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

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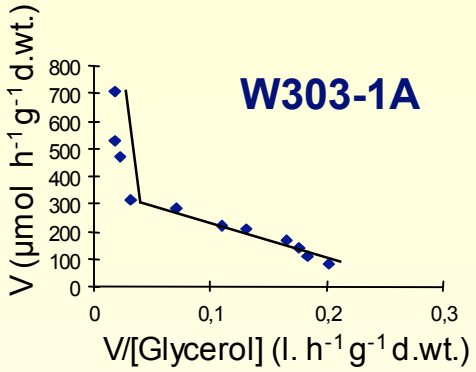
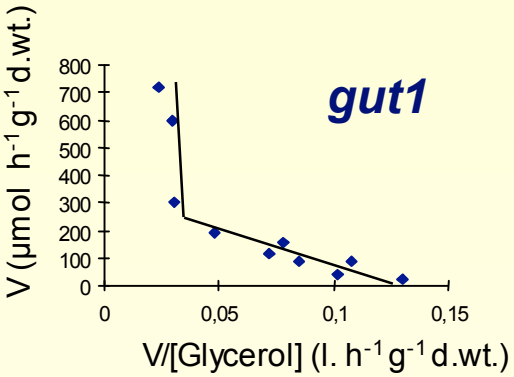
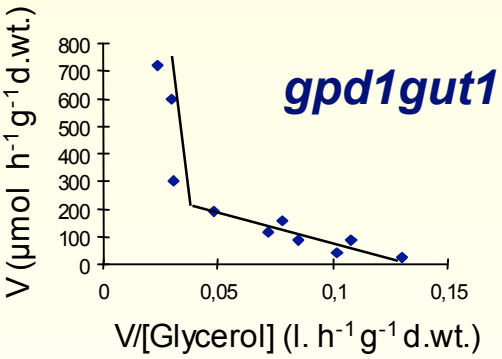
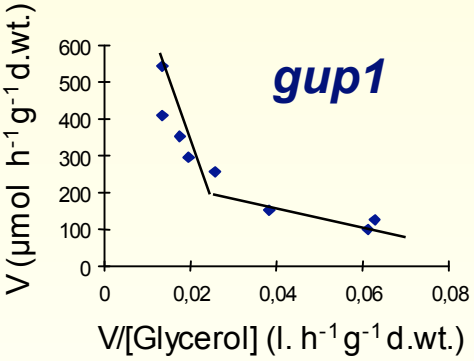
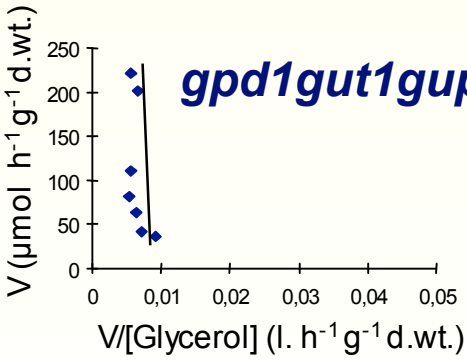
ETHANOL-GROWN CELLS



