Note

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

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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 (Marg er 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 (Ozcan and Johnston 1995).

We previously isolated mutants of *S. cerevisiae*, designated *gsf* (glucose signaling factor), 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 Sau3AI 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; Ozcan 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...
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 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.

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 (hxtΔ; Liang and Gaber 1996). pHXT1/4 did not support growth of the hxtΔ 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 Gsf2 phenotype: SUC2 expression in hxt1Δ and hxt1Δ/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 Gsf2 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-
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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 hxtΔ 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 (Her skowitz 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**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>PS1450-2B</td>
<td>MATα GSF4-1 his3Δ-200 leu2Δ1 trp1Δ ura3Δ-52</td>
<td>This study</td>
</tr>
<tr>
<td>PS1451-5A</td>
<td>MATα his3Δ-200 leu2Δ1 trp1Δ ura3Δ-52</td>
<td>This study</td>
</tr>
<tr>
<td>PS1451-5D</td>
<td>MATα GSF4-1 his3Δ-200 leu2Δ1 trp1Δ ura3Δ-52</td>
<td>This study</td>
</tr>
<tr>
<td>HY133</td>
<td>MATα snf3Δ::HIS3 hxt1Δ::TRP1::hxt4Δ hxt2Δ::EU2 hxt3Δ::TRP1 hxt6::TRP1 gal2 his3Δ::LEU2 leu2Δ1 trp1Δ ura3Δ-52</td>
<td>Liang and Gaber (1996)</td>
</tr>
<tr>
<td>BY4742</td>
<td>MATα his3Δ leu2Δ lys2Δ ura3Δ</td>
<td>Research Genetics</td>
</tr>
<tr>
<td>11922</td>
<td>BY4742 hxt1Δ::kanMX4</td>
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</tr>
<tr>
<td>W303-1A</td>
<td>MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</td>
<td>Thomas and Rothstein (1989)</td>
</tr>
</tbody>
</table>

*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 (Marguer 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.

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