

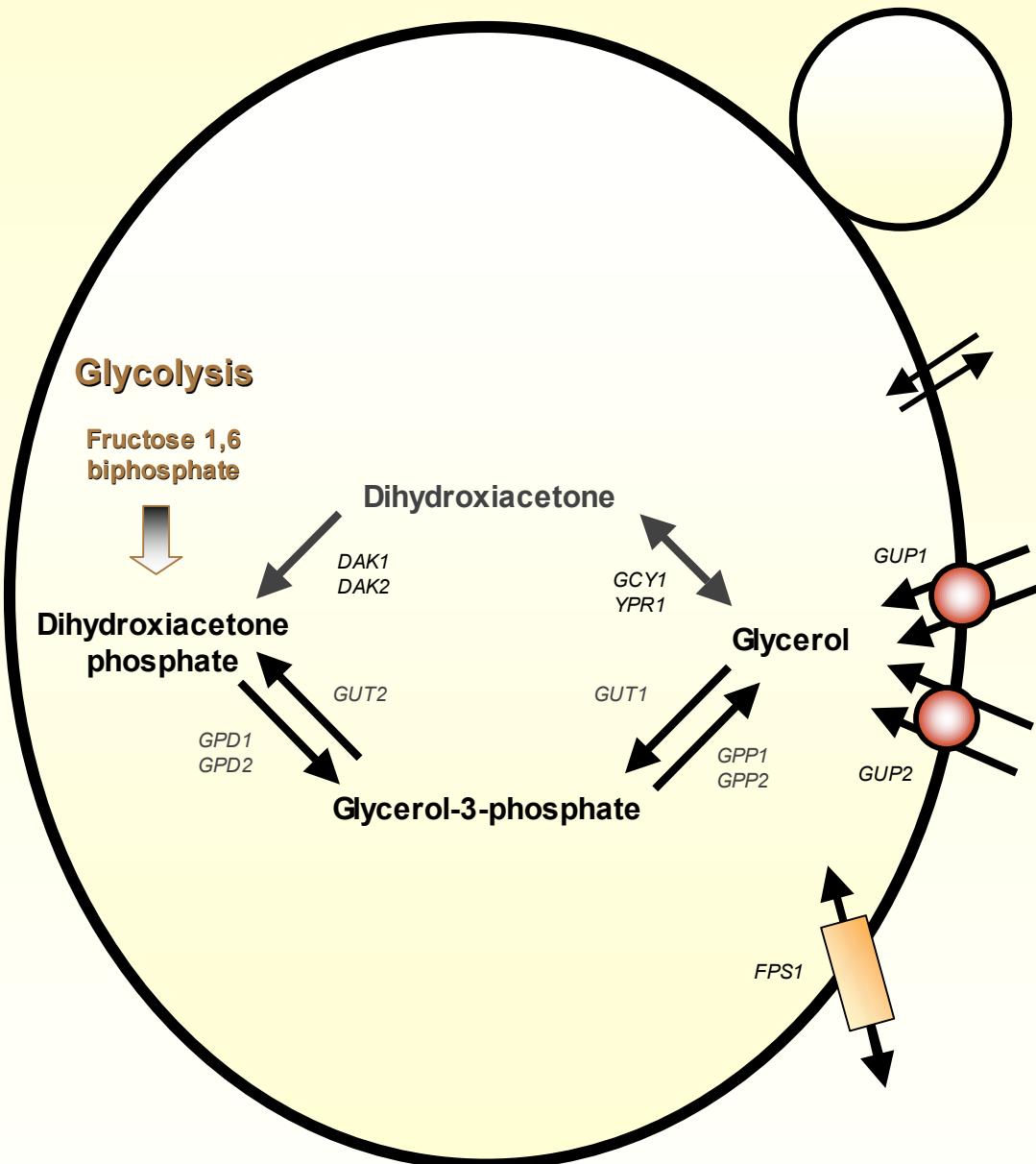
Expressão dos genes *GUP1* e *GUP2* de *Saccharomyces cerevisiae*: influência da fonte de carbono e do stresse salino

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Glycerol metabolic pathway in *Saccharomyces cerevisiae*



GROWTH CONDITIONS

Ethanol

(Exponential)