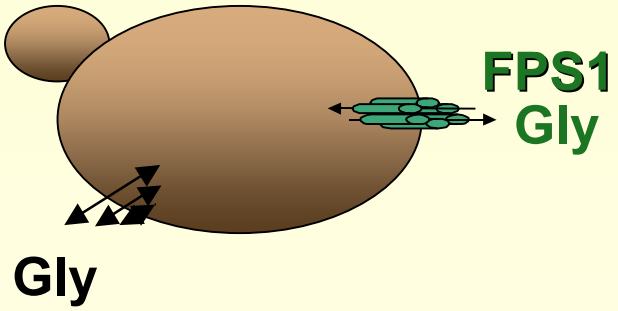








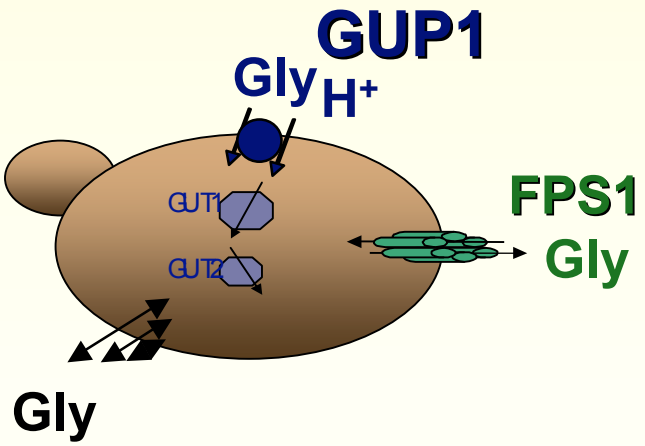
Cells under glucose repression



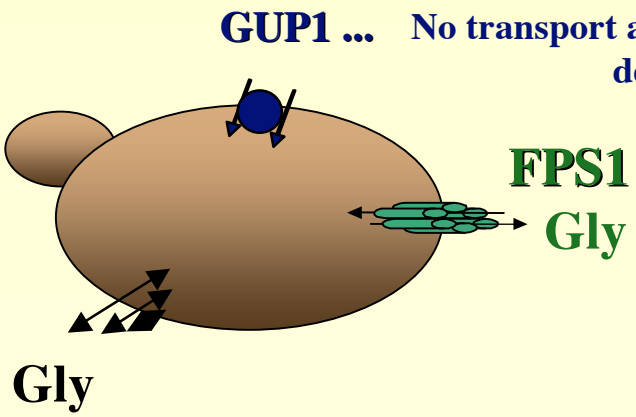
Induced cells

Growth on

- ethanol
- glycerol
- acetic acid



Cells under glucose repression

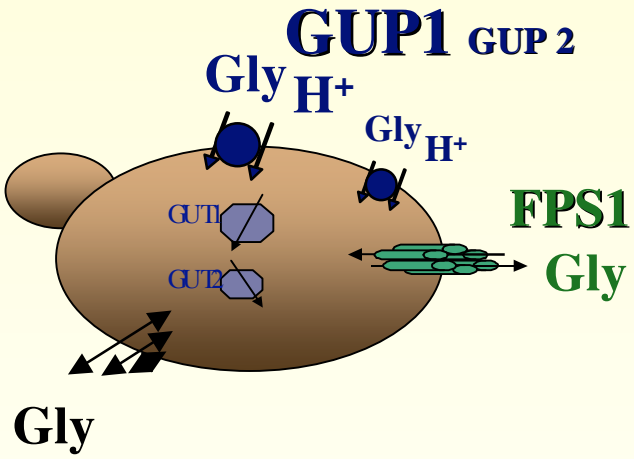


Induced cells

Growth on

ethanol

glycerol

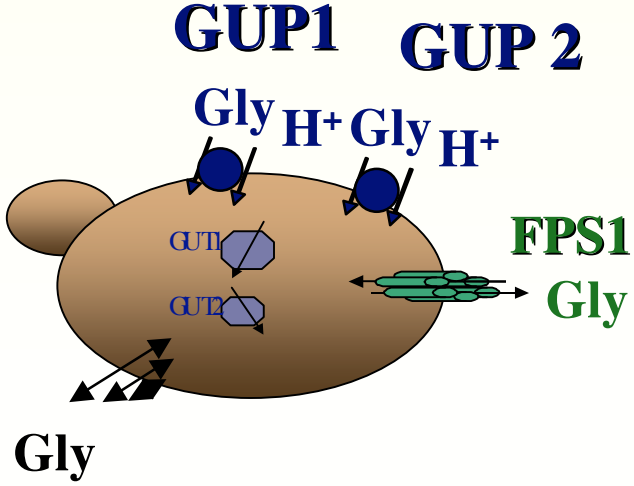


Induced cells

Growth on

heavy salt stress with externally added glycerol

GLYCEROL



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)	Active transport H^+ uptake
wt	6.5 ± 4.1 (5/2)	-	-
*gpd1*Δ*gpd2*Δ	4.8 ± 0.2 (2/1)	630 ± 18 (3)	+
*gup1*Δ	4.5 ± 3.1 (9/3)	-	-
*gut1*Δ	4.1 ± 2.9 (5/2)	-	-
*gup1*Δ *gut1*Δ	3.5 ± 1.8 (10/2)	-	-
*gpd1*Δ *gut1*Δ	6.7 ± 1.7 (3/1)	165 ± 9 (2)	+
*gpd1*Δ *gup1*Δ	5.8 ± 4.0 (9/2)	182 ± 0.5 (2)	+
*gup1*Δ *gup2*Δ	4.5 ± 1.6 (6/2)	-	-
*gpd1*Δ *gup1*Δ *gup2*Δ	2.1 ± 0.8 (9/3)	-	-
*gpd1*Δ *gpd2*Δ *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

