Note

A Glucose Transporter Chimera Confers a Dominant Negative Glucose Starvation Phenotype in *Saccharomyces cerevisiae*

Peter W. Sherwood,[1] Iskra Katic, Pascual Sanz[2] and Marian Carlson

Department of Genetics and Development, Columbia University, New York, New York 10032

Manuscript received January 5, 2000
Accepted for publication February 24, 2000

ABSTRACT

A family of glucose transporters mediates glucose uptake in *Saccharomyces cerevisiae*. We show that the dominant mutation *GSF4-1*, which impairs glucose repression of *SUC2*, results in a nonfunctional chimera of the transporters Hxt1p and Hxt4p. Hxt1/4p inhibits the function of wild-type glucose transporters. Similar mutations may facilitate analysis of the major facilitator superfamily.

THE extracellular concentration of glucose strongly influences gene expression and cell physiology in many organisms. In eukaryotes, a family of glucose transporters with 12 transmembrane domains mediates glucose uptake via facilitated diffusion. These transporters, encoded by the *GLUT* genes in mammals and by the *HXT* genes in *Saccharomyces cerevisiae* (Mueckler *et al.* 1985; Bisson *et al.* 1993; Mueckler 1994; Kruckeberg 1996; Boles and Hollenberg 1997; Charron *et al.* 1999; Ozcan and Johnston 1999), are members of the sugar permease family within the major facilitator superfamily (Marger and Saier 1993; Nelissen *et al.* 1997).

The yeast hexose transporters have diverse kinetic properties and patterns of expression (Bisson *et al.* 1993; Kruckeberg 1996; Boles and Hollenberg 1997; Reifenberger *et al.* 1997; Ozcan and Johnston 1999). Six genes (*HXT1–HXT4*, *HXT6*, *HXT7*) encode the major proteins responsible for glucose transport. Their transcription is regulated by the concentration of glucose in the environment, and the different genes are induced and/or repressed by different levels of glucose (Özcan and Johnston 1995).

We previously isolated mutants of *S. cerevisiae*, designated *gsf* (*g*lucose *s*ignaling *f*actor), that are defective in glucose repression of *SUC2* transcription (Sherwood and Carlson 1997). Defects in glucose transport can confer this phenotype (Sherwood and Carlson 1999). Here we have characterized the dominant mutation *GSF4-1*. During growth on high concentrations of glucose, the *GSF4-1* mutant expresses *SUC2* at levels 20-fold higher than the wild type and exhibits a His^+ phenotype when bearing the reporter *SUC2::HIS3*, a fusion of the *SUC2* promoter to *HIS3* (Sherwood and Carlson 1997). We show here that *GSF4-1* results in synthesis of a nonfunctional chimera of the transporters Hxt1p and Hxt4p (Hxt1/4p).

We cloned the *GSF4-1* locus from a library of genomic DNA of the mutant PS1450-2B (Table 1). The library contained partial *Sau*3AI DNA fragments in the centromeric vector pRS316 (Sikorski and Hieter 1989). We selected a plasmid that conferred a His^+ phenotype to a glucose-grown wild-type strain (W303-1A) containing the *SUC2::HIS3* reporter (pSUC2::HIS3; Tu and Carlson 1994). The recovered plasmid (pC1) also conferred glucose-insensitive *SUC2* expression.

Plasmid pC1 bears a region of the genome that contains two glucose transporter genes, *HXT1* and *HXT4* (Figure 1a). However, in pC1 the 5′ two-thirds of *HXT1* is joined to the 3′ end of *HXT4*. Southern blot and genetic linkage analysis confirmed that this gene fusion is tightly linked to the *GSF4-1* mutation (data not shown).

