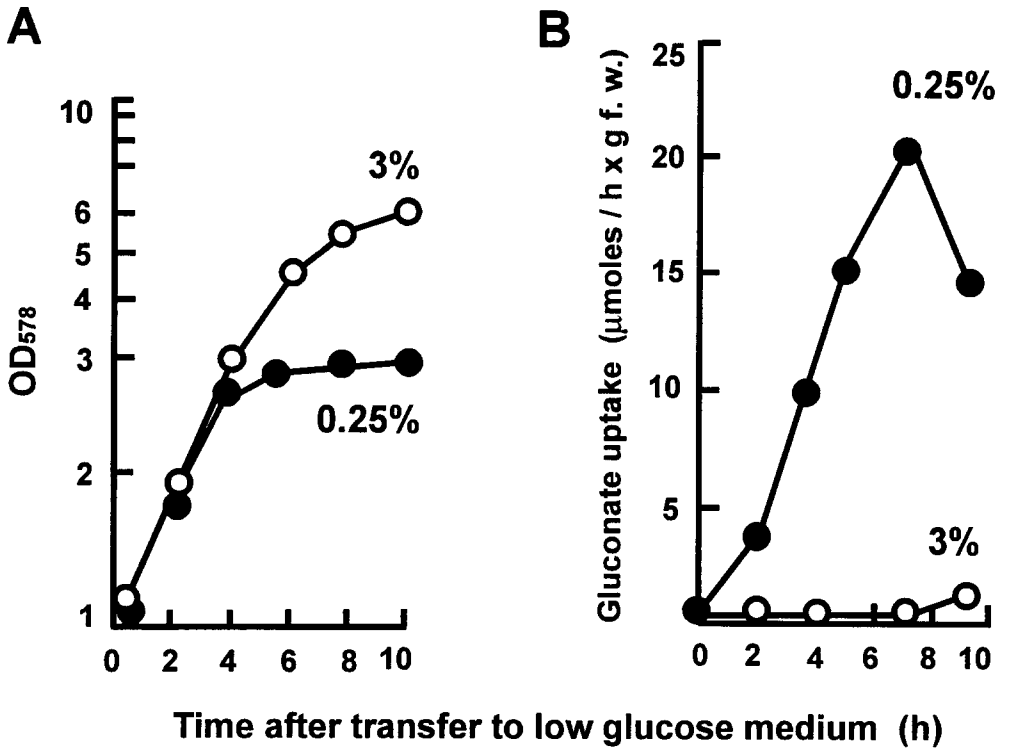

Fig. 1. Glucose depletion triggers gluconate uptake. Cells of the wild-type strain 972h$^-$ were grown in rich medium supplemented with 3% D-glucose. At an optical density of 1.0, one half of the culture was removed, washed and resuspended in a medium containing 0.25% D-glucose. (A) Growth of the cultures. (B) Initial velocities of gluconate uptake. f.w., fresh weight.

grow on gluconate-containing medium. The ability of glycerol-grown cells to transport gluconate supports the view that gluconate itself is not required for gluconate uptake. Contrastingly in *S. cerevisiae* cells, the induction of the maltose-H^+ symporter [23] and galactose uniporter [24] occurs only in the presence of the substrate, maltose or galactose, respectively.

In a previous report [10], the gluconate symport protein was shown to be constitutively expressed by the observation that glucose-grown fission yeast cells were able to take up gluconate. It has been observed that *S. pombe* cells do not transport gluconate during logarithmic growth on glucose, whereas the uptake activity appears when cells enter their stationary growth phase (Fig. 1). This contradicting report could perhaps be explained if stationary *S. pombe* cells were used to measure gluconate uptake.

The decrease in initial gluconate uptake rate after a starvation period of 7 h (Fig. 1B) may be due to reduction of the proton-motive force generated by the plasma membrane H^+-ATPase [25] indicating that ATP has been exhausted.

3.2. *Addition of D-glucose to glycerol-grown cells causes an immediate but reversible loss of gluconate uptake*

The addition of glucose to maltose-fermenting *S. cerevisiae* cells results in irreversible inactivation of the maltose-H^+ symport protein in less than 2 h [6,7]. To investigate whether the *S. pombe* gluconate symporter is also subjected to such regulation, glucose was added to glycerol-grown cells during the gluconate uptake measurements. As demonstrated in Fig. 2, addition of 10 mM D-glucose triggers the loss of gluconate uptake within 1 min, a period of time completely different from that reported for the inactivation of the *S. cerevisiae* maltose transporter. This rapid down-regulation can be achieved not only by D-glucose but also by its non-metabolizable analogues 6-deoxy-D-glucose (6-DG) and 3-*O*-methyl-D-glucose (3-OMG), whereas L-glucose is unable to cause this inhibition. The same regulation was observed when cells were cultivated on gluconate instead of glycerol (not shown). The down-regulation of gluconate transport was shown to be transient (Fig. 3). In addition, gluconate uptake is rapidly restored when glucose is removed from the buffer.

