RESEARCH ARTICLE

Restricted sugar uptake by sugar-induced internalization of the yeast lactose/galactose permease Lac12

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Kluyveromyces lactis; lactose permease; Lac12; post-translational control; catabolite inactivation; sugar transport.

Abstract

Kluyveromyces lactis Lac12 permease mediates lactose and low-affinity galactose transports. In this study we investigated the effects of carbon sources on internalization of Lac12 using a *LAC12–GFP* fusion construct. When galactose- or lactose-grown cells are shifted to a fresh sugar medium, Lac12–GFP is removed from the plasma membrane and is localized intracellularly. Surprisingly, either galactose or lactose in the new media caused the internalization, and cells responded differently to these two sugars. Our results reveal that this process is dependent on sugar species and also sugar concentration. Lac12–GFP internalization causes reduction of [C^{14}]lactose uptake rates and also occurs in a Kl*snf1* mutant strain; it is therefore independent of KlSnf1 activity. We suggest that glucose-6-phosphate is the intracellular signal, as internalization was induced by 2-deoxyglucose, and inhibition of phosphoglucomutase by lithium prevented galactose- but not lactose- or glucose-induced internalization. Lac12–GFP internalization was not triggered by 6-deoxyglucose, and was irreversible in the absence of protein synthesis.

Introduction

The yeast *Kluyveromyces lactis* is one of the few yeasts capable of assimilating the milk sugar lactose. Among its biotechnological applications is the fermentation of lactose present in cheese whey, an abundant residue from the dairy industry. It is also claimed to be a potential host for heterologous protein synthesis, and has emerged as a model for nonconventional yeasts (Rubio-Texeira, 2006). Compared with *Saccharomyces cerevisiae*, *K. lactis* is able to use a more diverse range of sugars, such as pentoses and disaccharides as cellobiose, in addition to lactose (Freer, 1991; Breunig *et al.*, 2000).

Lactose metabolism in *K. lactis* is mediated by the proteins Lac12 and Lac4, which promote lactose transport and hydrolysis, respectively. It has been suggested that fermentative utilization of sugars by *Kluyveromyces* is limited by transport capacity (Milkowski *et al.*, 2001). Consequently, improvement of *K. lactis* fermentative potential might be reached by modulating sugar uptake.

Lac12 permease mediates both lactose and low-affinity galactose transport by a proton symport mechanism (Dickson & Barr, 1983; Wiedemuth & Breunig, 2005). *LAC12* is regulated at the transcriptional level and coregulated with the galactose metabolic *GAL* genes (Riley *et al.*, 1987; Chang & Dickson, 1988). In addition, the activity and stability of Lac12 may be regulated (Wiedemuth & Breunig, 2005). In *S. cerevisiae*, fermentable carbon sources can cause irreversible inactivation of transporters. Glucose-induced catabolite inactivation occurs when *S. cerevisiae* is shifted from alternative carbon sources to glucose, and involves internalization by endocytosis and vacuolar proteolysis. Addition of glucose to galactose-growing *S. cerevisiae* triggers monoubiquitination of the major galactose transporter, Gal2, followed by internalization and vacuolar degradation (Horak & Wolf, 1997).

There are two signaling pathways that stimulate the glucose-induced inactivation, elucidated by studies of *S. cerevisiae* maltose permease Mal61: one is mediated by the extracellular glucose sensor Rgt2 and causes Mal61 proteolysis independently of glucose transport; the other

pathway is dependent on transport and stimulates Mal61 proteolysis and an inhibition of maltose transport that is faster than can be explained by proteolysis alone (Medintz *et al.*, 1996; Jiang *et al.*, 1997). The transport-dependent pathway is stimulated by the initial steps of sugar metabolism including transport and phosphorylation. However, it has been shown that the signaling of the process is not glucose-specific and can be produced by fast transport and phosphorylation of many other sugars. For example, maltose can trigger inactivation of its own permease (Jiang *et al.*, 2000).

The main transcriptional regulation by glucose in *S. cerevisiae* depends on the trimeric serine/threonine Snf1 kinase, the central mediator of release from catabolic repression of genes related to alternative carbon sources metabolism (reviewed in Hedbacker & Carlson, 2008). Mutant *snf1 K. lactis* cells presented lower growth rates in glucose, sucrose, lactose and galactose, and no growth was obtained in raffinose, sorbitol or maltose (Dong & Dickson, 1997). Furthermore, deletion of Kl*SNF1* causes a deficiency in the accumulation of Lac12 at the cytoplasmic membrane; it has also been shown that this deficiency is not primarily due to reduction of *LAC12* transcription, but to alteration of the subcellular distribution of the permease (Wiedemuth & Breunig, 2005). These data indicate a role of KlSnf1 in post-translational regulation in *K. lactis*.

The post-translational control of *K. lactis* lactose permease in response to glucose or other sugars has not been studied in detail. In this study we demonstrate that Lac12 is post-translationally regulated by changing its subcellular localization in response not only to glucose, but also to lactose and galactose.

