

Catabolite inactivation of the high-affinity hexose transporters Hxt6 and Hxt7 of *Saccharomyces cerevisiae* occurs in the vacuole after internalization by endocytosis

Stefanie Krampe, Olaf Stamm, Cornelis P. Hollenberg, Eckhard Boles*

Institut für Mikrobiologie, Heinrich-Heine-Universität, Universitätsstr. 1, Geb. 26.12.01, D-40225 Düsseldorf, Germany

Received 3 November 1998

Abstract After addition of high concentrations of glucose, rates of high-affinity glucose uptake in *Saccharomyces cerevisiae* decrease rapidly. We found that the high-affinity hexose transporters Hxt6 and Hxt7 are subject to glucose-induced proteolytic degradation (catabolite inactivation). Degradation occurs in the vacuole, as Hxt6/7 were stabilized in proteinase A-deficient mutant cells. Degradation was independent of the proteasome. The half-life of Hxt6 and Hxt7 strongly increased in *end4*, *ren1* and *act1* mutant strains, indicating that the proteins are delivered to the vacuole by endocytosis. Moreover, both proteins were also stabilized in mutants defective in ubiquitination. However, the initial signal that triggers catabolite inactivation is not relayed via the glucose sensors Snf3 and Rgt2.

© 1998 Federation of European Biochemical Societies.

Key words: Sugar transport; Glucose; Endocytosis; Vacuole; Ubiquitination; Yeast

1. Introduction

Several strategies are used by *Saccharomyces cerevisiae* to accommodate the activities of the hexose uptake system to different environmental conditions [1]. Glucose itself is the most important regulator and affects hexose transport activities at several levels. On the one hand, the extracellular concentration of glucose tightly controls transcription of various *HXT* genes encoding glucose transporters with different kinetic characteristics [2,3]. On the other hand, the intracellular glucose concentration seems to exert feedback regulation on glucose transport activities [4]. Moreover, posttranscriptional mechanisms control the actual number of transporter proteins in the plasma membrane [1].

One as yet insufficiently investigated aspect of regulation of hexose transport concerns the stability of the transporter proteins. It is known that under certain starvation conditions high- and low-affinity components of the hexose uptake system are specifically inactivated [5]. Moreover, a component of high-affinity glucose uptake is subject to catabolite inactivation, a degradation process that is triggered by the addition of high concentrations of glucose to derepressed yeast cells [5,6]. Here, we show that the high-affinity glucose transporters Hxt6 and Hxt7 are subject to glucose-induced degradation in the vacuole.

2. Materials and methods

2.1. Yeast strains, media and growth conditions

The genotypes of the yeast strains used in this work are listed in Table 1. Rich media were based on 1% yeast extract and 2% peptone (YEP), minimal media consisted of 0.17% Difco yeast nitrogen base without amino acids and ammonium (YNB), supplemented with various carbon sources. Yeast cells were grown aerobically at 25°C (temperature-sensitive mutants) or 30°C on a rotary shaker. Cell growth was monitored by measuring optical densities at 600 nm (OD₆₀₀). For Western analysis of Hxt6/7 and for glucose uptake measurements, wild-type and mutant strains were grown to an OD₆₀₀ of 1.0–1.5 in YEP medium with 3% raffinose (*HXT6/7*-inducing conditions). To induce inactivation, cells were harvested during exponential growth, washed with YNB medium and resuspended in YNB medium with 5% glucose at 30°C or 37°C (temperature-sensitive mutants). We routinely used nitrogen-free medium, as these conditions slightly accelerate the inactivation process ([7]; data not shown). Moreover, dilution of the proteins by cell growth is avoided. Glucose uptake was assayed as described previously [8] with modifications according to Walsh et al. [9].

2.2. Preparation of Hxt6/7 and Hxt1 antibodies

To prepare antibodies directed against Hxt6 and 7, a PCR-amplified DNA fragment encoding amino acids 17–47 of the hydrophilic N-terminal region of Hxt6/7 was fused in frame to the C-terminus of glutathione-S-transferase (GST) in pGEX-3X (Pharmacia). This region exhibits no similarities to the other hexose transporters. Likewise, for the preparation of antibodies against Hxt1, a DNA fragment encoding amino acids 17–49 of Hxt1 was fused to the C-terminus of GST. The Hxt6/7-GST and Hxt1-GST fusion proteins were expressed in *Escherichia coli*, purified by affinity chromatography using glutathione-Sepharose columns, and used for the immunization of rabbits by standard injection regimens (Eurogentec). The antisera were purified by affinity chromatography using the Hxt1-GST and Hxt6/7-GST fusion proteins coupled to Affi-gel-15 (Bio-Rad) columns.