GROWTH CONDITIONS

Glucose

(Exponential)

Assay / Strain	Glycerol kinase (mU/mg prot)	Active transport
wt	3.2 ± 1.1 (6/2)	-
*gpd1*Δ*gpd2*Δ	5.4 ± 1.3 (5/2)	-
*gup1*Δ	3.4 ± 2.8 (5/2)	-
*gut1*Δ	0.9 ± 0.2 (2/1)	-
*gup1*Δ *gut1*Δ	5.3 ± 2.5 (8/2)	-
*gpd1*Δ *gut1*Δ	3.7 ± 2.8 (7/3)	-
*gpd1*Δ *gup1*Δ	3.7 ± 1.9 (7/2)	-
*gpd1*Δ *gup1*Δ*gut1*Δ	nd	-
*gup1*Δ *gup2*Δ	4.5 ± 2.6 (6/2)	-
*gpd1*Δ *gup1*Δ*gup2*Δ	2.3 ± 0.0 (9/3)	-
*gpd1*Δ*gpd2*Δ*gup1*Δ	1.6 ± 1.0 (9/3)	-

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

GROWTH CONDITIONS

Ethanol

(Exponential)

Assay / Strain	Glycerol kinase (mU/mg prot)	Active transport Vmax (µmols h-1 g-1)
wt	66.1 ± 4.1 (6/2)	277 ± 26 (3)
*gpd1*Δ*gpd2*Δ	55.8 ± 11.4 (9/3)	237 ± 30 (3)
*gup1*Δ	43.1 ± 5.8 (9/3)	181 ± 12 (4)
*gut1*Δ	nd	205 ± 17 (4)
*gup1*Δ *gut1*Δ	nd	-
*gpd1*Δ *gut1*Δ	4.1 (1/1)	248 ± 36 (4)
*gpd1*Δ *gup1*Δ	nd	nd
*gpd1*Δ *gup1*Δ*gut1*Δ	nd	-
*gup1*Δ *gup2*Δ	48.9 ± 13.5 (6/3)	169 ± 17 (3)
*gut1*Δ *gup1*Δ*gup2*Δ	nd	-
*gpd1*Δ*gut1*Δ*gup1*Δ	nd	-

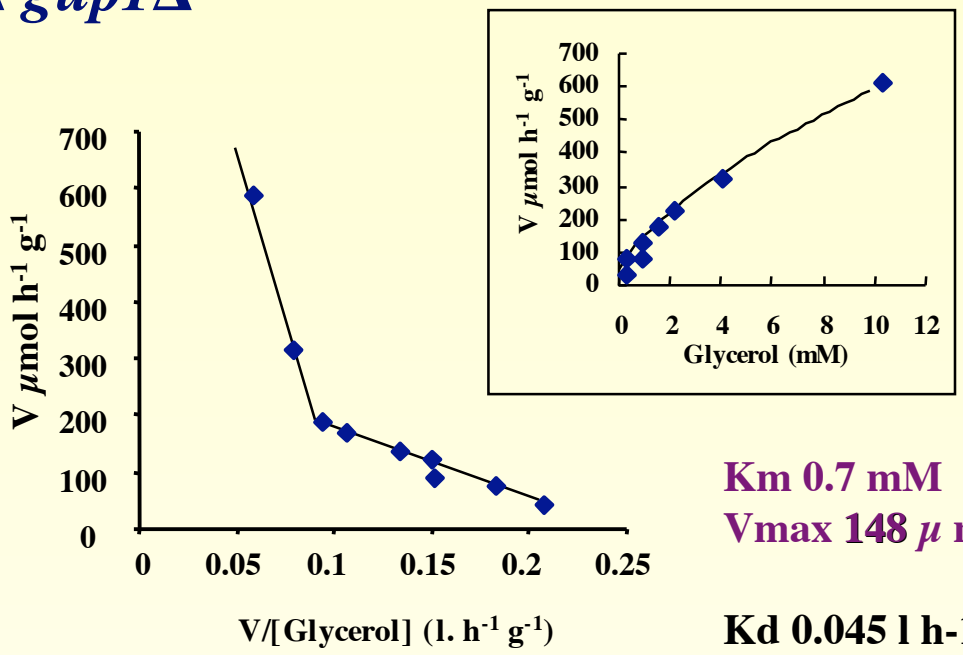
Vmax Determined using one glycerol concentration in the range of active transport Vmax (2mM