Materials and methods

Yeast strains and growth conditions

The *K. lactis* strains used in this work were JA6-LAC12GFP (*MATα ade1-600 adeT-600 ura3-12 trp1-11 LAC9-2 LAC12-EGFP*) and JSD1R-LAC12GFP (*MATα ade1-600 adeT-600 ura3-12 trp1-11 LAC9-2* Kl*snf1*::*ura3 LAC12-EGFP*) (Wiedemuth & Breunig, 2005). In both strains, Lac12–GFP fusion protein is expressed under control of its own *LAC12* promoter. Cells were grown in synthetic complete (SC) medium (0.67% w/v yeast nitrogen base supplemented with amino acids and nucleobases) containing 2% (w/v) galactose or lactose as carbon and energy sources. Cultures were maintained in rotatory shaker at 200 r.p.m., and 30 °C.

Effects of carbon source concentration and presence of 2-deoxy-D-glucose (2-DG) and 6-deoxy-D-glucose (6-DG) on Lac12–GFP localization

Kluyveromyces lactis JA6-LAC12GFP was precultured in SC medium containing 2% galactose or lactose to late log phase ($OD_{600\,nm}$ 3–4). Cells were then harvested by centrifugation, washed with sterile water and resuspended in SC media with 0.1%, 0.5% or 2% galactose, glucose or lactose. The non-fermentable carbon source glycerol 3% (v/v) was used as a negative control of the inactivation process. In addition, cells were resuspended in SC medium with 2% of the nonmetabolizable glucose analogues 2-DG or 6-DG to determine the minimal number of metabolic steps required for inactivation of Lac12 permease. 2-DG and 6-DG were obtained from Sigma Chemical Company. Fluorescence images from preculture cells (0 h) were taken before washing and resuspension.

Fluorescence microscopy

At selected time intervals, the localization of the permease was observed by the presence of fluorescence in cellular compartments, as both strains contain the chimerical gene *LAC12–GFP*. The microscope was an Olympus BX50 with an oil immersion objective (× 100).

Measurement of [C^{14}]lactose uptake rates

The initial uptake rates of [D-glucose-1-^{14}C] lactose were measured as previously described elsewhere (Loureiro-Dias & Peinado, 1984). Cell suspension (20 μL; about 0.6 mg dry weight), 20 μL Tris/citrate buffer (pH 5.0, 100 mM) and 10 μL of a solution of [D-glucose-1-^{14}C] lactose (Amersham) were incubated at 28 °C for 10 s (the range of final concentrations of lactose was 0.05 to 5 mM and the specific activity was about 50 GBq mol^{-1}). Incorporation was stopped by addition of 5 mL ice-cold water. Cells were immediately filtered and washed with ice-cold water on GF/C Whatman glass-fiber filters. Radioactivity was counted in a liquid scintillation system (Beckman LS 6000SC). Controls were prepared by addition of 5 mL cold water before addition of labeled lactose. To check whether the measurements were good estimates of initial uptake rates, linearity of incorporation with time for periods up to 40 s was confirmed. All assays were done in duplicate. Final lactose concentration utilized was 0.20 mM.

Effect of LiCl on *K. lactis* growth and on Lac12–GFP internalization

Kluyveromyces lactis was precultured in SC medium containing 2% galactose to late log phase. Cells were harvested and diluted for plating in YP 2% galactose or glucose agar media containing 0 or 30 mM LiCl to investigate the effect of phosphoglucomutase inhibition on *K. lactis* growth in galactose. Colonies were examined after 48 h incubation at 30 °C. To test the effect of lithium on Lac12–GFP internalization, galactose-grown cells were shifted to SC medium containing 2% galactose, lactose or glucose, and 30 mM

LiCl. Samples were taken and cellular fluorescence was observed.

Results

Lac12–GFP localization is altered during *K. lactis* growth

The subcellular localization of Lac12–GFP was analyzed by fluorescence microscopy during 12 h growth with samples being taken each hour. Figure 1 shows yeast cells sampled after 2 and 4 h at log growth phase, and after 10 and 12 h at reduced growth rates. The images reveal permease internalization within the first hours in fresh medium. At later time points the intracellular fluorescence was reduced, whereas a strong fluorescence signal in the plasma membrane reappeared.

Galactose and lactose caused internalization of Lac12–GFP permease, even though these sugars are substrates for the permease. In lactose, the cells emitted fluorescence from intracellular compartments in all samples taken from the cultures during 12 h growth (Fig. 1a). The punctuated staining may reflect fragmented vacuoles or intracellular vesicles. When cells were grown in galactose, practically no internal fluorescence was observed after 10 h and the signal at the plasma membrane was very intense (Fig. 1b).

To analyze the influence of sugar concentration on Lac12–GFP internalization, galactose-grown cells were shifted to fresh media containing galactose or lactose at concentrations of 0.1%, 0.5% or 2% (Fig. 2). In 0.1% and 0.5% galactose, most of the fluorescence signal remained at the membrane after shifting and only a slight intracellular signal appeared; 2% galactose caused a strong internalization. Lactose caused rapid internalization of the permease even at a concentration of 0.1%. Glycerol, which is a nonfermentable carbon source, did not cause internalization of the permease.