2.3. Preparation of crude extracts and Western blot analysis

2.3.1. Preparation of total protein extracts. Cells were harvested by centrifugation, and washed once with 10 mM Na₂SO₄/NaF solution. Cells were resuspended in 100 µl of lysis buffer (0.3 M sorbitol, 50 mM MOPS, 10 mM Na₂SO₄, pH 7.5; plus protease inhibitor cocktail tablets, Boehringer Mannheim). Cells were broken by shaking the suspension with glass beads (ϕ=0.45 mm), 200 µl of sample buffer was added (4% SDS, 20% glycerol, 125 mM Tris-HCl, 20 mM DTT, pH 6.8) and proteins were denatured by incubating the suspensions for 5 min at 95°C. Insoluble cell components were removed by centrifugation.

2.3.2. Preparation of membrane fractions. Cells were harvested by centrifugation, washed twice with water and frozen quickly. Glass beads, 200 µl of Tris Mix (0.1 M Tris-HCl, pH 7.5, 0.15 mM NaCl, 5 mM EDTA) with 0.2 mM PMSF was added and the cell suspension was shaken on a vibrax for 3 min. After addition of 200 µl of Tris Mix, the supernatant was centrifuged for 45 min at 12 000 × g. The pellet was quickly rinsed with 400 µl of Tris Mix containing 5 M urea and incubated for 30 min on ice. The membrane fraction was centrifuged for 45 min at 12 000 × g. Then 320 µl of Tris Mix was added to the pellet. Proteins were precipitated by adding 80 µl of 50% trichloroacetic acid and the samples were incubated for 30 min on ice. The precipitates were collected by centrifugation for 30 min at 12 000 × g.

*Corresponding author. Fax: (49) (211) 811 5370.

E-mail: boles@uni-duesseldorf.de

Dedicated to the memory of Professor Manfred Rizzi.

Table 1
Yeast strains

Strain	Genotype	Source
CMY 1001	<i>MATa MAL61/HA MAL12 MAL13 GAL⁺ leu2 ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200</i>	C. Michels
CMY 1003	<i>ren1Δ::LEU2</i> , isogenic to CMY1001	C. Michels
DBY 1689	<i>MATa, his4-619</i>	D. Botstein
DBY 4878	<i>MATa, his4-619, act1-1</i> , isogenic to DBY1689	D. Botstein
RKY#534	<i>MATa bar1-1 leu2 his4 ura3</i>	R. Kölling
RKY#536	<i>MATa bar1-1 leu2 his4 ura3 end4</i> , isogenic to RKY#534	R. Kölling
RKY#649	<i>MATa ura3 leu2-3, 112 his3-11,15 GAL⁺ CAN^s</i>	R. Kölling
RKY#927	<i>MATa ura3 leu2-3,112 his3-11,15 GAL⁺ CAN^s pre1-1 pre2-2</i> , isogenic to RKY#649	R. Kölling
RKY#826	<i>MATa ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 lys2-801</i>	R. Kölling
RKY#969	<i>MATa ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 lys2-801 Δpep4::URA3</i> , isogenic to RKY#826	R. Kölling
CEN.PK2-1C	<i>MATa leu2-3,112 ura3-52 trp1-289 his3-Δ1 MAL2-8^c SUC2</i>	K.D. Entian
CEN.PK2-1D	<i>MATa leu2-3,112 ura3-52 trp1-289 his3-Δ1 MAL2-8^c SUC2</i>	K.D. Entian
SKY1	<i>MATa leu2-3,112 ura3-52 trp1-289 his3Δ1 MAL2-8^c SUC2 Δsnf3::kanMX</i> , isogenic to CEN.PK2-1C	This study
SKY2	<i>MATa leu2-3, 112 ura3-52 trp1-289 his3-Δ1 MAL2-8^c SUC2 Δrgt2::kanMX</i> , isogenic to CEN.PK2-1D	This study
23344c	<i>MATa ura3</i>	B. André
27038a	<i>MATa np1 ura3</i> , isogenic to 23344c	B. André
27002d	<i>MATa np2 ura3</i> , isogenic to 23344c	B. André
RE700A	<i>MATa ura3-52 Δhxt1 Δhxt2 Δhxt3 Δhxt4 Δhxt5 Δhxt6 Δhxt7</i>	E. Reifenberger

and pellets were diluted in 100 μl of sample buffer (0.4 M Tris-HCl, 225 mM NaCl, 7.5 mM EDTA).

