Glycerol metabolism and transport activity regulation in *Saccharomyces cerevisiae*

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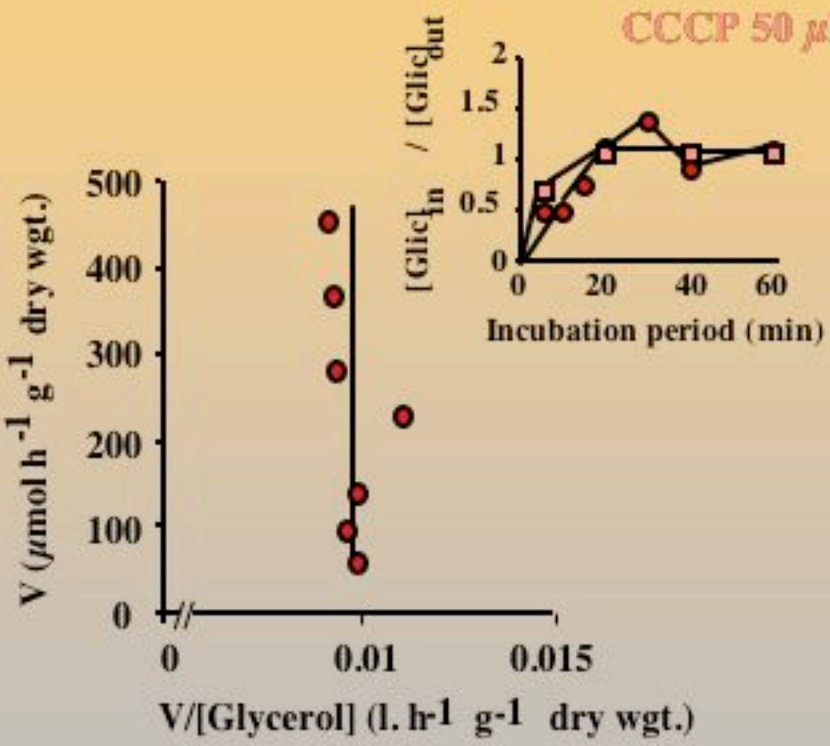
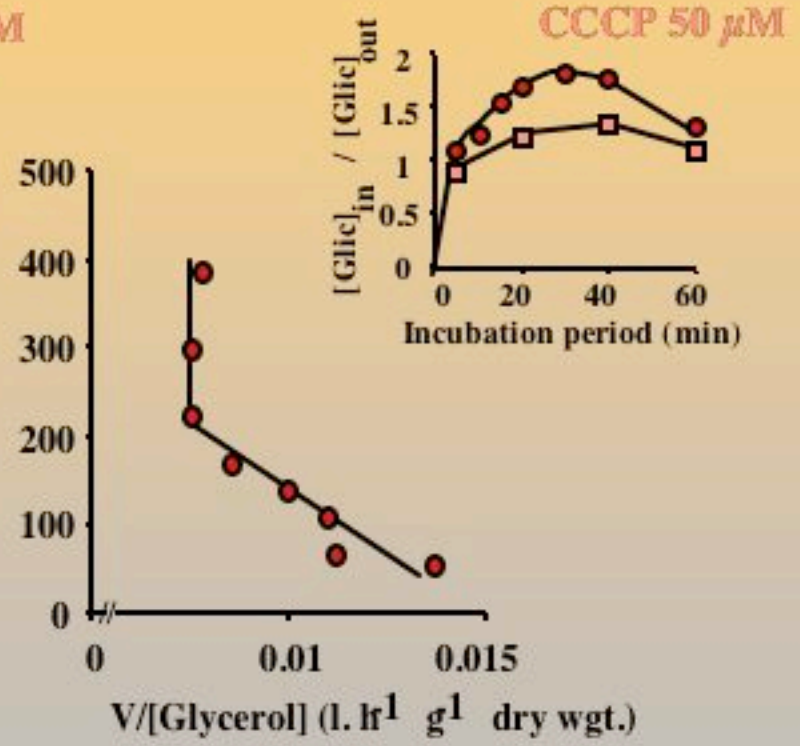
gut 1 Δ