Permease internalization is followed by reduction of lactose uptake rates

Galactose-grown cells were harvested at log phase and inoculated into fresh media containing 0.1% or 2% galactose. Subcellular Lac12–GFP distribution was then followed by fluorescence microscopy. Samples were taken after 1 and 3 h of inoculation for analysis of [C^{14}]lactose uptake rates. In 0.1% sugar, the transport rate and the fluorescence signal were not reduced after 3 h. In 2% galactose, the rates were consistent with the phenomenon of internalization of the permease as presented in Fig. 1: samples of yeast cells at time zero (preculture) showed higher transport activity, whereas after 3 h in fresh medium the transport rate decreased (Table 1). Lactose-grown cells also presented an intense reduction in lactose uptake 3 h after transfer to fresh 2% lactose medium.

Lactose uptake rates from galactose-grown cells were higher than those from cells lactose-grown (Table 1, 'preculture' line). This was expected as the fluorescence signal at the plasma membrane is more intense in galactose- than in lactose-grown cells (Fig. 1).

Blocking phosphoglucomutase inhibits galactose-, but not lactose- or glucose-induced internalization

To address the question whether galactose-induced internalization requires galactose metabolism, we tested the influence of lithium on yeast growth and on Lac12 internalization. Lithium is known to inhibit phosphoglucomutase (Masuda *et al.*, 2001), which is essential for galactose metabolism, as it catalyzes the interconversion of glucose-1-phosphate into glucose-6-phosphate (G6P). In *S. cerevisiae*

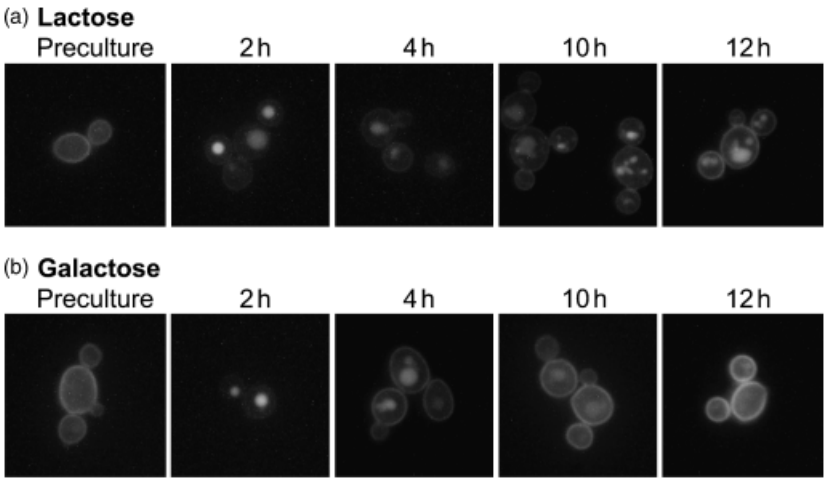

Fig. 1. Subcellular localization of the permease Lac12–GFP during growth of *Kluyveromyces lactis* in lactose and galactose. Yeast cells were pregrown in SC medium plus 2% lactose (a) or 2% galactose (b) and shifted to fresh lactose (a) or galactose (b) media, for an initial $OD_{600\,nm}$ of 0.2. Lac12–GFP fluorescence was analyzed at indicated times with a fluorescence microscope.

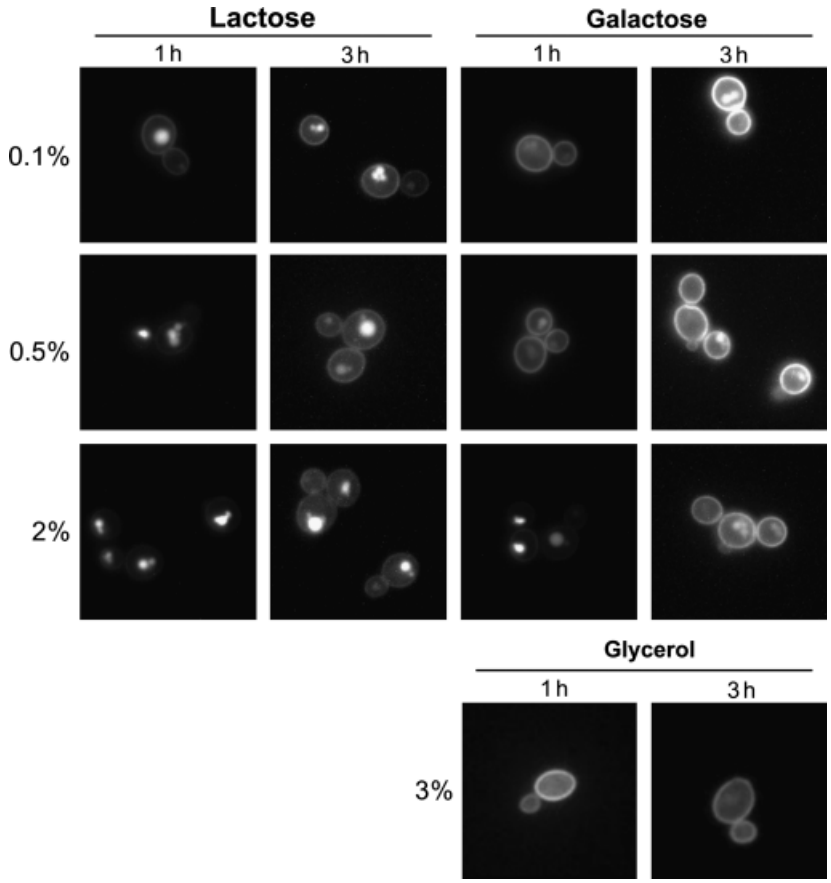

Fig. 2. Subcellular localization of the permease Lac12–GFP in SC media containing 0.1, 0.5 or 2% galactose or lactose, or 3% glycerol. Pregrown cells in SC 2% galactose medium were shifted to the specified carbon concentration. Lac12–GFP fluorescence was analyzed as in Fig. 1.