Equal amounts of protein were loaded into each lane of a standard 10% acrylamide gel. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by standard methods. The proteins were then blotted onto a PVDF membrane (Millipore). The filters were incubated in phosphate-buffered saline (PBSTB buffer: 50 mM K₂PO₄, pH 5.7, 100 mM NaCl, 0.1% Tween 20, 10% BSA) by shaking for 2 h at room temperature. Antiserum against the yeast hexose transporters (diluted 1:500 in PBSTB buffer) was added, and the filter was incubated on a rotary shaker overnight at room temperature. After washing 3 times in PBST (50 mM K₂PO₄, pH 5.7, 100 mM NaCl, 0.1% Tween 20) goat anti-rabbit peroxidase conjugate (Bio-Rad), diluted 1:10 000, was used as secondary antibody. The yeast hexose transporters were visualized using NBT/BCIP dye detection.

2.4. Construction of *snf3* and *rgt2* deletion mutant strains

Yeast strains lacking the glucose sensor genes *SNF3* and *RGT2* were constructed by the 'short flanking homology PCR' technique and the *loxP::kanMX::loxP/Cre*-recombinase system [10]. The primers used for the construction of the *SNF3* replacement PCR constructs were: (1) 5'-ATGGATCCTAATAGTAACAGTTCTAGCGG-AAACATTACGCCAAGAGAAAATTCGTACGCTGCAGGTCGAC-3', and (2) 5'-TATTTCAAATCATTATTTTTCATTTCACAGGTTGATTAGTGGCGTTTTTCGCATAGGCCACTAGTGGATCTG-3'. The primers used for the construction of the *RGT2* replacement PCR constructs were: (1) 5'-ATGAACGATAGCCAAAACCTGCCTACGAC-AGAGGGAAAGAAAATAGTCATTTTCGTACGCTGCAGGTCGAC-3' and (2) 5'-GGGGGAAGTGTATTGGCTGTGCTTACTAGCGAGG-TCACTCGTCCAATTGCATAGGCCACTAGTGGATCTG-3' (homology to the sugar transporter genes is in bold; homology to *kanMX* cassette is in italics). Yeast transformation was as described [11]. The transformants were plated on YEP/2% maltose medium supplemented with G418 (200 mg/l). The correct replacements were confirmed by PCR.

2.5. Construction of HXT plasmids

Three different 2μ-based multicopy plasmids were constructed for the constitutive expression of *HXT1* or *HXT7*, or the simultaneous expression of *HXT1* and *HXT7*. To construct the plasmid YEAHXT1, a 4.4-kb *Bam*HI fragment containing the *ADH1promoter-HXT1* fusion construct from plasmid YIpAPHXT1 [3] was re-cloned into YEplac195 [12]. For plasmid YEpkHXT7, a 2.5-kb *Sph*I/*Xba*I fragment from plasmid p21-PST containing the *HXT7* gene with only 480 bp of its promoter region [13] was re-cloned into YEplac195. The plasmid YkH7AH1 contains a 4.4-kb *Bam*HI fragment comprising the *ADH1promoter-HXT1* fusion, cloned into the vector YEpkHXT7.

3. Results and discussion

3.1. Glucose-induced inactivation of high-affinity glucose transporters *Hxt6* and *Hxt7*

Yeast cells were grown in rich medium with raffinose, harvested and incubated for up to 5 h in nitrogen starvation medium, supplemented either with 5% glucose or, as a control, 3% raffinose (due to the extracellular hydrolysis of raffinose by invertase, this carbon source is used to ensure a constant supply of low concentrations of glucose). Under both conditions, glucose uptake kinetics may be described by a biphasic system with a high- and a low-affinity component (Fig. 1). However, in the cells incubated with 5% glucose the glucose transport capacity of the high-affinity component was reduced by more than 50% as compared to the cells incubated with raffinose. Moreover, high-affinity glucose uptake kinetics were shifted to significantly higher *K_m* values. These observations confirm previous findings [6,14] that a component of high-affinity glucose transport in *S. cerevisiae* is subject to glucose-induced inactivation.