Strain	Active transport Vmax (μ moles h-1 g-1)
W303-1A	277 ± 26 (3)
<i>gpd1 gpd2</i>	237 ± 30 (3)
<i>gup1</i>	181 ± 12 (4)
<i>gut1</i>	205 ± 17 (4)
<i>gup1 gut1</i>	-
<i>gpd1 gut1</i>	248 ± 36 (4)
<i>gpd1 gup1</i>	nd
<i>gpd1 gup1 gut1</i>	-
<i>gup1 gup2</i>	169 ± 17 (3)
<i>gut1 gup1 gup2</i>	-
<i>gpd1 gut1 gup1</i>	-

Vmax Determined using one glycerol concentration in the range of active transport Vmax (2mM)

(-) < 50 μ moles h-1 g-1

(.../...) Number of replicates / number of independent batches of cells



GROWTH CONDITIONS

Glucose + 1M NaCl + 15mM Gly
(Exponential)

Strain	Active transport	
	^{14}C Gly uptake Vmax ($\mu\text{moles h}^{-1} \text{g}^{-1}$)	H+ uptake
W303-1A	-	-
<i>gpd1 gpd2</i>	630 ± 18 (3)	+
<i>gup1</i>	-	-
<i>gut1</i>	-	-
<i>gup1 gut1</i>	-	-
<i>gpd1 gut1</i>	165 ± 9 (2)	+
<i>gpd1 gup1</i>	182 ± 0.5 (2)	+
<i>gup1 gup2</i>	-	-
<i>gpd1 gup1 gup2</i>	-	-
<i>gpd1 gpd2 gup1</i>	379 ± 37 (2)	+

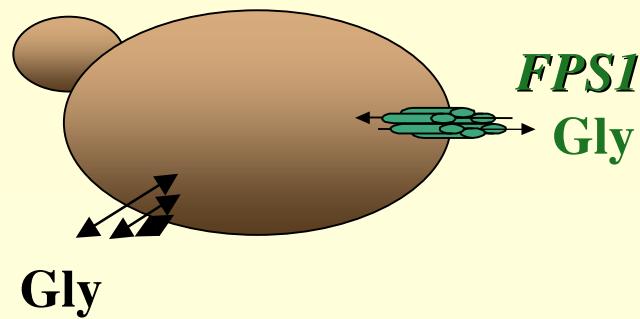
Vmax Determined using one glycerol concentration in the range of active transport Vmax (2mM)

(-) < 50 $\mu\text{moles h}^{-1} \text{g}^{-1}$

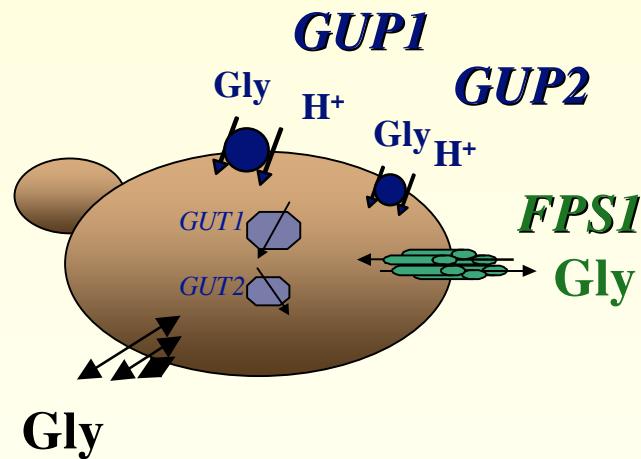
(.../..) Number of replicates / number of independent batches of cells