To investigate whether the uptake of glucose triggers the down-regulation of gluconate uptake, a mutant strain (YGS-B25), which has lost its ability to take up monosaccharides [20,21], was used. In this mutant strain, D-glucose was unable to induce reversible inhibition of the gluconate transporter (Fig. 4). Therefore, in the absence of glucose transport, external glucose cannot act as an inhibitor of gluconate uptake. Since down-regulation can be restored in this glucose-transport deficient strain (YGS-B25) by the expression of an algal glucose-H^+ symport protein [21] (not shown), it is suggested that the fission yeast glucose transport system itself is not involved in the observed regulation of gluconate uptake. In contrast, for the *S. cerevisiae* glucose carriers a function in glucose sensing has been postulated [8].

The ability of the non-metabolizable glucose analogues to cause this down-regulation (Fig. 2) suggests that glucose itself is the signal recognized by the cells. The higher concentrations of the analogues needed to trigger the down-regulation as well as the delayed effectiveness of 3-OMG can be explained by the fact that these sugars are poor substrates for the glucose uniporter in fission yeast cells [22,26]. The K_m values for 6-DG (45 mM) and 3-OMG (178 mM) are both significantly larger than that for D-glucose (3 mM) [26].

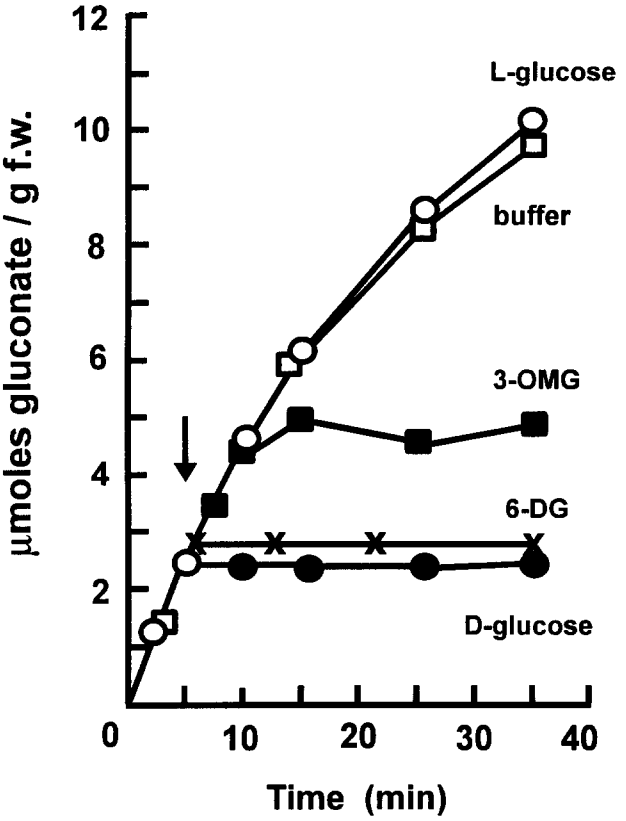

Fig. 2. D-Glucose and its non-metabolizable analogues inactivate gluconate uptake. Glycerol-grown wild-type cells were incubated in the presence of labeled gluconate and after 5 min (arrow) the indicated sugars were added. Final concentrations: D-glucose=10 mM (●); L-glucose (○)=3-OMG (■)=6-DG (×)=125mM. Buffer (□) was added instead of sugar.