Hxt1p and Hxt4p are 73% identical and 81% similar, but their kinetic properties and patterns of expression are distinct. Hxt1p is a major low-affinity (K_m = 100 mm), high-capacity transporter that is expressed exclusively when glucose is abundant (>200 mm); Hxt4p is an intermediate-affinity transporter that is expressed when glucose levels are low (Lewis and Bisson 1991; Ko *et al.* 1993; Özcan and Johnston 1995; Reifenberger *et al.* 1997). Hxt1p, together with the low-affinity transporter Hxt3p, is responsible for glucose uptake in cells growing on high concentrations of glucose. The fusion

Corresponding author: Marian Carlson, Columbia University, 701 W. 168th St., HHSC 922, New York, NY 10032.
E-mail: mbc1@columbia.edu

[1]*Present address:* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

[2]*Present address:* Instituto de Biomedicina de Valencia (CSIC), Valencia 46010, Spain.

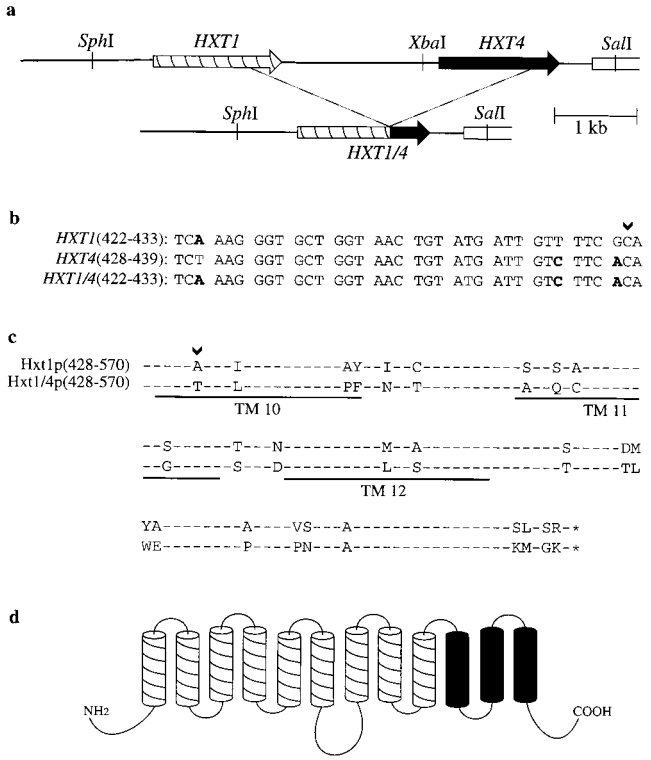

Figure 1.—Structure of the *GSF4-1* locus and the Hxt1/4p chimera. (a) Structure of the wild-type *HXT1-HXT4* locus (top) and the corresponding region of the *HXT1/4* (*GSF4-1*) locus in pC1 (bottom). Striped and solid arrows represent *HXT1* and *HXT4* sequences, respectively, and open bar represents an adjacent open reading frame. (b) Alignment of the *HXT1*, *HXT4*, and *HXT1/4* sequences in the region corresponding to the *HXT1/4* junction. Positions of diagnostic nucleotide similarities (boldface) and codon 433 of *HXT1* and *HXT1/4* (arrowhead) are indicated. (c) Alignment of the C-terminal 140 residues of Hxt1p and the Hxt1/4p chimera indicating identical residues (−) and amino acid substitutions (single letter code). Positions of residue 433 of Hxt1p and Hxt1/4p (arrowhead) and predicted transmembrane (TM) domains 10 to 12 (thick underline) are indicated. (d) Predicted membrane topology of the Hxt1/4p chimera. Striped and solid regions represent Hxt1p and Hxt4p sequences, respectively.