Cells under glucose repression

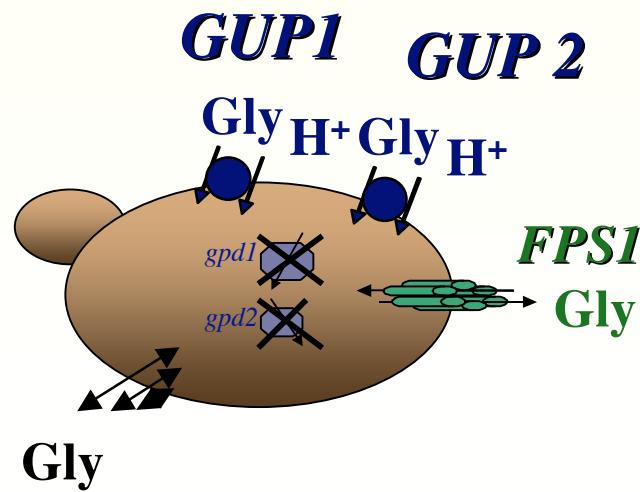


Induced cells
Growth on
ethanol
glycerol



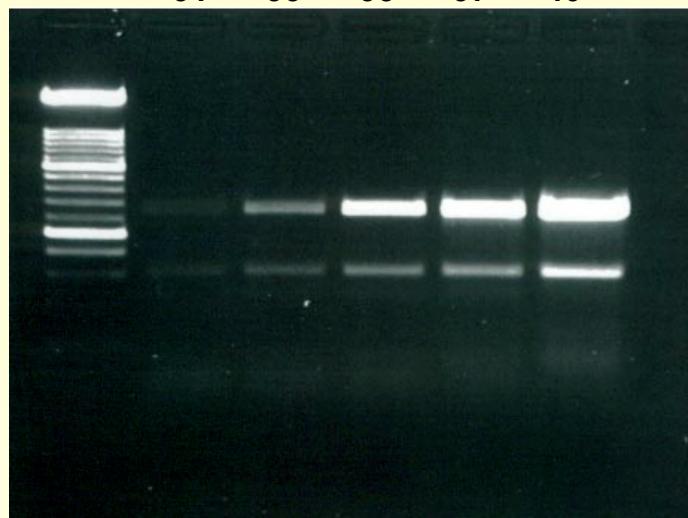
Induced cells
Growth on
heavy salt stress
with
externally added
glycerol

GLYCEROL →

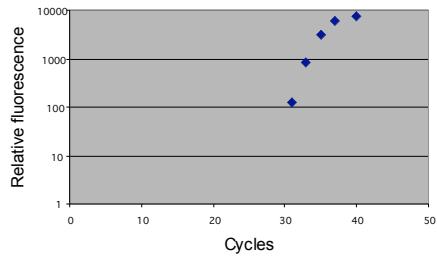


Cycle#

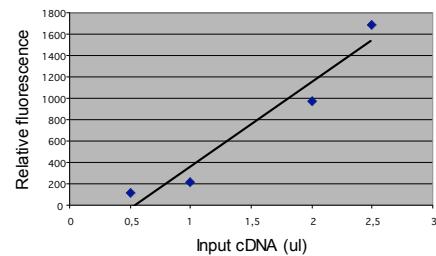
31 33 35 37 40



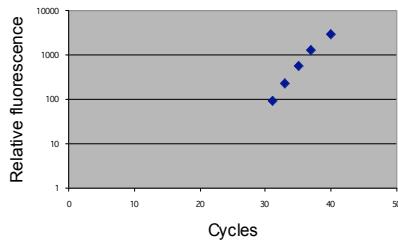
Exponential phase of amplification of GUP1



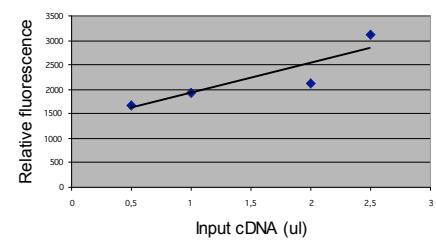
RT-PCR dynamic range for GUP1



Exponential phase of amplification of 18S rRNA internal standard



RT-PCR dynamic range for 18S rRNA internal standard



GUP1 mRNA relative amounts

Growth conditions/carbon sources

Strains	Glucose (fermentative metabolism)	Glucose (respiratory metabolism)	Glucose + NaCl 1M	Glucose + NaCl 1M + glycerol 15mM	Ethanol	Glycerol
W303-1A parental strain	1.76±0.25 (3)	1.29±0.43 (3)	1.49±0.38 (4)	1.20±0.26 (3)	1.21±0.14 (4)	0.97±0.26 (3)
YSH642 <i>gpd1gpd2</i>	1.31±0.13 (3)	nd	1.35±0.39 (3)	1.61±0.15 (3)	nd	nd