gut 2 Δ

Glucose grown cells





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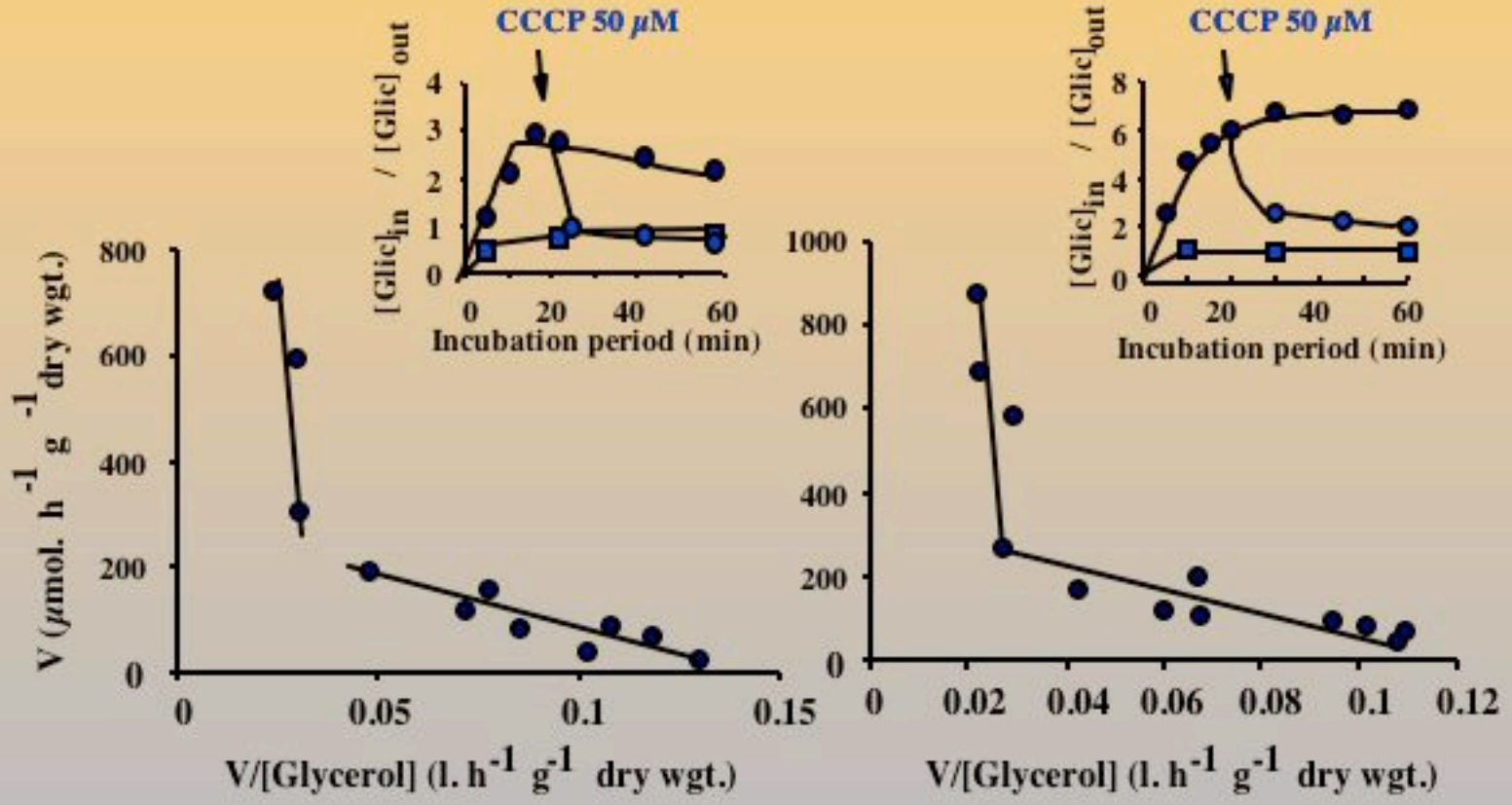
gut 1 Δ