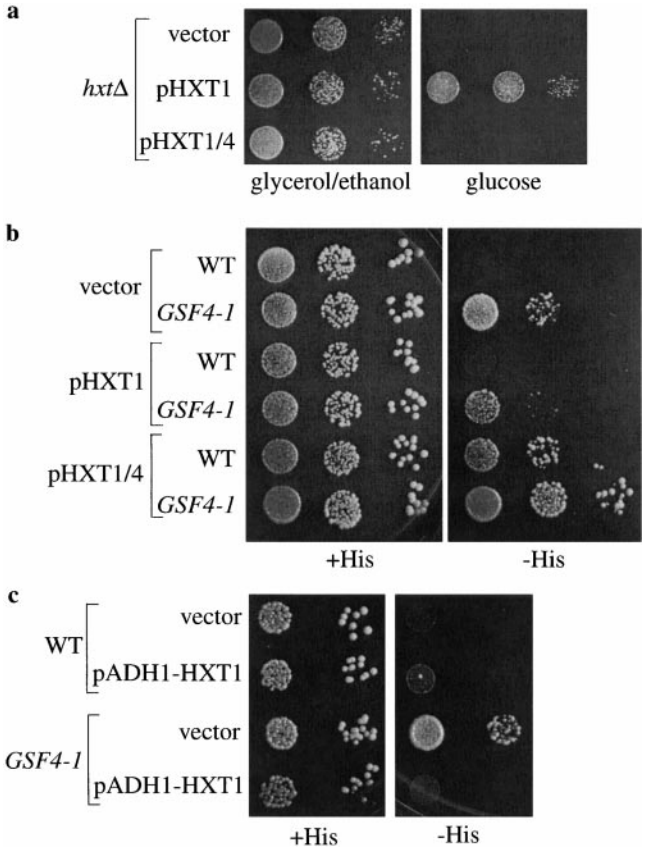

Figure 2.—Effect of mutation and altered dosage of hexose transporter genes on glucose utilization and *SUC2::HIS3* expression. (a) A strain lacking all major glucose transporter genes (*hxt*Δ) was transformed with the vector pRS316 or its derivatives pHXT1 and pHXT1/4, expressing Hxt1p or Hxt1/4p from the *HXT1* promoter, respectively. To assess the ability of Hxt1p and Hxt1/4p to restore glucose transporter function, 10-fold serial dilutions of cells were spotted onto selective synthetic media containing 5% glycerol/2% ethanol or 5% glucose. (b) Wild-type and *GSF4-1* strains containing pSUC2::HIS3 were transformed with the indicated plasmids. To monitor expression of the *SUC2::HIS3* reporter, 10-fold serial dilutions of cells were spotted onto selective synthetic medium plus 2% glucose containing (+His) or lacking (−His) histidine. (c) Wild-type and *GSF4-1* strains containing pSUC2::HIS3 were transformed with the vector pSK134 or pADH1::HXT1, which expresses Hxt1p from the *ADH1* promoter. *SUC2::HIS3* expression was monitored as in b. Plates were incubated aerobically at 30°, except that the plate containing 5% glucose in a was incubated anaerobically.

of Hxt1p and Hxt4p sequences occurs at the beginning of the tenth transmembrane segment, on the basis of the predicted membrane topology of glucose transporters (Marger and Saier 1993). The sequence of the chimera differs from that of Hxt1p at 27 positions in the 140-residue C-terminal region where Hxt4p residues replace those of Hxt1p (Figure 1).

We next tested whether pC1, renamed pHXT1/4, restores glucose transport to a strain deleted for the six *HXT* genes that encode the major glucose transporters and *SNF3* (*hxt*Δ; Liang and Gaber 1996). pHXT1/4 did not support growth of the *hxt*Δ strain on medium containing 5% glucose, suggesting that the Hxt1/4p chimera is nonfunctional for glucose transport (Figure 2a). The dominant *GSF4-1* mutant phenotype is not due to loss of Hxt1p function because deletion of *HXT1* does not confer a Gsf$^-$ phenotype: *SUC2* expression in *hxt1*Δ and *hxt1*Δ/*HXT1* strains is properly glucose repressed (∼1 unit of invertase activity in these and wild-type strains). Moreover, a C-terminal truncation of Hxt1p (without fusion to Hxt4p) also did not confer a Gsf$^-$ phenotype: deletion of codons 428 through 570 in pHXT1/4 did not affect *SUC2::HIS3* expression (data not shown). Thus, the presence of the nonfunctional Hxt1/4p fusion protein is required for the dominant negative mutant phenotype.