Hxt6 and *Hxt7* are known to be the hexose transporters with the highest affinities for glucose [3]. Because of the high similarity between these proteins [13] it is not possible to distinguish between *Hxt6* and *Hxt7* in Western analyses. Raffinose-grown wild-type yeast cells were subject to catabolite inactivation conditions, harvested at different times, and the relative amount of *Hxt6/7* protein was determined by Western analysis with *Hxt6/7*-specific antibodies. Rapid degradation of the *Hxt6/7* proteins was observed under catabolite inactivation conditions (Fig. 2). The half-life of *Hxt6/7* hexose transporters in 5% glucose medium without ammonium was about 45 min. Thus, glucose-induced loss of high-affinity glucose transport activity parallels the decline in *Hxt6/7* protein levels.

3.2. Degradation of *Hxt6/7* occurs in the vacuole after internalization by endocytosis

Yeast cells have two major protein degradation pathways, the cytoplasmic proteasome complex and the vacuole [15,16]. To investigate the route of glucose-induced degradation of

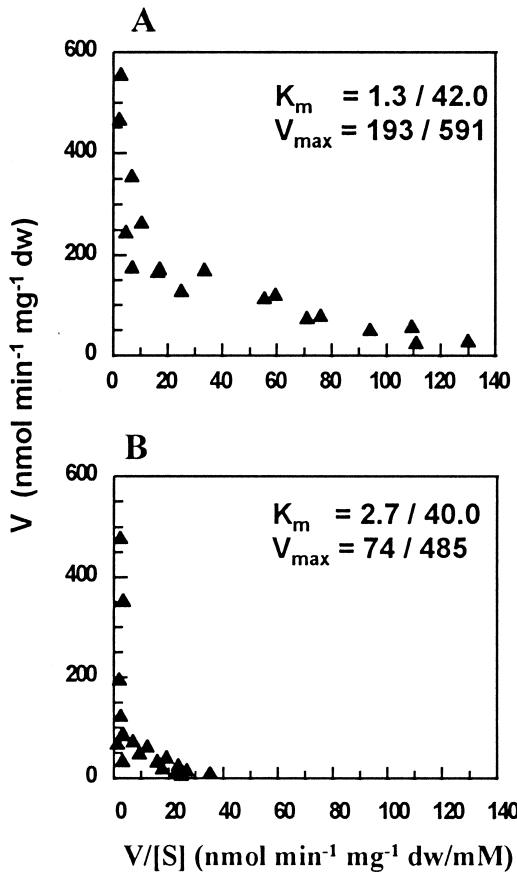


Fig. 1. Eadie-Hofstee plots of glucose uptake kinetics in *S. cerevisiae* cells. Strain RKY#826 was grown in YEP with 3% raffinose, and harvested during exponential growth. The cells were washed and transferred to ammonium-free YNB medium containing (A) 3% raffinose or (B) 5% glucose (catabolite inactivation conditions). After incubation for 5 h at 30°C, glucose uptake activities were determined. The results from three independent experiments are presented. K_m in mM, V_{max} in nmol min⁻¹ mg⁻¹ dw. dw = dry weight.

Hxt6/7 we examined the stability of both proteins in mutant strains affected in either proteasomal or vacuolar proteolytic activity. The essential genes *PRE1* and *PRE2* encode β -subunits of the proteasome responsible for the chymotrypsin-like activity of the enzyme complex [17,18]. The stability of Hxt6/7 was not affected in a temperature-sensitive *pre1-1 pre2-2* mutant strain at the restrictive temperature of 37°C (Fig. 2). Pep4

is the master protease in the vacuolar lumen, and its loss essentially results in a proteolytically inactive vacuole [19,20]. In contrast to the corresponding wild-type strain, Hxt6 and Hxt7 were stable for more than 6 h in a *pep4* deletion mutant strain under inactivation conditions (Fig. 2). These data demonstrate that cellular turnover of Hxt6/7 mainly depends on vacuolar proteolysis and not on the cytoplasmic proteasome.