gut 2 Δ

Ethanol grown cells



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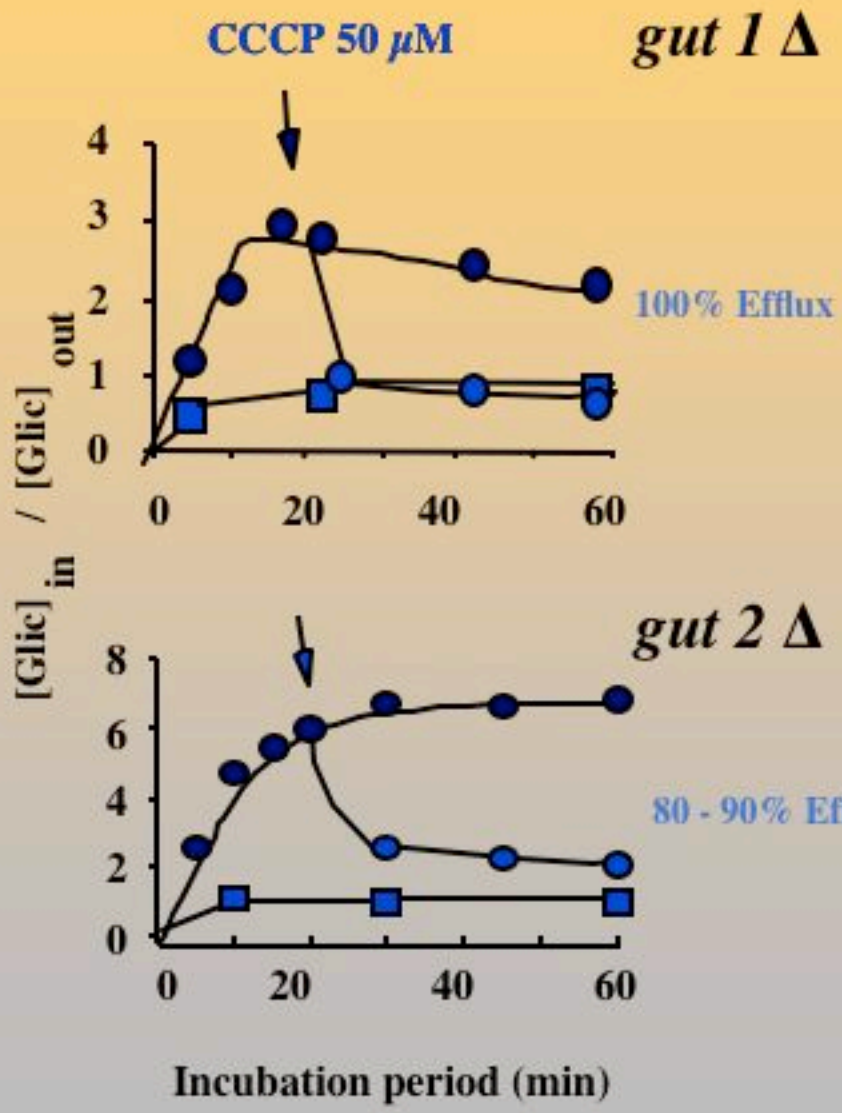
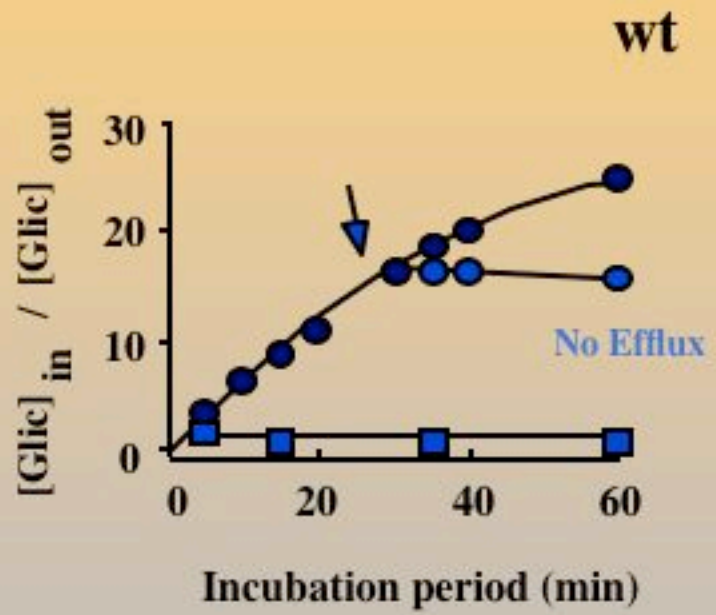
Ethanol grown cells





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Glycerol tansport kinetic parameters