To determine whether this dominant negative pheno-

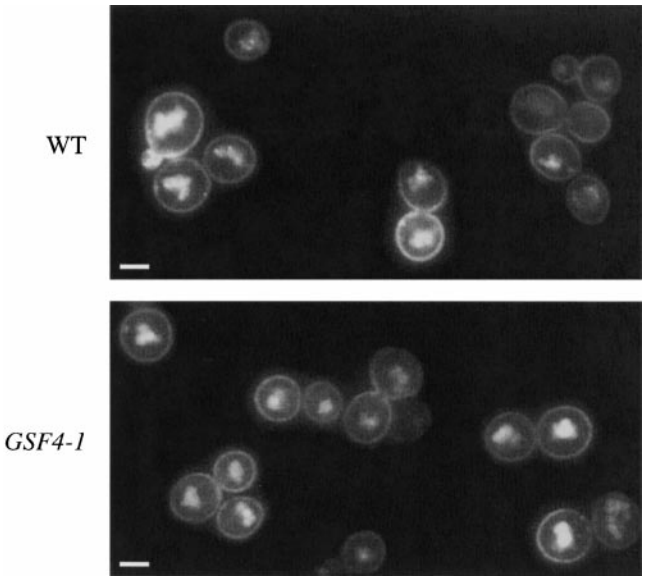

Figure 3.—Localization of Hxt1-GFP in wild-type and *GSF4-1* strains. Strains PS1451-5A and PS1451-5D were transformed with the centromeric plasmid pHXT1-GFP, which expresses Hxt1-GFP from the *HXT1* promoter (Sherwood and Carlson 1999). Transformants were grown to midlogarithmic phase in SC-Leu + 5% glycerol/2% ethanol medium, glucose was added to 4% to induce Hxt1-GFP synthesis (Özcan and Johnston 1995; Sherwood and Carlson 1999), and aliquots were harvested 90 min after glucose addition. GFP autofluorescence was visualized in unfixed cells using a Nikon Eclipse E800 microscope. Images were captured using a digital camera (Orca-100, Inovision, Durham, NC) and imaging system (Openlab 2.0.5, Improvision) and were converted to Adobe Photoshop files for processing. Exposure time was 0.7 sec. Bar, 2.5 μm.

type reflects a competition between nonfunctional Hxt1/4p and wild-type glucose transporters, we altered the relative dosage of the *HXT1* and *HXT1/4* (*GSF4-1*) genes. Expression of Hxt1p from the centromeric plasmid pHXT1 partially suppressed the His^+ phenotype of the *GSF4-1* strain bearing *SUC2::HIS3* (Figure 2b, rows 2 and 4). Conversely, the additional expression of Hxt1/4p from plasmid pHXT1/4 enhanced the mutant phenotype (Figure 2b, rows 2 and 6). Moreover, expression of Hxt1p from the strong *ADH1* promoter on multicopy plasmid pADH1-HXT1 completely suppressed the mutant phenotype of a *GSF4-1* strain (Figure 2c, rows 3 and 4). Thus, there is a correlation between the relative dosage of the mutant and wild-type alleles and the severity of the mutant phenotype. These findings suggest that the nonfunctional Hxt1/4p chimera interferes with the synthesis or function of wild-type glucose transporters. At least one other transporter besides Hxt1p must be affected because the *GSF4-1* mutant lacks Hxt1p.

We considered the possibility that the expression of Hxt1/4p interferes with the localization of glucose transporters to the plasma membrane because *gsf2* mutations cause a defect in protein trafficking of Hxt1p (Sherwood and Carlson 1999). We examined an Hxt1p-green fluorescent protein (GFP) fusion protein expressed from the *HXT1* promoter. Hxt1-GFP is efficiently localized to the plasma membrane in wild-type cells and restores glucose transport as effectively as native Hxt1p to an *hxt*Δ strain (Sherwood and Carlson 1999). The Hxt1/4p mutant protein did not appear to affect the localization of Hxt1-GFP to the cell periphery (Figure 3).