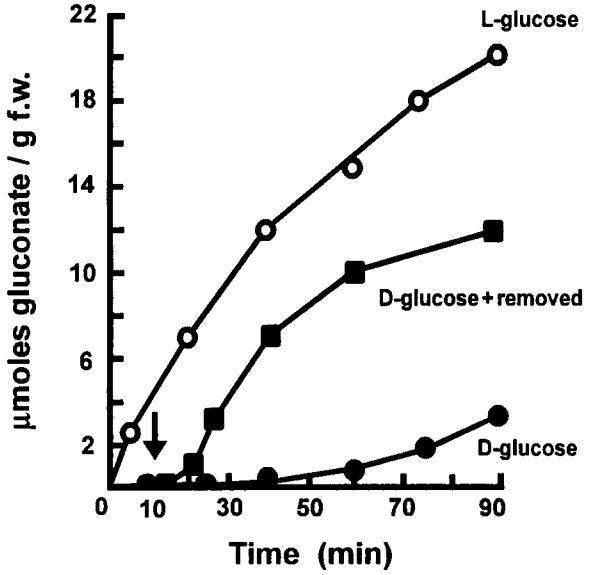

Fig. 3. Down-regulation of the gluconate transporter is reversible. Glycerol-grown wild-type cells were incubated with labeled gluconate in the presence of either 10 mM L-glucose (○) or 10 mM D-glucose (●). To a third cell suspension only 10 mM D-glucose was added (■). After 10 min (arrow) cells were quickly washed and resuspended in glucose-free buffer containing labeled gluconate.

3.3. Down-regulation is also triggered by extracellular cAMP

For *S. pombe* [15], as well as *S. cerevisiae* [27], it has been demonstrated that the cytosolic cAMP level increases, rising to a peak within approx. 1 min, upon addition of D-glucose to glycerol-grown cells. The coincidence between the occurrence of the cAMP signal and the kinetics of the down-regulation led to the hypothesis that cAMP is involved in this phenomenon. In fission yeast, cAMP-dependent regulation can be triggered by the addition of cAMP (5–50 mM) to the medium [11,14,16,28].

As demonstrated in Fig. 5, extracellular cAMP is also able to trigger the transient loss of gluconate uptake. Although this observation indicates that glucose and its analogues operate via this second messenger, it cannot be excluded that extracellular cAMP may act in a different way. For instance, *S. cerevisiae* cells have an extracellular protein which binds cAMP with high affinity and may be involved in glucose sensing [29].

3.4. The cAMP-dependent protein kinase is not involved in the gluconate uptake inactivation

A model explaining the reversible down-regulation of the gluconate symport protein may include phosphorylation events carried out by a cAMP-activated protein kinase. Haploid fission yeast cells have only one allele (*PKA1*) encoding this enzyme [16], whereas *S. cerevisiae* cells possess three functional redundant gene products for cAMP-activated kinase

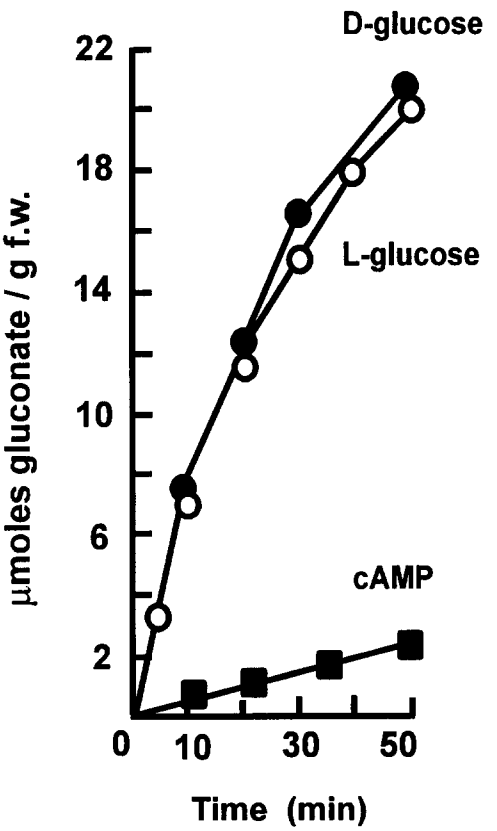

Fig. 4. D-Glucose must be taken up to cause symporter inactivation. Glycerol-grown cells of the mutant strain YGS-B25 lacking the endogenous glucose uniporter were incubated with labeled gluconate in the presence of either: 10 mM L-glucose (○), 10 mM D-glucose (●), or 35 mM cAMP (■).

expression [30]. To examine the involvement of this kinase, gluconate transport measurements were performed in the kinase-deficient strain JZ633 [16]. As shown in Fig. 6, both D-glucose and cAMP are still able to trigger the loss of gluconate uptake. It is concluded, therefore, that the kinase PKA1 does not play a role in down-regulation of gluconate transport.