Glucose grown cells

Growth phase	Exponential			Pre-stationary		
Strain	Km (mM)	Vmax (μmol h^{-1} g dwt^{-1})	Kd ($l\,h^{-1}$ g d wt^{-1})	Km (mM)	Vmax (μmol h^{-1} g dwt^{-1})	Kd ($l\,h^{-1}$ g d wt^{-1})
wt IGC 3507	—	—	0.005±0.001(5)	7.8±2.2(3)	91±31(3)	0.006±0.001(5)
*gut1*Δ	—	—	0.010±0.002(2)	—	—	0.009±0 (2)
*gut2*Δ	Nd	Nd	Nd	7.0±7.5(3)	94±29(3)	0.005(3)

In red - Lages and Lucas 1997

— Absence of saturation kinetics

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Kinetic parameters from glycerol tranport

Mutants from glycerol metabolic pathway

Ethanol grown cells

Strain	Km (mM)	Vmax (μmol.h^{-1}.g dwt^{-1})	Kd (μmol.h^{-1}.g dwt^{-1})
wt 3507	1.1 ± 0.3(5)	310±92(5)	N.d.
wt W303	2.0±0.4 (3)	253±46(3)	0.006 (2)
	1.0 ± 0.4(3)	242±50(3)	0.017±0.003 (3)
*gut1*Δ	1.0 ± 0.3(3)	165±25(3)	0.019± 0.001(3)
*gut2*Δ	1.5 ± 0.7(3)	212±48(3)	0.008± 0.002(3)
*gpp1*Δ	1.6 ± 0.7(3)	326±62(3)	0.011± 0.002(3)
*gpp2*Δ	1.9 ± 0.2(2)	318±20(2)	0.009± 0.001(2)
*gpp1*Δ*gpp2*Δ	1.3 ± 0.3(3)	226±27(3)	0.009± 0.001(3)
*gpd1*Δ	1.3 ± 0.3(2)	256±27(2)	0.008± 0.001(2)
*gpd2*Δ	1.2 (1)	252 (1)	0.009(1)
*gpd1*Δ*gpd2*Δ	1.6 ± 0.4(3)	237±30(3)	0.013± 0.001(3)
Mean values	1.4 ± 0.4* (22)	267±73* (19)	0.012±0.004* (22)

±50%

* Except wt 3507 and W303 *gut1*Δ

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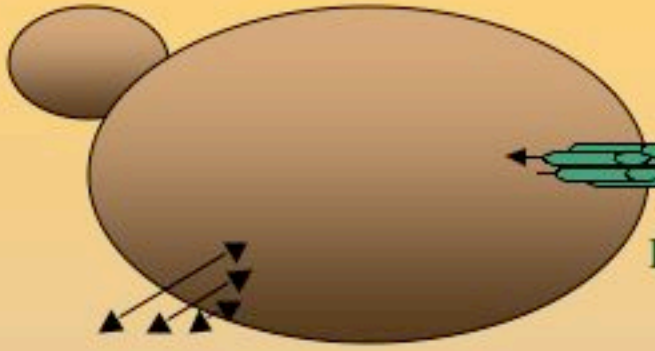
Non-induced cells - Glucose



LOWER AFFINITY

Gly

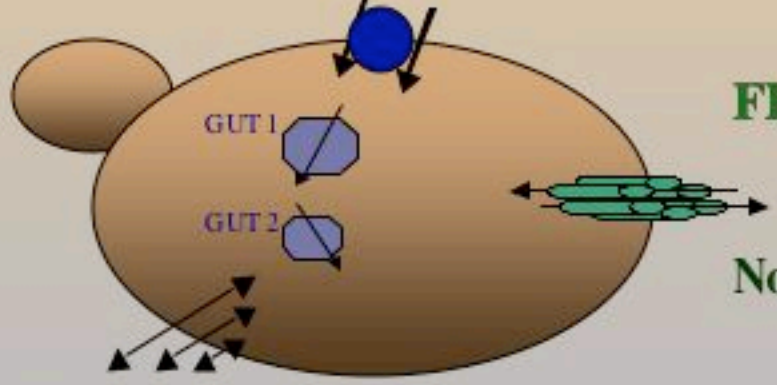
HIGHER AFFINITY

GUP

Gly

H^+

Induced cells - Ethanol Glycerol



FPS1

Gly ?