Together, these results suggest that Hxt1/4p actively interferes with the function of other members of the glucose transporter family, including Hxt1p and most likely Hxt3p, which also contributes to glucose uptake during growth on high glucose. The direct relationship between the relative dosage of the mutant and wild-type alleles and the severity of the mutant phenotype suggests that the nonfunctional Hxt1/4p chimera competes with wild-type glucose transporters.

A likely explanation for the dominant negative effect of *GSF4-1* is that Hxt1/4p directly inhibits the function of glucose transport proteins. Dominant negative mutations often inhibit the function of oligomeric complexes (Herskowitz 1987), and studies indicate an oligomeric structure for the glucose transporter Glut1p, which is 47% similar and 26% identical to Hxt1p. Glut1p is ca-

TABLE 1

List of *S. cerevisiae* strains used in this study

Strain	Genotype[a]	Source
PS1450-2B	*MAT*α *GSF4-1 his3*Δ*-200 leu2-*Δ*1 trp1*Δ *ura3-52*	This study
PS1451-5A	*MAT***a** *his3*Δ*-200 leu2-*Δ*1 trp1*Δ *ura3-52*	This study
PS1451-5D	*MAT***a** *GSF4-1 his3*Δ*-200 leu2-*Δ*1 trp1*Δ *ura3-52*	This study
HY133	*MAT***a** *snf3*Δ*::HIS3 hxt1*Δ*::TRP1::hxt4*Δ *hxt2*Δ*::LEU2 hxt3*Δ*::TRP1 hxt6/7*Δ*::TRP1 gal2 his3*Δ*-200 leu2-*Δ*1 trp1*Δ *ura3-52*	Liang and Gaber (1996)
BY4742	*MAT*α *his3*Δ *leu2*Δ *lys2*Δ *ura3*Δ	Research Genetics
11922	BY4742 *hxt1*Δ*::kanMX4*	Research Genetics
W303-1A	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	Thomas and Rothstein (1989)

[a] All strains have the S288C genetic background except W303-1A.

pable of forming functional dimers and tetramers (Hebert and Carruthers 1991, 1992; Pessino *et al.* 1991; Zottola *et al.* 1995). We propose that, in a *GSF4-1* mutant, the Hxt1/4p chimeric protein interacts with wild-type glucose transporter subunits to create nonfunctional complexes. The number of functional complexes in the plasma membrane is thus decreased, leading to inhibition of glucose transport and glucose starvation phenotypes.

The conservation of structure and function between yeast and mammalian glucose transporters suggests that similar dominant negative chimeras will inform investigations of glucose transport in other eukaryotes. Moreover, glucose transporters belong to the major facilitator superfamily, which includes proteins with 12 transmembrane domains that function as sugar, amino acid, ammonia, phosphate, calcium, sulfate, purine, and multidrug transporters (Marger and Saier 1993; Nelissen *et al.* 1997). Thus, chimeric proteins like the one characterized here may prove useful for the study, and possibly the genetic manipulation, of a wide variety of transport systems.

We thank F. Chang, K. Ferenbacher, and P. Tran for assistance with microscopy and A. Mitchell, R. Rothstein, and L. Symington for valuable discussions. This work was supported by National Institutes of Health (NIH) grant GM34095 to M.C. P.W.S. was supported by a Damon Runyon-Walter Winchell Postdoctoral Fellowship and NIH/NIAID training grant 2T32A107161.

LITERATURE CITED

Bisson, L. F., D. M. Coons, A. L. Kruckeberg and D. A. Lewis, 1993 Yeast sugar transporters. Crit. Rev. Biochem. Mol. Biol. **28:** 259–308.

Boles, E., and C. P. Hollenberg, 1997 The molecular genetics of hexose transport in yeasts. FEMS Microbiol. Rev. **21:** 85–111.

