Regulation of glycerol transport genes *GUP1* and *GUP2* in *Saccharomyces cerevisiae*

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Two highly homologous genes related to a phenotype of salt stress tolerance were identified in *Saccharomyces cerevisiae*. These genes were named *GUP1* and *GUP2* from glycerol uptake (Holst *et al*, 2000).
Upon salt stress, a strain lacking the capacity to sinthesize glycerol (*gpd1gpd2*) (figure 1) is able to increase intracellular levels of glycerol by taking it up from the medium. Accordingly, the presence of small amounts of glycerol in the medium decreased osmosensitivity in this strain. Based on these findings, yeast genomic fragments cut out from an mTn-*lacZ/LEU2*-mutagenized plasmid library were used to transform a *gpd1gpd2* strain with subsequent secreening for decreased osmotolerance of the transformants. This screening was performed in two independent steps:

- a first screen for mutants unable to grow on glycerol as sole carbon and energy source
- a second screen for mutants that had lost the ability to survive under severe salt stress even when glycerol was added to the medium.

A transformant mutagenized in the ORF *YGL084c* suggested to encode a multimembrane-spanning protein (Nelissen *et al*, 1997) was isolated. A close homologue of *YGL084c*, *YPL189w* was identified by BLAST searches with 57% sequence identity and 77% similarity at aminoacid sequence level.

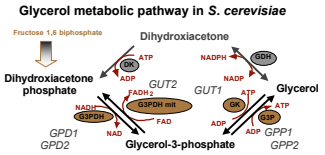

Figure 1

Previous results from glycerol transport physiological characterization on wild type strains enabled the elaboration of a regulation model as presented in Figure 2.
Taking this model in consideration, and in spite of the screening test having been done on glucose-grown cells in the presence of NaCl and glycerol, transport ability was tested in ethanol-grown cells in which case an interference of the glycerol kinase activity on transport measurements was detected, being the saturation kinetics present in *gup1* deleted strains attributed to a residual uptake, not active as such, but pulled by an active catabolism of glycerol under derepressing growth conditions (Figure 2, Table 1). Only the combination of *gup1* and *gut1* deletions eliminated the saturable glycerol uptake branch and further deletion of the *GUP1* homologue (*GUP2*, *YPL189w*) did not change the uptake results.
The phenotype in ethanol-grown cells suggested that *GUP1* is the gene responsible for the proton symport described before (Lages and Lucas, 1997).

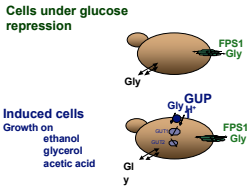

Figure 2

GROWTH CONDITIONS — **Ethanol** (Exponential)

Strain	Assay: Glycerol kinase (mU/mg prot)	Active transport Vmax (µmols h-1 g-1)
wt	66.1 ± 4.1 (6/2)	277 ± 26 (3)
gpd1gpd2	55.8 ± 11.4 (9/3)	237 ± 30 (3)
gup1	43.1 ± 5.8 (8/3)	181 ± 12 (4)
gut1	nd	205 ± 17 (4)
gup1 gut1	nd	-
gpd1 gut1	4.1 (1/1)	248 ± 36 (4)
gpd1 gup1	nd	-
gpd1 gup1gut1	nd	-
gup1 gup2	48.9 ± 13.5 (6/3)	169 ± 17 (3)
gut1 gup1gup2	nd	-
gpd1gut1gup1	nd	-

Vmax Determined using one glycerol concentration in the range of active transport Vmax (2mM)
(-) ~50µmoles h-1 g-1
(../..) Number of replicates / number of independent batches of cells

Table 1

The model presented above suggests *GUP1* to be under glucose repression. However, the mutant screening that lead to *GUP1* identification was performed in media containing glucose as carbon and energy source supplemented with NaCl and glycerol. Therefore, the glycerol transport study was extended to cells grown in this medium and, as a control, in cells grown on glucose as sole carbon and energy source (Table 3).
Surprisingly, *gpd1gpd2* strain displayed a strong uptake which, once determined as a total kinetic study revealed a Vmax more than five times higher than wild type cells grown in ethanol (Table 2). This transport activity displayed proton uptake, and accumulation capacity, and protonophore sensivity characteristic of symports. Furthermore, the Km value was very close to the one attributed to *GUP1* activity (Lages and Lucas, 1997).
One of the possibilities to explain these results was to attribute to *GUP1* homologue the responsability for this active uptake. As shown in Table 2, deletion in *gpd1* or *gpd1gpd2* allowed the detection of transport beyond passive diffusion substrate entry level. Further *gup1* deletion in this genetic background reduced the activity to approximately 50%. On the other hand, *gup2* deletion also affects glycerol transport by abolishing any measurable transport in a *gpd1gup1* genetic background. Furthermore, when *gpd2* is deleted in a strain already partially defficient in glycerol synthesis because of *gpd1* deletion, the *GUP2*-dependent transport capacity went up by a factor of 2.

- These results suggest that *GUP1*-dependent and *GUP2*-dependent glycerol uptake in glucose-grown cells are tightly controlled and need the extreme conditions of strong osmotic stress combined with glycerol synthesis impairment to be detectable.