Not detected

Gly

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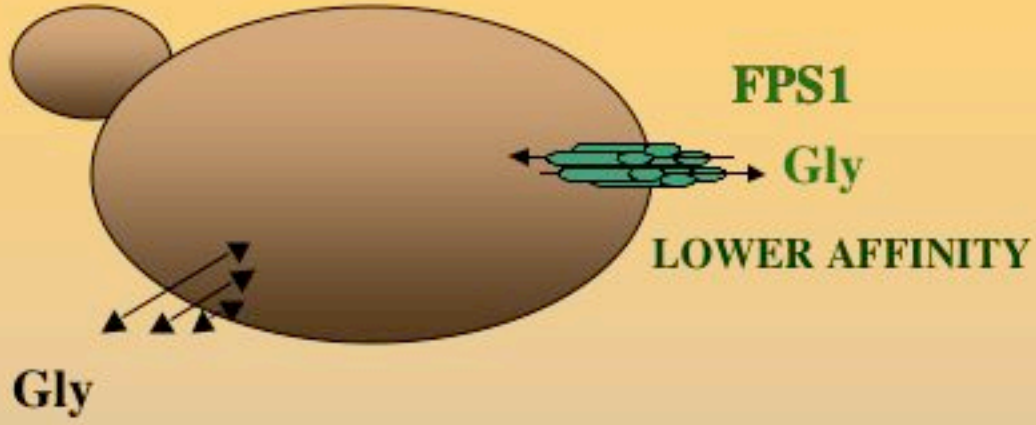
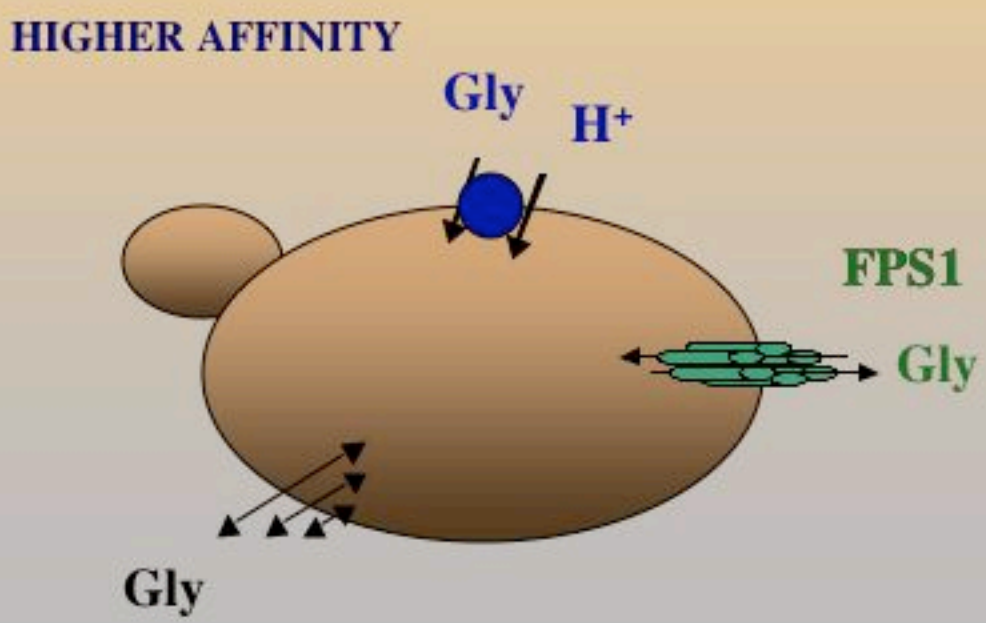
Non-induced cells - Glucose