Table 1. [C^{14}]lactose uptake rates (μmol h^{-1} g^{-1} dry weight) in *Kluyveromyces lactis* cells

	Lactose	Galactose	
0 h (pre-culture)	477.57	787.48	
	2% Lactose	2% Galactose	0.1% Galactose
1 h	317.68	735.51	677.33
3 h	202.77	487.00	835.37

Kluyveromyces lactis JA6-Lac12GFP was precultivated in SC 2% lactose or 2% galactose media. [C^{14}]lactose uptake rates were measured from precultures, and 1 and 3 h after transfer to the indicated media. The [C^{14}]lactose concentration utilized in this experiment was 0.2 mM.

grown in glucose, the lithium inhibitory concentration (IC_{50}) is 100 mM, whereas in galactose the IC_{50} is 6 mM (Bro *et al.*, 2003). Growth assays in the presence of 30 mM LiCl confirmed that lithium inhibits the growth of *K. lactis* in galactose but not in glucose medium (Fig. 3a).

The effect of LiCl on Lac12–GFP internalization was determined in galactose-grown cells shifted to fresh SC media containing 30 mM LiCl and 2% galactose, lactose or glucose. Lithium prevented galactose- but not lactose- or glucose-induced Lac12 internalization (Fig. 3b). In lactose, the enzymatic hydrolysis of the disaccharide releases intracellular glucose, which apparently can generate the signal for Lac12–GFP internalization. In contrast, no such signaling occurs when galactose channeling into glycolysis is blocked. This suggests that signaling requires the formation of G6P.

2-DG, but not 6-DG, induces Lac12–GFP internalization

To directly address the question of whether G6P is involved in internalization, *K. lactis* cells pregrown on galactose were shifted to fresh media containing 2% 2-DG, 6-DG or galactose. 2-DG is a glucose analogue that can be phosphorylated but not further metabolized via the glycolytic pathway beyond 2-DG-6-phosphate. 2-DG is known to cause catabolite repression in *S. cerevisiae* and growth inhibition in galactose, maltose, ethanol and lactate in *K. lactis* strains (Betina *et al.*, 2001; Brondijk *et al.*, 2001). 6-DG is a glucose analogue that is transported into the cell but cannot be phosphorylated (Bisson & Fraenkel, 1983). After 2 h in fresh media the reduction of the fluorescence signal at the plasma membrane in the presence of galactose (positive control) or 2-DG indicates that 2-DG can cause internalization of Lac12–GFP (Fig. 4). In 6-DG, practically no internalization was observed. This result is consistent with a role of G6P as a signaling molecule.