GROWTH CONDITIONS — **Glucose + 1M NaCl + 15mM Gly** (Exponential)

Strain	Assays: Glycerol kinase (mU/mg prot)	Active transport ^{14}[C]Gly uptake Vmax (µmoles h-1 g-1)	H+ uptake
wt	6.5 ± 4.1 (5/2)	-	-
gpd1gpd2	4.0 ± 0.2 (2/1)	630 ± 18 (3)	+
gup1	4.5 ± 3.1 (9/3)	-	-
gut1	4.1 ± 2.9 (5/2)	-	-
gup1 gut1	3.5 ± 1.8 (10/2)	-	-
gpd1 gut1	6.7 ± 1.7 (3/1)	165 ± 9 (2)	+
gpd1 gup1	5.8 ± 4.8 (9/2)	162 ± 0.5 (2)	+
gup1 gup2	4.5 ± 1.6 (6/2)	-	-
gpd1 gup1 gup2	2.1 ± 0.8 (9/3)	-	-
gpd1 gpd2 gup1	2.4 ± 0.3 (9/3)	379 ± 37 (2)	+

2x ~50%

Vmax Determined using one glycerol concentration in the range of active transport Vmax (2mM)
(-) ~ 50µmoles h-1 g-1
(../..) Number of replicates / number of independent batches of cells

Table 2

GROWTH CONDITIONS — **Glucose** (Exponential)

Strain	Assay: Glycerol kinase (mU/mg prot)	Active transport
wt	3.2 ± 1.1 (6/2)	-
gpd1gpd2	4.0 ± 1.3 (6/2)	-
gup1	3.5 ± 2.8 (6/2)	-
gut1	4.1 ± 0.2 (6/2)	-
gup1 gut1	3.5 ± 2.5 (6/2)	-
gpd1 gup1	5.8 ± 2.8 (6/2)	-
gup1 gup2	2.1 ± 1.9 (6/2)	-
gpd1 gup1 gup2	2.4 ± 2.6 (7/2)	-
gpd1 gut1	4.5 ± 2.6 (6/2)	-
gup1 gut1	3.5 ± 0.0 (2/1)	-
gpd1 gpd2 gup1	2.4 ± 1.0 (9/3)	-

Vmax Determined using one glycerol concentration in the range of active transport Vmax (2mM)
(../..) Number of replicates / number of independent batches of cells
(-) ~ 50µmoles h-1 g-1

Table 3

To elucidate the relation of glycerol transporters with osmotic adaptation, intracellular levels of several solutes were determined by HPLC in cells grown under salt stress with and without extracellular glycerol. Besides glycerol, trehalose and acetic acid were found and no other compound was detected (Figure 3). Trehalose levels increase when glycerol production is impaired but less dramatically if some external glycerol is available, suggesting that the cells use trehalose as a substitute for glycerol as a compatible solute. On the other hand, acetic acid levels in a *gpd1gpd2* strain match the levels of glycerol under the same conditions.
Surprisingly, the *gpd1gpd2* strain produces residual amounts of glycerol that might be due to an alternative biosynthesis pathway via dihydroxyacetone that was suggested to be present in *S. cerevisiae* (Norbeck and Blomberg, 1997; Blomberg, 1997). Nevertheless, the intracellular levels of glycerol when some external glycerol is available, strongly supports the increased expression of gene(s) encoding glycerol transporter(s).

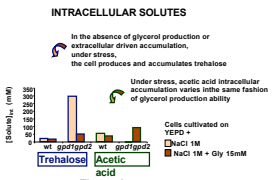

Figure 3

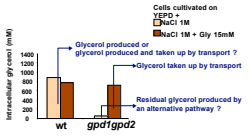

Figure 4

Relative quantification of *GUP1* mRNAs by RT-PCR showed that *GUP1* is expressed under all conditions tested (including glucose-grown cells) (Figure 5), indicating constitutive expression in opposition to what was determined with the physiological approach. Nevertheless, a marked difference in mRNA levels was detected in wild type and *gpd1gpd2* strains which is compatible with the higher transport activity detected in this strain under salt stress with external glycerol.
Concerning *GUP2* mRNAs, available data suggest lower expression than for *GUP1* on ethanol-grown cells and no detection on glucose-grown cells (Figure 7).

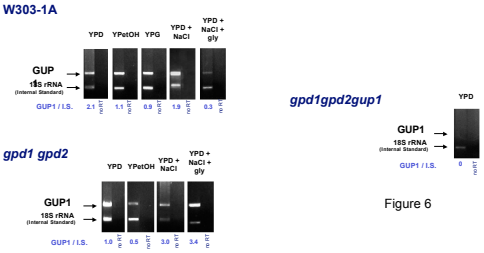

Figure 5

Figure 6

Figure 7

The following model is proposed based on available data that includes three distinct environmental conditions.

- Cells under glucose repression with passive and mediated (through *FPS1* channel) diffusions as the only measurable transport but in which *GUP1* is expressed, indicating that might be under post-translational control (Figure 8).
- Induced cells by growth on ethanol or glycerol with further detection of *GUP1* active transport activity as well as the activity of the enzymes of the glycerol catabolism (glycerol kinase encoded by *GUT1* and glycerol-3P-dehydrogenase encoded by *GUT2*) (Figure 9).
- Induced cells by growth on glucose with the presence of NaCl 1M and glycerol 15mM in which *GUP2* is also expressed in cells unable to sinthesize glycerol (Figure 10), being highly contributing for transport measurements and apparently controlled by glycerol synthesis regulation (Table 2).

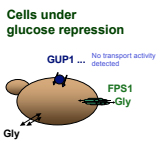

Figure 8

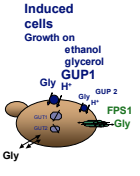

Figure 9

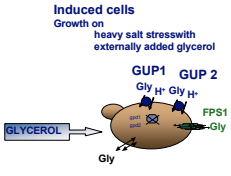

Figure 10

Close colaboration with B. Holst and M. Kielland-Brandt from Carlsberg Laboratory in Copenhagen, Denmark