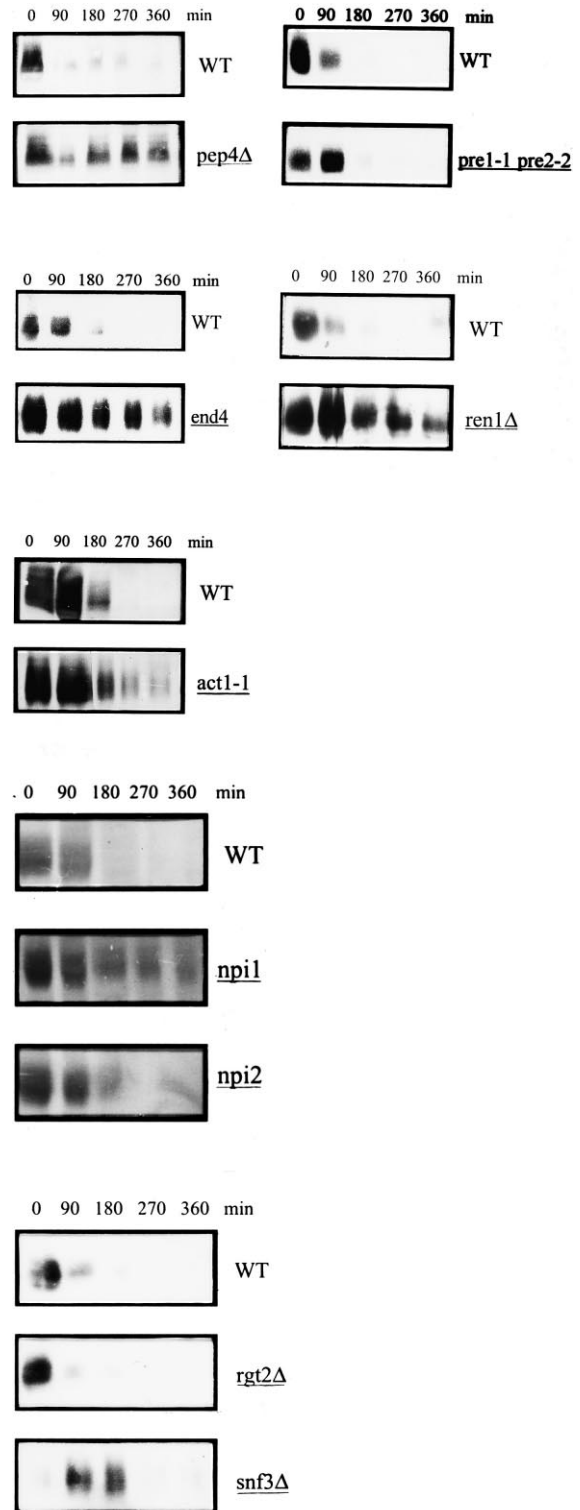


Fig. 2. Stability of Hxt6 and Hxt7 under catabolite inactivation conditions. The strains RKY#969 ($\Delta pep4$), RKY#927 (*pre1-1 pre2-2*), RKY#536 (*end4*), CMY1003 (*ren1*), DBY4878 (*act1-1*), 27038a (*np1*), 27002d (*np2*), SKY1 (*snf3*) and SKY2 (*rgt2*) and their isogenic wild-type strains RKY#826, RKY#649, RKY#534, CMY1001, DBY1869, 23344c, CEN.PK2-1C/D, respectively, were grown to the early exponential phase in YEP medium with 3% raffinose, at 25°C (RKY#927, RKY#649, RKY#536, RKY#534, DBY4878, DBY1869) or 30°C (other strains). Cells were shifted to nitrogen starvation medium containing 5% glucose, and incubated at 37°C (RKY#927, RKY#649, RKY#536, RKY#534, DBY4878, DBY1869) or 30°C (other strains). At the indicated times, cells were harvested, protein extracts (RKY#969, RKY#826, CMY strains, 27038a, 27002d, 23344c) or membrane fractions (RKY#927, RKY#649, RKY#536, RKY#534, DBY strains, CEN.PK strains) were prepared, and the proteins were subjected to Western analysis with Hxt6/7-specific antibodies. The experiments were repeated twice with similar results.

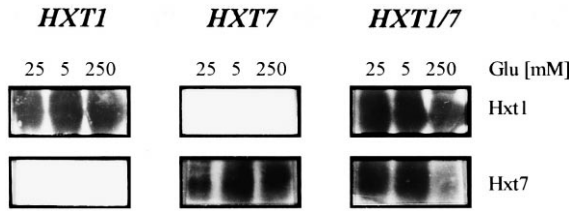


Fig. 3. Coexpression of Hxt1 and Hxt7. The strain RE700 (*hxt1-7*) was transformed with plasmids YEAHXT1 (*HXT1*), YEpkHXT7 (*HXT7*) and YkH7AH1 (*HXT1+HXT7*). Transformants were grown on selective medium with 25 mM glucose to the early exponential phase. Cells were harvested and shifted to selective medium with either 5 mM or 250 mM glucose. After 6 h cells were harvested, protein extracts were prepared, and the proteins were subjected to Western analysis with Hxt1- and Hxt6/7-specific antibodies.