(-) ≤ 50µmoles h-1 g-1

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

CELLS CULTIVATED ON YEPD + 1M NaCl + 15mM glycerol

gpd1Δ gup1Δ

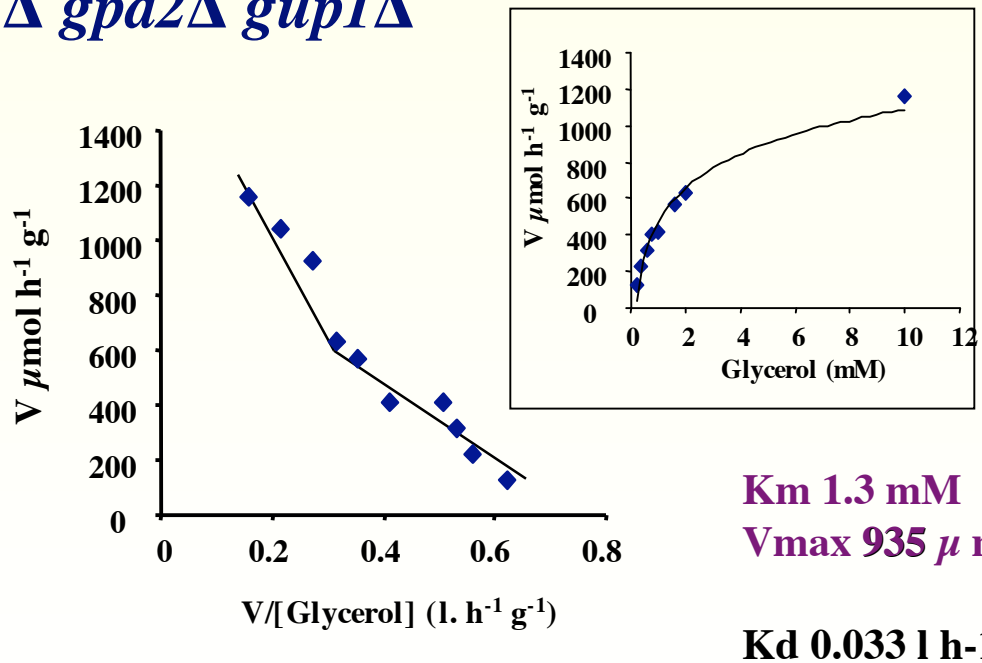


Km 0.7 mM
Vmax 148 *μ* mol h-1 g-1

Kd 0.045 l h-1 g-1

***μ*g** **0.22 h-1 (≈ wt)**
lag phase **≈42 h (≈ 2.5 x wt)**

gpd1Δ gpd2Δ gup1Δ



Km 1.3 mM
Vmax 935 *μ* mol h-1 g-1

Kd 0.033 l h-1 g-1

***μ*g** **0.17 h-1 (≈ *gpd1Δgpd2Δ*)**
lag phase **≈190 h (≈ 2.5 x *gpd1Δgpd2Δ*)**