An unusual property of the kinase-deficient strain (JZ633) is its strongly impaired growth on gluconate (not shown), indicating that the cells have lost their ability to metabolize this carbon source. Due to the absence of gluconate metabolism, the plateau shown in Fig. 6 may result from the accumulation of gluconate in the cytosol. However, the addition of 50 mM uncoupler (carbonyl cyanide *m*-chlorophenylhydrazone) did not result in efflux of gluconate (not shown).

3.5. Conclusions

What might be the physiological relevance of this regulation? Foury and Goffeau [31] reported that the uptake of several growth substrates, like amino acids or uridine, is markedly stimulated within 30 s after addition of cAMP to glycerol-grown fission yeast cells. Due to the observation that cAMP improves the extrusion of protons, they suggested that the stimulation of transport is indirectly achieved by increasing the driving force (proton-motive force) [25,31]. While speculative, it is possible that cAMP acts as a signal for the functional coordination of the proton-symport proteins upon addition of glucose to glucose-starved *S. pombe* cells. Since all proton-symport proteins will be stimulated by the increase in the proton-motive force, symport proteins like the gluconate-H^+ symporter not required for growth on glucose could be switched off via cAMP.

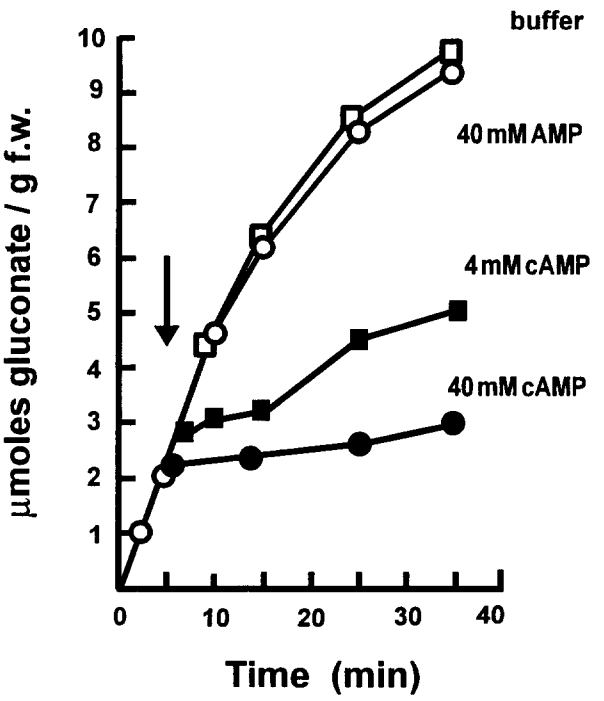

Fig. 5. Exogenous cAMP triggers the inactivation of the gluconate symporter. To glycerol-grown wild-type cells the indicated amounts of AMP (○) or cAMP (●,■) were added (arrow). Buffer (□) was added instead of a sugar nucleotide.

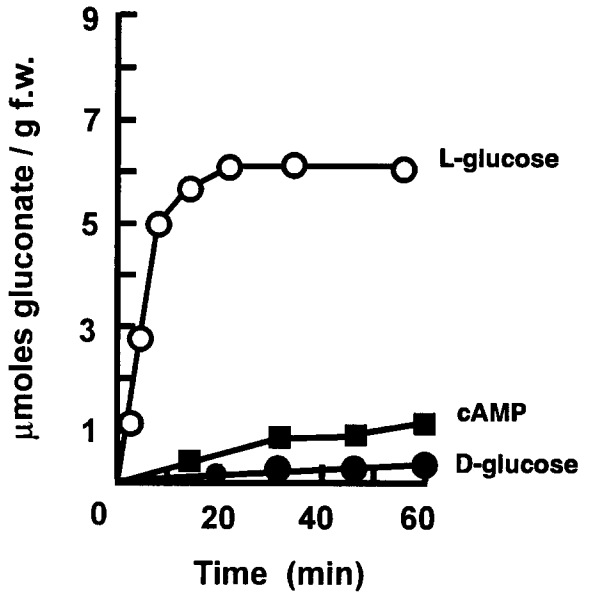

Fig. 6. The cAMP-dependent protein kinase (PKA1) is not involved in the down-regulation of gluconate uptake. Glycerol-grown cells of the mutant strain JZ633 carrying a disrupted allele of the cAMP-dependent protein kinase were incubated with labeled gluconate together with either: 10 mM L-glucose (○), 10 mM D-glucose (●), or 40 mM cAMP (■).

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