Degradation of Hxt6/7 in the vacuole implies that the proteins must first be delivered from the plasma membrane to the vacuole. The main route for degradation of plasma membrane proteins is via internalization by endocytosis and subsequent delivery to the vacuole [21–23]. Internalization of Hxt6/7 during catabolite inactivation conditions was studied with either *end4^{ts}*, *ren1 Δ* or *act1-1^{ts}* mutants, all defective in endocytosis [24–26]. As can be seen in Fig. 2, the hexose transporters Hxt6/7 were highly stabilized in all three mutant strains, as compared to the corresponding wild types. Our data suggest that under high glucose conditions the hexose transporters Hxt6/7 are rapidly internalized via endocytosis and transported to the vacuole by vesicular carriers.

Moreover, as degradation of Hxt6 and Hxt7 was substantially reduced in *npi1/rsp5* ubiquitin-protein ligase- and *npi2l/doa4* ubiquitin-protein hydrolase-deficient mutant strains [27,28] (Fig. 2), ubiquitination may also be involved in catabolite inactivation of the glucose transporters.

3.3. The glucose sensors Snf3 and Rgt2 are not required for glucose-induced degradation of Hxt6/7

Catabolite inactivation of Hxt6/7 is observed only in the presence of high concentrations of glucose. This implies the existence of a glucose sensing system which senses changes in the external glucose concentration, and finally triggers internalization and degradation of Hxt6/7. It has been shown that the glucose sensor Rgt2, but not Snf3, is involved in mediating the signal for glucose-induced degradation of the maltose permease [29]. In contrast, we could not observe an increase in the stability of Hxt6/7 in *rgt2* mutant cells (Fig. 2). Moreover, about 90 min after addition of 5% glucose to *snf3* mutant cells a rapid decline of Hxt6/7 protein levels sets in (Fig. 2). The low amount of Hxt6/7 protein after growth on a mixture of 2% maltose and 2% raffinose is consistent with the finding that low-glucose induced transcription of *HXT6* and *HXT7* is dependent on Snf3 [30]; R. Wiczorke and E. Boles, unpublished results). Our results indicate that neither of both glucose sensors is involved in triggering the catabolite inactivation signal of Hxt6/7.

3.4. High- and low-affinity glucose transporters can coexist in the cell

It has been speculated that the simultaneous presence of high- and low-affinity glucose transporter proteins might be mutually exclusive [31]. Therefore, it might be speculated that the inactivation of the high-affinity transporters Hxt6 and

Hxt7, after addition of high concentrations of glucose, is triggered by the high glucose-induced appearance of low-affinity glucose transporters, e.g. Hxt1 [2,3]. To investigate this possibility, we simultaneously expressed the low-affinity Hxt1 glucose transporter under transcriptional control of the constitutive *ADHI* promoter and the high-affinity Hxt7 transporter downstream of a truncated version of its own promoter that is no longer subject to glucose repression, in an *hxt1-7* glucose transporter-less strain [13]. Both proteins, Hxt1 and Hxt7, could be detected in the strain expressing both genes (Fig. 3), demonstrating that different types of glucose transporter proteins can coexist in the same cell.

3.5. Conclusions

On the basis of our results, Hxt6 and Hxt7 belong to the group of yeast membrane proteins whose stabilities are regulated by the physiological state of the cells and/or the presence or absence of specific ligands. To date, this group of proteins includes transporters that mediate uptake of maltose, galactose, amino acids, inositol, uracil, a-factor as well as the α - and α -factor receptors [7,21–23]. However, the significance of the rapid degradation of high-affinity glucose transporters is not obvious. Theoretically, high-affinity transporters should also contribute to the transport of glucose even at high concentrations. Characterization of yeast cells that constitutively express high-affinity glucose transporters under conditions in which internalization and proteolytic degradation are blocked should reveal the physiological significance of glucose-induced inactivation of high-affinity glucose transport.