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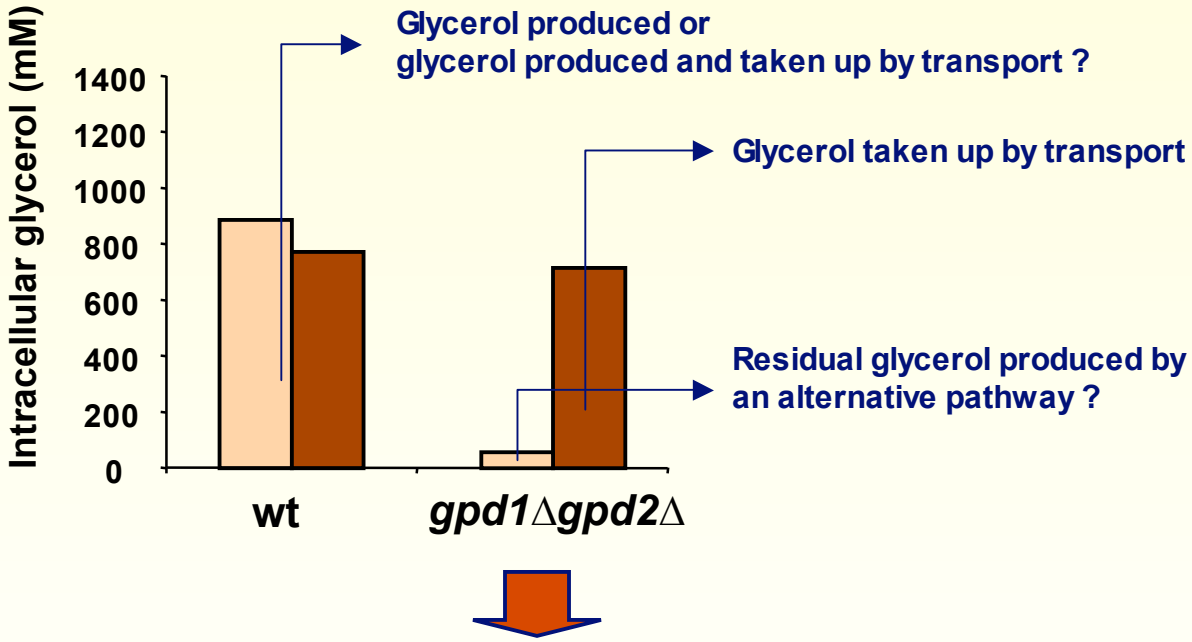
INTRACELLULAR SOLUTES

Under salt stress

Cells cultivated on YEPD +

- NaCl 1M
- NaCl 1M + Gly 15mM



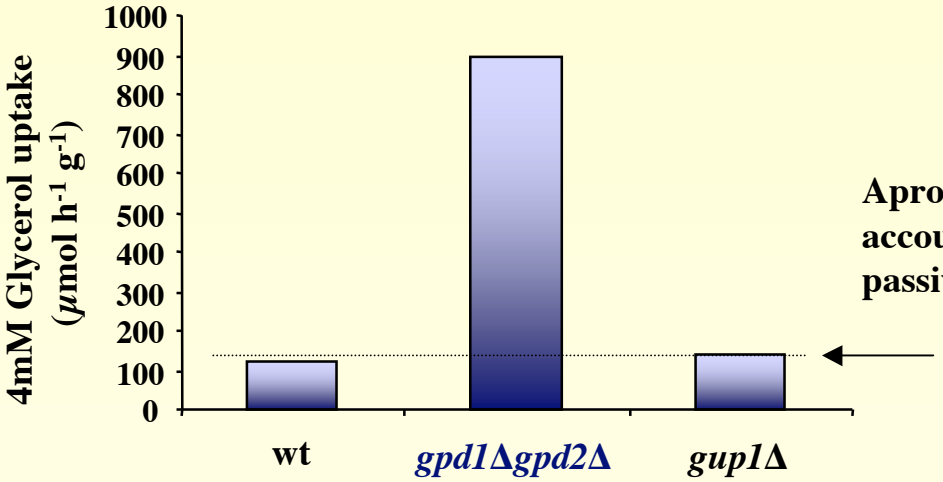
Compatible with a very high transport activity (Vmax)

Compatible with the transporter(s) gene(s) expression being

- dependent on glycerol production impairment
- dependent on heavy salt stress
- increased by external glycerol presence
- increased by acetic acid production