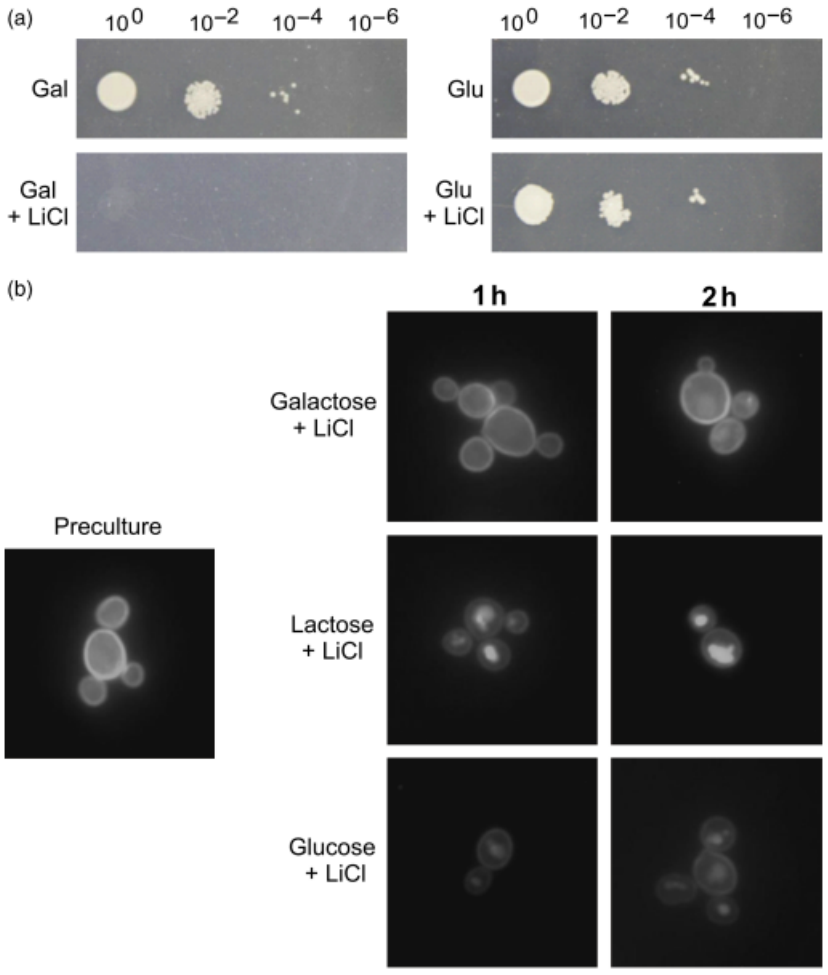

Fig. 3. Effect of LiCl on *Kluyveromyces lactis* growth and on Lac12–GFP internalization. (a) Pregrown cells in SC 2% galactose medium were harvested at log phase and diluted for plating in YP 2% galactose (Gal) or 2% glucose (Glu) agar media, containing 0 or 30 mM LiCl. Growth was documented after 48 h. (b) Pregrown cells at log phase in SC 2% galactose medium were shifted to fresh SC media with 2% of the indicated sugars plus 30 mM LiCl. Lac12–GFP fluorescence was analyzed after 1 and 2 h as in Fig. 1.

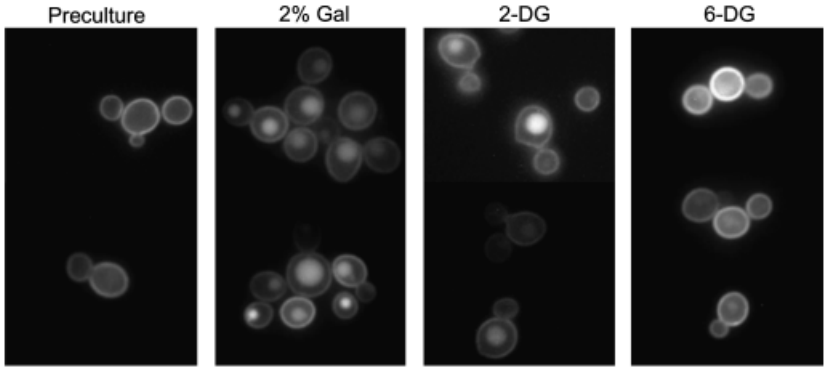

Fig. 4. The effect of the glucose analogues 2-deoxyglucose (2-DG) and 6-deoxyglucose (6-DG) on Lac12–GFP localization. Pregrown cells in SC 2% galactose medium were shifted to SC medium with 2% 2-DG, 6-DG or galactose (Gal). Lac12–GFP fluorescence was analyzed as in Fig. 1. Images were obtained after 2 h in fresh media.

Lac12 internalization is independent of KlSnf1 activity

The *K. lactis* protein kinase Snf1 has been implicated in post-translational regulation of the lactose permease and was proposed to contribute to persistence of Lac12–GFP in the plasma membrane (Wiedemuth & Breunig, 2005). To test whether Lac12 internalization is also dependent on KlSnf1 activity, the *K. lactis snf1* mutant strain JSD1R-LAC12GFP was grown in SC 2% galactose to late log phase and shifted to fresh SC 2% galactose or 3% glycerol media. After 1 and 2 h (Fig. 5), Lac12–GFP internalization occurred in 2% galactose medium, but no internalization was observed in glycerol, similar to results obtained with strain