Charron, M. J., E. B. Katz and A. L. Olson, 1999 GLUT4 gene regulation and manipulation. J. Biol. Chem. **274:** 3253–3256.

Hebert, D. N., and A. Carruthers, 1991 Cholate-solubilized erythrocyte glucose transporters exist as a mixture of homodimers and homotetramers. Biochemistry **30:** 4654–4658.

Hebert, D. N., and A. Carruthers, 1992 Glucose transporter oligomeric structure determines transporter function. J. Biol. Chem. **267:** 23829–23838.

Herskowitz, I., 1987 Functional inactivation of genes by dominant negative mutations. Nature **329:** 219–222.

Ko, C. H., H. Liang and R. F. Gaber, 1993 Roles of multiple glucose transporters in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 638–648.

Kruckeberg, A. L., 1996 The hexose transporter family of *Saccharomyces cerevisiae*. Arch. Microbiol. **166:** 283–292.

Lewis, D. A., and L. F. Bisson, 1991 The *HXT1* gene product of *Saccharomyces cerevisiae* is a new member of the family of hexose transporters. Mol. Cell. Biol. **11:** 3804–3813.

Liang, H., and R. F. Gaber, 1996 A novel signal transduction pathway in *Saccharomyces cerevisiae* defined by Snf3-regulated expression of *HXT6*. Mol. Biol. Cell **7:** 1953–1966.

Marger, M. D., and M. H. Saier, 1993 A major superfamily of transmembrane facilitators that catalyse uniport, symport, and antiport. Trends Biochem. Sci. **18:** 13–20.

Mueckler, M., 1994 Facilitative glucose transporters. Eur. J. Biochem. **219:** 713–725.

Mueckler, M., C. Caruso, S. A. Baldwin, M. Panico, I. Blench *et al.*, 1985 Sequence and structure of a human glucose transporter. Science **229:** 941–945.

Nelissen, B., R. De Wachter and A. Goffeau, 1997 Classification of all putative permeases and other membrane plurispanners of the major facilitator superfamily encoded by the complete genome of *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. **21:** 113–134.

Özcan, S., and M. Johnston, 1995 Three different regulatory mechanisms enable yeast hexose transporter (*HXT*) genes to be induced by different levels of glucose. Mol. Cell. Biol. **15:** 1564–1572.

Ozcan, S., and M. Johnston, 1999 Function and regulation of yeast hexose transporters. Microbiol. Mol. Biol. Rev. **63:** 554–569.

Pessino, A., D. N. Hebert, C. W. Woon, S. A. Harrison, B. M. Clancy *et al.*, 1991 Evidence that functional erythrocyte-type glucose transporters are oligomers. J. Biol. Chem. **266:** 20213–20217.

Reifenberger, E., E. Boles and M. Ciriacy, 1997 Kinetic characterization of individual hexose transporters of *Saccharomyces cerevisiae* and their relation to the triggering mechanisms of glucose repression. Eur. J. Biochem. **245:** 324–333.

Sherwood, P. W., and M. Carlson, 1997 Mutations in *GSF1* and *GSF2* alter glucose signaling in *Saccharomyces cerevisiae*. Genetics **147:** 557–566.

Sherwood, P. W., and M. Carlson, 1999 Efficient export of the glucose transporter Hxt1p from the endoplasmic reticulum requires Gsf2p. Proc. Natl. Acad. Sci. USA **96:** 7415–7420.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Thomas, B. J., and R. Rothstein, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

Tu, J., and M. Carlson, 1994 The GLC7 type 1 protein phosphatase is required for glucose repression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:** 6789–6796.

Zottola, R. J., E. K. Cloherty, P. E. Coderre, A. Hansen, D. N. Hebert *et al.*, 1995 Glucose transporter function is controlled by transporter oligomeric structure: a single, intramolecular disulfide promotes GLUT1 tetramerization. Biochemistry **34:** 9734–9747.

Communicating editor: M. Johnston