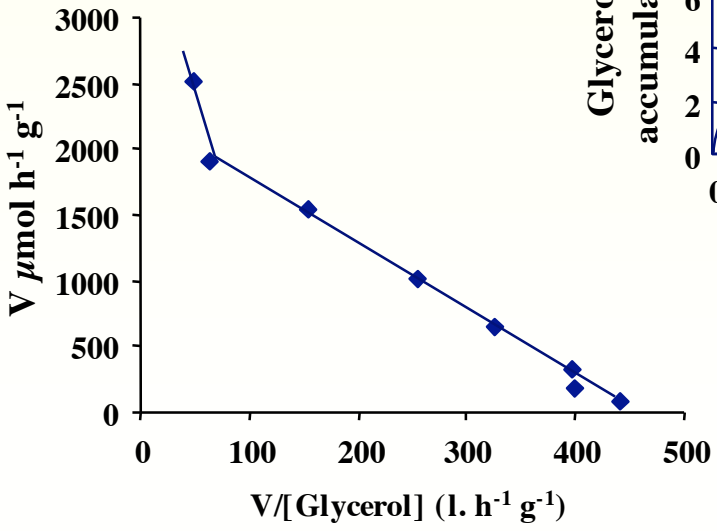
?

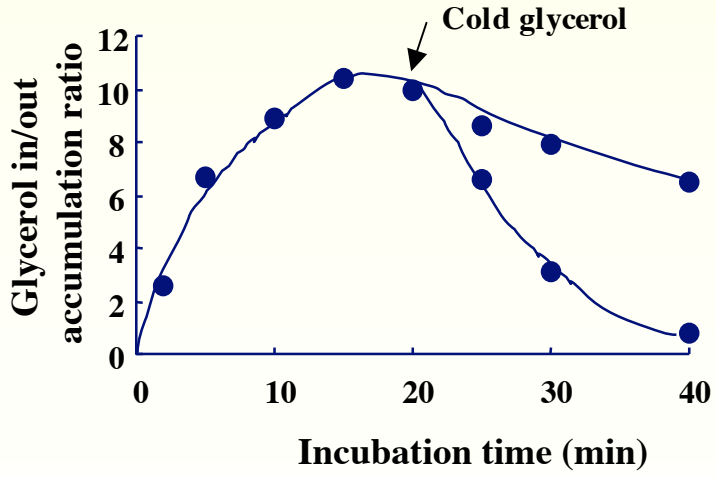
CELLS CULTIVATED ON YEPD + NaCl 1 + Gly 15mM



Km 3.1 mM
Vmax 1659 μ mol h-1 g-1

Kd 0.018 l h-1 g-1





★ ***GUP1* is active in ethanol growing cells**

★ *GUP1* is indispensible for cells to grow on glycerol

★ ***GUP1* deletion increases lag phase**
without affecting μg on glucose and salt

★ ***GUP1* is constutivelly expressed**

★ ***GUP2* activity is only apparent in cells**
growing on glucose under heavy salt stress
combined with glycerol production impairment

★ *GUP2* deletion does not affect growth on
either glucose or non-fermentable carbon sources

Both genes are expressed in ethanol grown cells,
although the level of expression of
GUP1* is apparently much higher than *GUP2

★ ***GUP2* expression - ? is it under**

- glucose repression **or**
- induction by

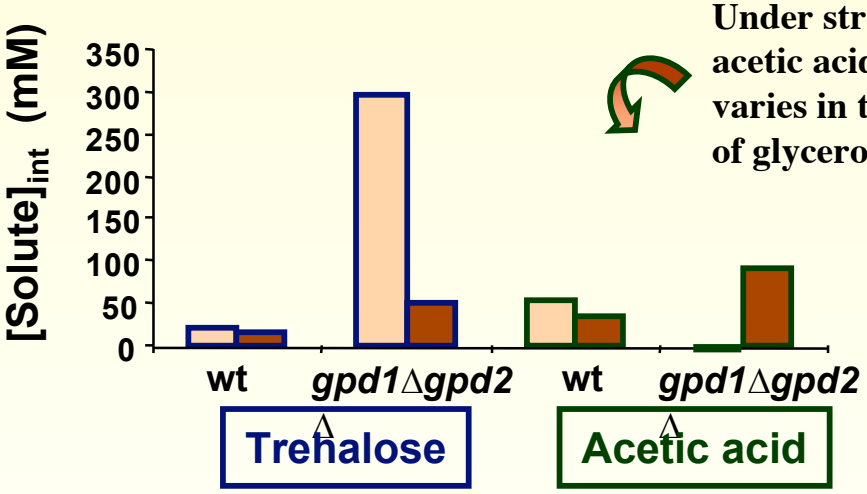
salt stress or
secondary metabolite (like acetic acid)