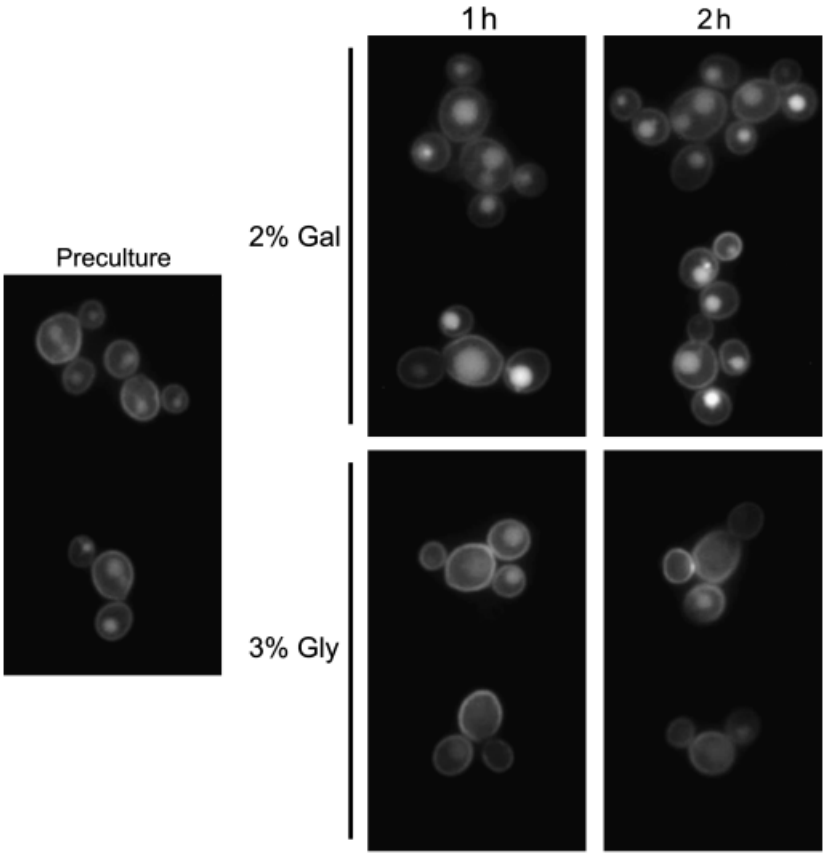

Fig. 5. Lac12 internalization in Kl*snf1* mutant strain. Cells were pregrown in SC 2% galactose to late log phase and shifted to fresh SC 2% galactose (Gal) or SC 3% glycerol (Gly) media. At indicated times, samples were collected and analyzed as in Fig. 1.

JA6-LAC12GFP. Thus, Snf1 signaling is apparently not required for internalization.

Return of Lac12–GFP to the plasma membrane is dependent on protein synthesis

Kluyveromyces lactis cells pregrown in galactose and transferred to fresh galactose medium show internalization of Lac12–GFP in the first hours and an increase of the fluorescence signal at the plasma membrane after some hours. To test whether this increase was due to *de novo* protein synthesis, galactose-grown cells were shifted to fresh SC 2% galactose, or SC 2% galactose+hygromycin (final concentration 200 $\mu g\,mL^{-1}$). Hygromycin is an inhibitor of protein synthesis that affects mRNA translation. On hour after transfer, Lac12–GFP internalization was observed in both media; after 4 h, the fluorescence signal had returned to the plasma membrane only in the medium without hygromycin (Fig. 6). This pattern also occurred in lactose. As a control, galactose-grown cells were also shifted to SC 0.1% galactose or SC 0.1% galactose+hygromycin; no internalization occurred in these media (data not shown), indicating that hygromycin did not cause the internalization in 2% sugar. This indicates that the return of the fluorescence to plasma membrane is dependent on protein synthesis – possibly *de novo* synthesis of Lac12–GFP.

Discussion

Kluyveromyces lactis, unlike *S. cerevisiae*, is one of the few yeasts capable of assimilating the milk sugar lactose, and this physiological ability determines many of the biotechnological applications of this yeast. The aim of this research was to investigate the post-translational control of the *K. lactis* lactose/galactose permease Lac12.

Our data showed that high concentrations of fermentable sugars induce internalization of the permease. Even the substrates of the permease, lactose and galactose, had such an effect. In galactose a higher concentration (> 0.5%) of the sugar was required before internalization was observed, whereas in lactose a concentration of 0.1% was sufficient for internalization. It is likely that efficient intracellular accumulation of lactose against a concentration gradient causes this higher sensitivity, whereas galactose might leak out via transporters of the diffusion facilitator family.

The gradual return of the fluorescence signal to the plasma membrane, observed while culture approached the deceleration growth phase in either galactose or lactose supports the