() number of independent assays
nd not determined



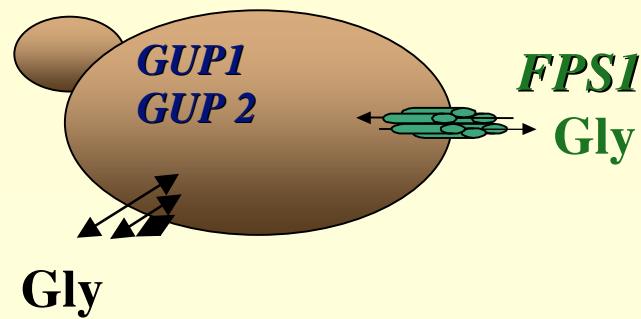
GUP2 mRNA relative amounts

Strains	Growth conditions					
	Glucose (fermentative metabolism)	Glucose (respiratory metabolism)	Glucose + NaCl 1M	Glucose + NaCl 1M + glycerol 15mM	Ethanol	Glycerol
W303-1A	0.91±0.12 (3)	0.66±0.21 (3)	1.39 (1)	1.36±0.10 (2)	1.20±0.33 (3)	nd
YSH642 <i>gpd1gpd2</i>	1.57±0.24 (3)	nd	1.35±0.46 (2)	2.04±0.44 (3)	nd	nd

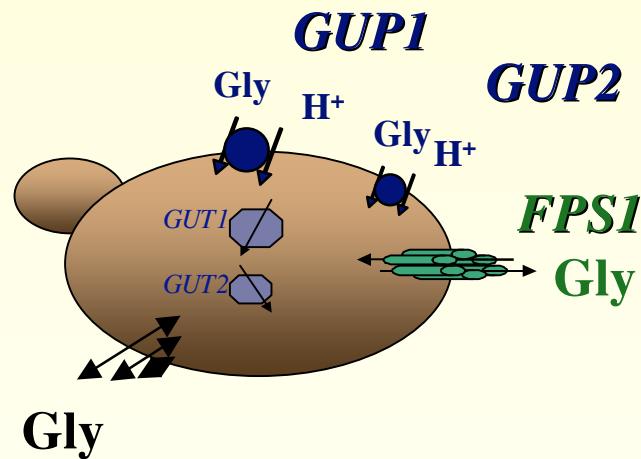
() number of independent assays
nd not determined



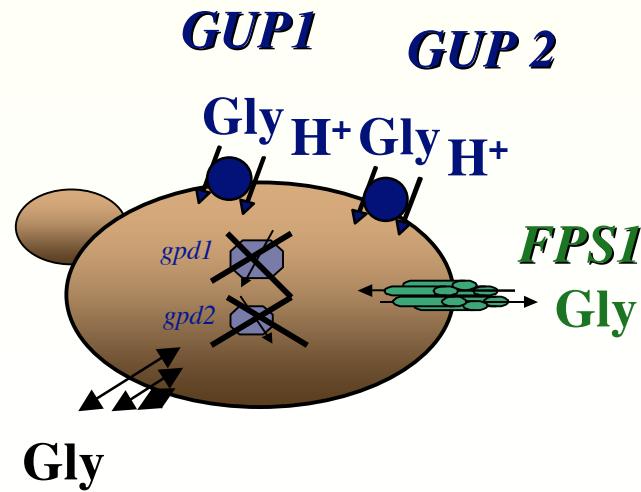
Cells under glucose repression



Induced cells
Growth on
ethanol
glycerol



Induced cells
Growth on
heavy salt stress
with or without
externally added
glycerol



Synthesis of results

GUP1 and *GUP2* mRNAs are always present regardless the carbon and energy source (fermentable or not fermentable)

GUP1 mRNA levels are not affected by carbon and energy source or salt stress

Transcription of *GUP2* is increased with the inability to synthesize glycerol

GUP2 mRNA levels increase with salt stress together with, apparently, the simultaneous presence of glycerol

Conclusions

Expression of *GUP1* and *GUP2* are regulated by post-transcriptional mechanisms

***GUP2* transcription:**

**differently regulated from *GUP1*
GPD1 and *GPD2* involved
involved in salt stress response**