INTRACELLULAR SOLUTES

Under salt stress

In the absence of glycerol production or extracellular driven accumulation, under stress, the cell produces and accumulates trehalose

Under stress, acetic acid intracellular accumulation varies in the inverse fashion of glycerol production ability



Cells cultivated on YEPD +

NaCl 1M

NaCl 1M + Gly 15mM

★ **The strong molecular homology between GUP1 and GUP2**

- 57% identity 77% similarity

★ **The apparent similarity of behaviour in terms of transport**

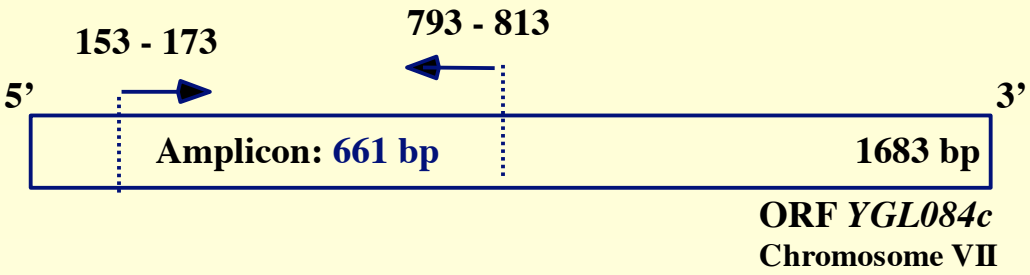
which renders GUP1- and GUP2-dependent glycerol transport physiologically indistinguisheable

- glycerol driven H+ uptake
- Km
- asccumulation capacity

GUP 2 to be, together with GUP1,
the genes responsible for glycerol active uptake
in *Saccharomyces cerevisiae*