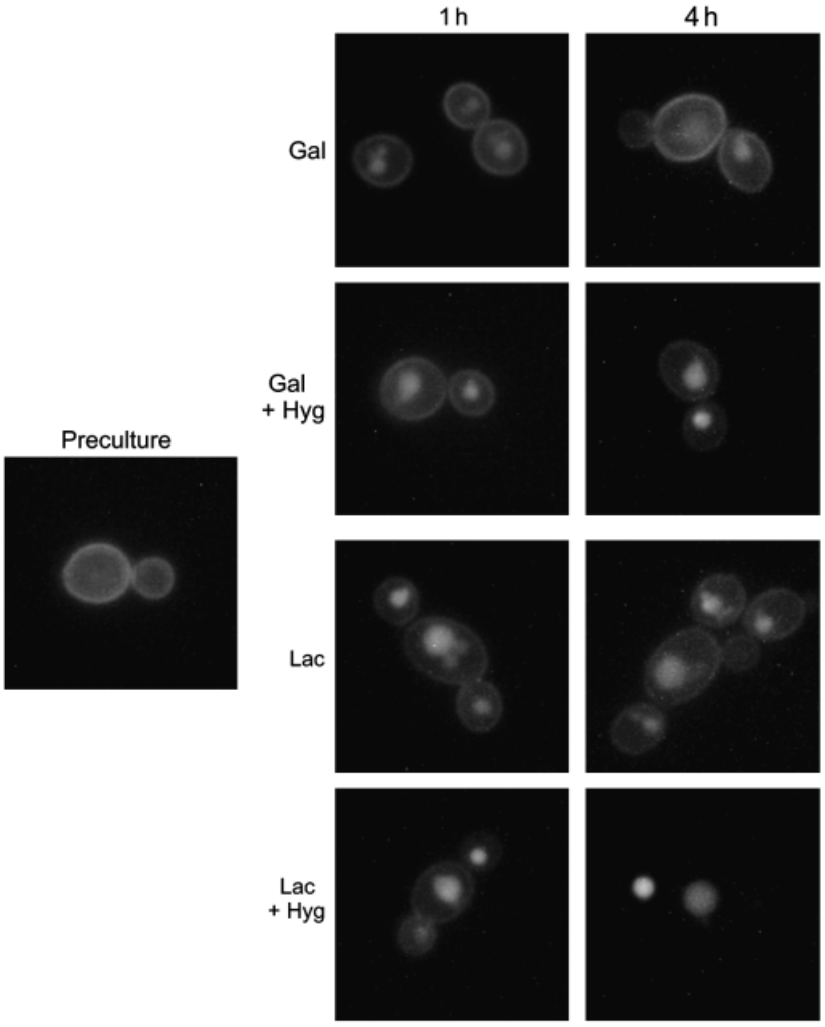

Fig. 6. Effect of protein synthesis impairment on Lac12–GFP internalization. Galactose-grown *Kluyveromyces lactis* cells were shifted to fresh SC galactose (Gal) or SC galactose + hygromycin (Hyg). Lactose-grown cells were shifted to fresh SC lactose (Lac) or SC lactose + hygromycin. Sugar concentration was 2% and hygromycin final concentration was 200 μg mL^{-1}. At indicated times, samples were collected and analyzed as in Fig. 1.

view that localization of the permease at the cell periphery is a response to the reduction in sugar concentration.

In cells cultured in lactose, fluorescence was detected in intracellular compartments during all phases of growth, whereas in galactose the intracellular fluorescence disappeared at the end of the log phase. While this might reflect differences in sugar consumption it could also result from the necessity to restrict lactose uptake. It is crucial for cell survival to prevent accumulation of intracellular lactose as shown by lethality of a β-galactosidase mutant exposed to lactose (Lodi & Donnini, 2005). Thus, permease internalization may be a mechanism to prevent excess sugar uptake that is not balanced by sugar consumption.

Consistently, [C^{14}]lactose uptake rates were reduced in the first hours of cell shifting to 2% sugar medium, paralleling the internalization of Lac12–GFP. Further, [C^{14}]lactose uptake measurements also confirmed that lactose causes more intense internalization than galactose and that 0.1% galactose is not sufficient to trigger the process.

Galactose is metabolized via the Leloir pathway, whose confluence with the glycolytic pathway occurs at the conversion of glucose-1-phosphate to G6P, catalyzed by phosphoglucomutase. G6P is already known to be a signal for catabolite repression in *S. cerevisiae* (Meijer *et al.*, 1998), and our results suggest that G6P is also the signaling molecule for internalization. The first evidence for this is that 2-DG but not 6-DG caused Lac12–GFP internalization. In *S. cerevisiae*, inactivation of maltose permease Mal61 is also caused by addition of 2-DG (Harma *et al.*, 2001). 2-DG is a nonmetabolizable glucose analogue that is transported and phosphorylated in the cell but does not proceed along the glycolytic pathway and so accumulates as 2-DG-6-phosphate (Pardo *et al.*, 1991). 2-DG is highly toxic to *S. cerevisiae*, and also causes growth inhibition in galactose, maltose, ethanol and lactate in *K. lactis* strains (Betina *et al.*, 2001; Brondijk *et al.*, 2001). The finding that lithium, which is known to inhibit phosphoglucomutase activity, blocked internalization by galactose but not by lactose or glucose, is a

second piece of evidence for signaling by G6P. In glucose and lactose media, intracellular G6P is formed by phosphorylation of glucose, taken up from the medium or released by lactose hydrolysis, respectively. The occurrence of internalization in these two sugars confirms that none of the possible indirect effects of lithium affected the cellular capability to promote internalization of the permease. We thus propose that galactose did not trigger internalization because G6P formation was impaired. The loss of phosphoglucomutase activity in *S. cerevisiae*, by mutation or lithium inhibition, generates a considerable increase in the G1P/G6P ratio in cells growing on galactose (Bro *et al.*, 2003; Csutora *et al.*, 2005).

The protein kinase Snf1 is one of the central regulators of carbon metabolism in *S. cerevisiae*, triggering activation of genes associated with alternative carbon sources metabolism. Mutant *snf1 K. lactis* cells expressing *LAC12-GFP* showed much less fluorescence at the plasma membrane and more fluorescence in internal vesicles (Wiedemuth & Breunig, 2005), indicating that the KlSnf1 kinase has an important post-translational role in the location of permease in the cells. We therefore analyzed whether Lac12 internalization is also dependent on KlSnf1 activity but found no influence of the *Klsnf1* mutation. This does not exclude that KlSnf1 contributes to regulation of Lac12 localization.

It appears that the observed return of the fluorescence to the plasma membrane after the permease internalization is dependent on protein synthesis, as it did not occur in cells transferred to 2% galactose or lactose when hygromycin, an inhibitor of protein synthesis, was present. Cells transferred to 0.1% galactose with or without hygromycin showed no internalization, leading to the conclusion that it was not the inhibitor that caused Lac12–GFP internalization. When the sugar concentration decreased with time during cultivation, the fluorescence signal at the membrane gradually increased. It is possible that this increase reflects *de novo* synthesis of Lac12–GFP, which is no longer subject to internalization because of reduced G6P formation rates.

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References

Betina S, Goffrini P, Ferrero I & Wésolowski-Louvel M (2001) *RAG4* gene encodes a glucose sensor in *Kluyveromyces lactis*. *Genetics* **158**: 541–548.