Acknowledgements: We thank B. André, R. Kölling, E. Reifenberger, C. Michels, K.-D. Entian and David Botstein for kind provision of yeast strains. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (BO 151/1-1) to E.B.

References

- [1] Boles, E. and Hollenberg, C.P. (1997) FEMS Microbiol. Rev. 21, 85–111.
- [2] Özcan, S. and Johnston, M. (1995) Mol. Cell. Biol. 15, 1564–1572.
- [3] Reifenberger, E., Boles, E. and Ciriacy, M. (1997) Eur. J. Biochem. 245, 324–333.
- [4] Teusink, B., Diderich, J.A., Westerhoff, H.V., van Dam, K. and Walsh, M.C. (1998) J. Bacteriol. 180, 556–562.
- [5] Busturia, A. and Lagunas, R. (1986) J. Gen. Microbiol. 132, 379–385.
- [6] Ramos, J., Szkutnicka, K. and Cirillo, V.P. (1988) J. Bacteriol. 170, 5375–5377.
- [7] Horak, J. and Wolf, D.H. (1997) J. Bacteriol. 179, 1541–1549.
- [8] Özcan, S., Freidel, K., Leuker, A. and Ciriacy, M. (1993) J. Bacteriol. 175, 5520–5528.
- [9] Walsh, M.C., Smits, H.P., Scholte, M. and van Dam, K. (1994) J. Bacteriol. 176, 953–958.
- [10] Güldener, U., Heck, S., Fiedler, T., Beinhauer, J. and Hegemann, J.H. (1996) Nucleic Acids Res. 24, 2519–2524.
- [11] Dohmen, R.J., Strasser, A.W.M., Höner, C.B. and Hollenberg, C.P. (1991) Yeast 7, 691–692.
- [12] Gietz, R.D. and Sugino, A. (1988) Gene 74, 527–534.
- [13] Reifenberger, E., Freidel, K. and Ciriacy, M. (1995) Mol. Microbiol. 16, 157–167.
- [14] Busturia, A. and Lagunas, R. (1985) Biochim. Biophys. Acta 820, 324–325.
- [15] Hochstrasser, M. (1995) Curr. Opin. Cell. Biol. 7, 215–223.
- [16] Ciechanover, A. (1994) Cell 79, 13–21.
- [17] Heinemeyer, W., Kleinschmidt, J.A., Saidowsky, J., Escher, C. and Wolf, D.H. (1991) EMBO J. 10, 555–562.

- [18] Heinemeyer, W., Gruhler, A., Mohrle, V., Mahe, Y. and Wolf, D.H. (1993) *J. Biol. Chem.* 268, 5115–5120.
- [19] Hemmings, B.A., Zubenko, G.S., Hasilik, A. and Jones, E.W. (1981) *Proc. Natl. Acad. Sci. USA* 78, 435–439.
- [20] Ammerer, G., Hunter, C.P., Rothman, J.H., Saari, G.C., Valls, L.A. and Stevens, T.H. (1986) *Mol. Cell. Biol.* 7, 2490–2499.
- [21] Kölling, R. and Hollenberg, C.P. (1994) *EMBO J.* 13, 3261–3271.
- [22] Hicke, L. (1997) *FASEB J.* 11, 1215–1226.
- [23] Wendland, B., Emr, S.D. and Riezman, H. (1998) *Curr. Opin. Cell Biol.* 10, 513–522.
- [24] Raths, S., Rohrer, J., Crausaz, F. and Riezmann, H. (1993) *J. Cell Biol.* 120, 55–65.
- [25] Davis, N.G., Horecka, J.L. and Sprague, G.F. (1993) *J. Cell Biol.* 122, 53–65.
- [26] Novick, P., Osmond, B.C. and Botstein, D. (1989) *Genetics* 121, 659–674.
- [27] Hein, C., Springael, J.Y., Volland, C., Haguenaer-Tsapis, R. and André, B. (1995) *Mol. Microbiol.* 18, 77–87.
- [28] Papa, F.R. and Hochstrasser, M. (1993) *Nature* 366, 313–319.
- [29] Jiang, H., Medintz, I. and Michels, C.A. (1997) *Mol. Biol. Cell* 8, 1293–1304.
- [30] Liang, H. and Gaber, R.F. (1996) *Mol. Biol. Cell* 7, 1953–1966.
- [31] Reifenberger, E. (1995) PhD thesis, Heinrich-Heine-Universität, Düsseldorf.