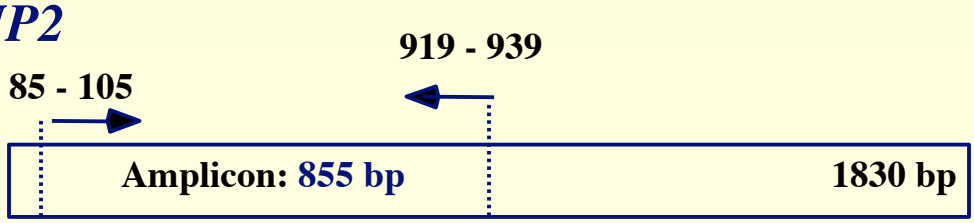
differently regulated

GUP1



ORF *YGL084c*
Chromosome VII

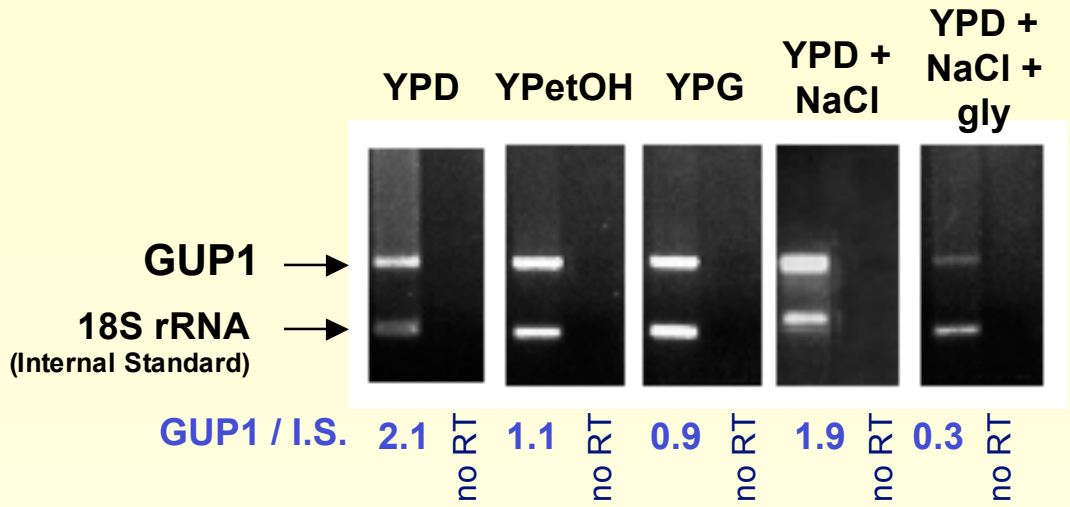
GUP2



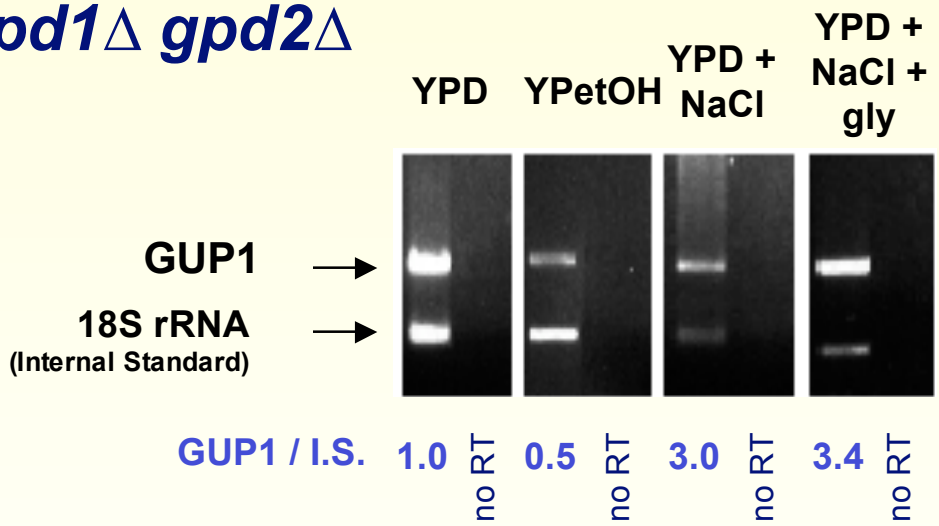
ORF *YPL189w*
Chromosome XVI

Primers	Tm (°C)	%GC	Ta (°C)	Length (b)
GUP1 left primer	60,0	50,0	55	20
GUP1 right primer	60,1	50,0	55	20
GUP2 left primer	59,7	40,0	55	20
GUP2 right primer	59,8	45,0	55	20
18S rRNA primer pair			55-68	

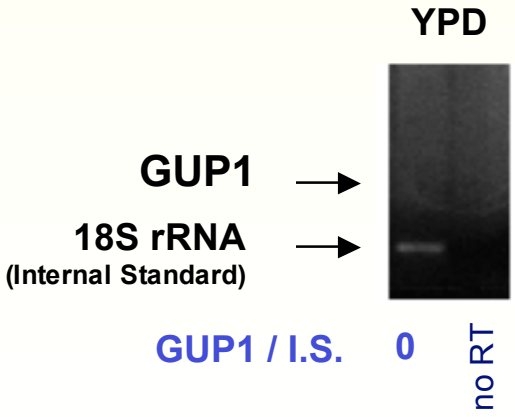
W303-1A



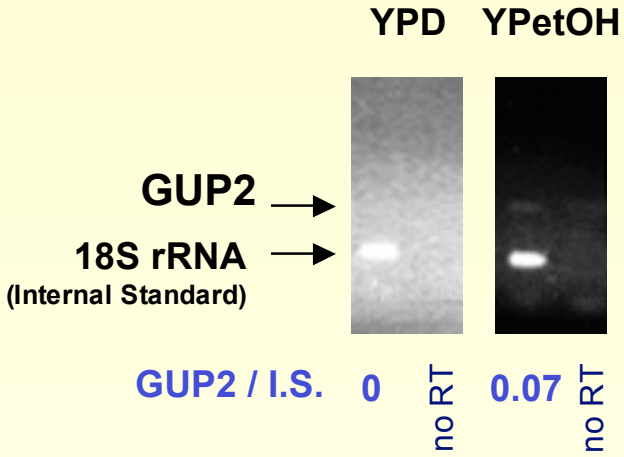
gpd1Δ gpd2Δ



gpd1 Δgpd2 Δgup1Δ



W303-1A



gpd1Δgpd2Δ