Bisson LF & Fraenkel DG (1983) Transport of 6-deoxyglucose in *Saccharomyces cerevisiae*. *J Bacteriol* **155**: 995–1000.

Breunig KD, Bolotin–Fukuhara M, Bianchi MM *et al.* (2000) Regulation of primary carbon metabolism in *Kluyveromyces lactis*. *Enzyme Microb Tech* **26**: 771–780.

Bro C, Regenberg B, Lagniel G, Labarre J, Montero-Lomelí M & Nielsen J (2003) Transcriptional, proteomic, and metabolic responses to lithium in galactose-grown yeast cells. *J Biol Chem* **278**: 32141–32149.

Brondijk TH, Konings WN & Poolman B (2001) Regulation of maltose transport in *Saccharomyces cerevisiae*. *Arch Microbiol* **176**: 96–105.

Chang Y & Dickson RC (1988) Primary structure of the lactose permease gene from the yeast *Kluyveromyces lactis*. Presence of an unusual transcript structure. *J Biol Chem* **263**: 16696–16703.

Csutora P, Strassz A, Boldizsár F, Németh P, Sipos K, Aiello DP, Bedwell DM & Miseta A (2005) Inhibition of phosphoglucomutase activity by lithium alters cellular calcium homeostasis and signaling in *Saccharomyces cerevisiae*. *Am J Physiol Cell Ph* **289**: C58–C67.

Dickson RC & Barr K (1983) Characterization of lactose transport in *Kluyveromyces lactis*. *J Bacteriol* **154**: 1245–1251.

Dong J & Dickson RC (1997) Glucose represses the lactose-galactose regulon in *Kluyveromyces lactis* through a *SNF1* and *MIG1*-dependent pathway that modulates galactokinase (*GAL1*) gene expression. *Nucleic Acids Res* **25**: 3657–3664.

Freer SN (1991) Fermentation and aerobic metabolism of cellodextrins by yeasts. *App Environ Microb* **57**: 655–659.

Harma T, Brondijk C, Konings WN & Poolman B (2001) Regulation of maltose transport in *Saccharomyces cerevisiae*. *Arch Microbiol* **176**: 96–105.

Hedbacker K & Carlson M (2008) SNF1/AMPK pathways in yeast. *Front Biosci* **13**: 2408–2420.

Horak J & Wolf DH (1997) Catabolite inactivation of the galactose transporter in the yeast *Saccharomyces cerevisiae*: ubiquitination, endocytosis, and degradation in the vacuole. *J Bacteriol* **179**: 1541–1549.

Jiang H, Medintz I & Michels CA (1997) Two glucose sensing/signaling pathways stimulate glucose-induced inactivation of maltose permease in *Saccharomyces*. *Mol Biol Cell* **8**: 1293–1304.

Jiang H, Medintz I, Zhang B & Michels C (2000) Metabolic signals trigger glucose-induced inactivation of maltose permease in *Saccharomyces*. *J Bacteriol* **182**: 647–654.

Lodi T & Donnini C (2005) Lactose-induced cell death of β-galactosidase mutants in *Kluyveromyces lactis*. *FEMS Yeast Res* **5**: 727–734.

Loureiro-Dias MC & Peinado JM (1984) Transport of maltose in *Saccharomyces cerevisiae*. Effect of pH and potassium ions. *Biochem J* **222**: 293–298.

Masuda CA, Xavier MA, Mattos KA, Galina A & Montero-Lomeli M (2001) Phosphoglucomutase is an *in vivo* lithium target in yeast. *J Biol Chem* **276**: 37794–37801.

Medintz I, Jiang H, Han E, Cui W & Michels CA (1996) Characterization of the glucose-induced inactivation of maltose permease in *Saccharomyces cerevisiae*. *J Bacteriol* **178**: 2245–2254.

Meijer MMC, Boonstra J, Verkleij AJ & Verrips CT (1998) Glucose repression in *Saccharomyces cerevisiae* is related to the glucose concentration rather than the glucose flux. *J Biol Chem* **273**: 24102–24107.

Milkowski C, Krampe S, Weirich J, Hasse V, Boles E & Breunig KD (2001) Feedback regulation of glucose transporter gene transcription in *Kluyveromyces lactis* by glucose uptake. *J Bacteriol* **183**: 5223–5229.

Pardo LA, Sánchez LM, Lazo PS & Ramos S (1991) *In vitro* activation of the *Saccharomyces cerevisiae* Ras/adenylate cyclase system by glucose and some of its analogues. *FEBS* **290**: 43–48.

Riley MI, Sreekrishna K, Bhairi S & Dickson RC (1987) Isolation and characterization of mutants of *Kluyveromyces lactis* defective in lactose transport. *Mol Gen Genet* **208**: 145–151.

Rubio-Texeira M (2006) Endless versatility in the biotechnological applications of *Kluyveromyces* LAC genes. *Biotech Adv* **28**: 212–225.

Wiedemuth C & Breunig KD (2005) Role of Snf1p in regulation of intracellular sorting of the lactose and galactose transporter Lac12p in *Kluyveromyces lactis*. *Eukaryot Cell* **4**: 716–721.