Induced cells - Ethanol Glycerol

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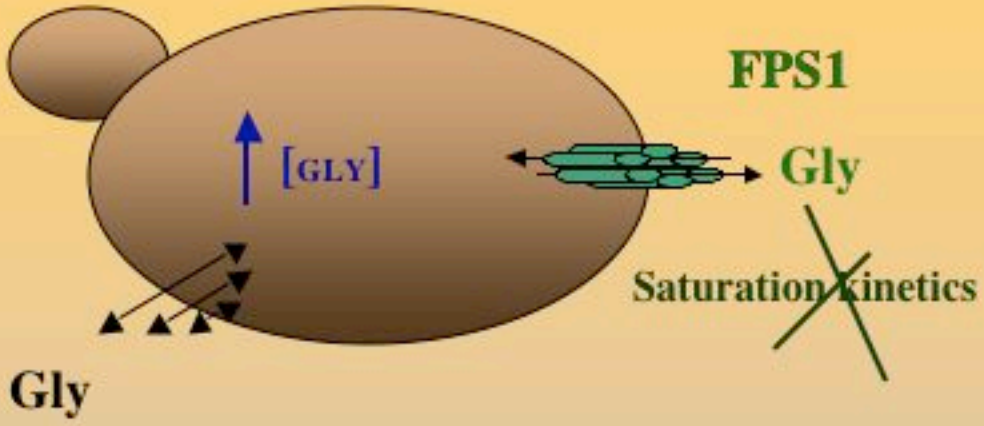
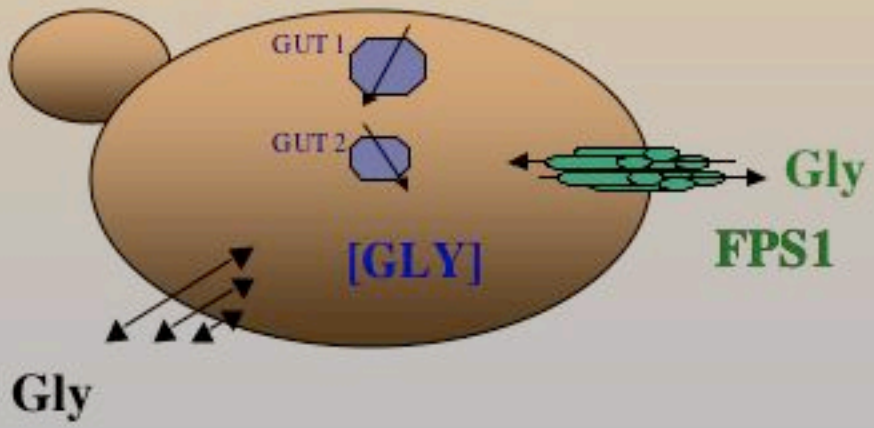
Non-induced cells

Exponentially growing
Glucose present
Catabolic repression



Saturation kinetics



Non-induced cells

Pre-stacionary
Glucose exhausted
Ethanol present
Partial derepression

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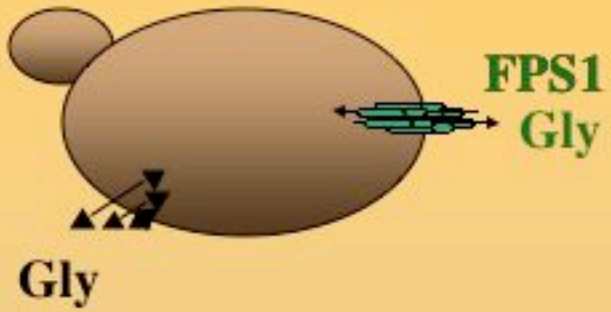
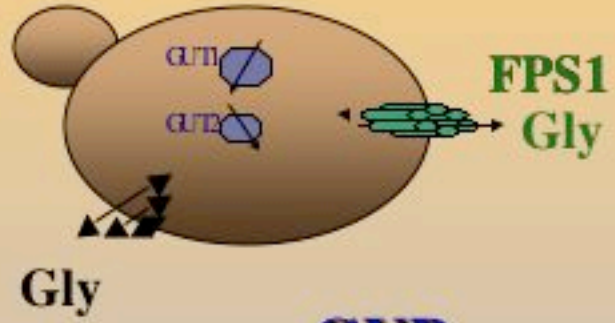
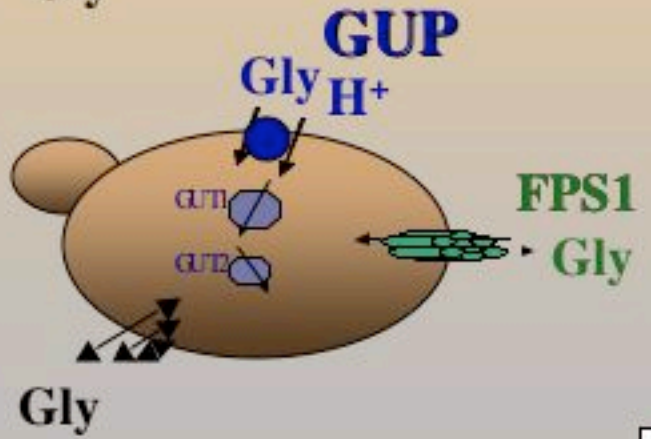
Cells under glucose repression



Derepressed cells



Induced cells



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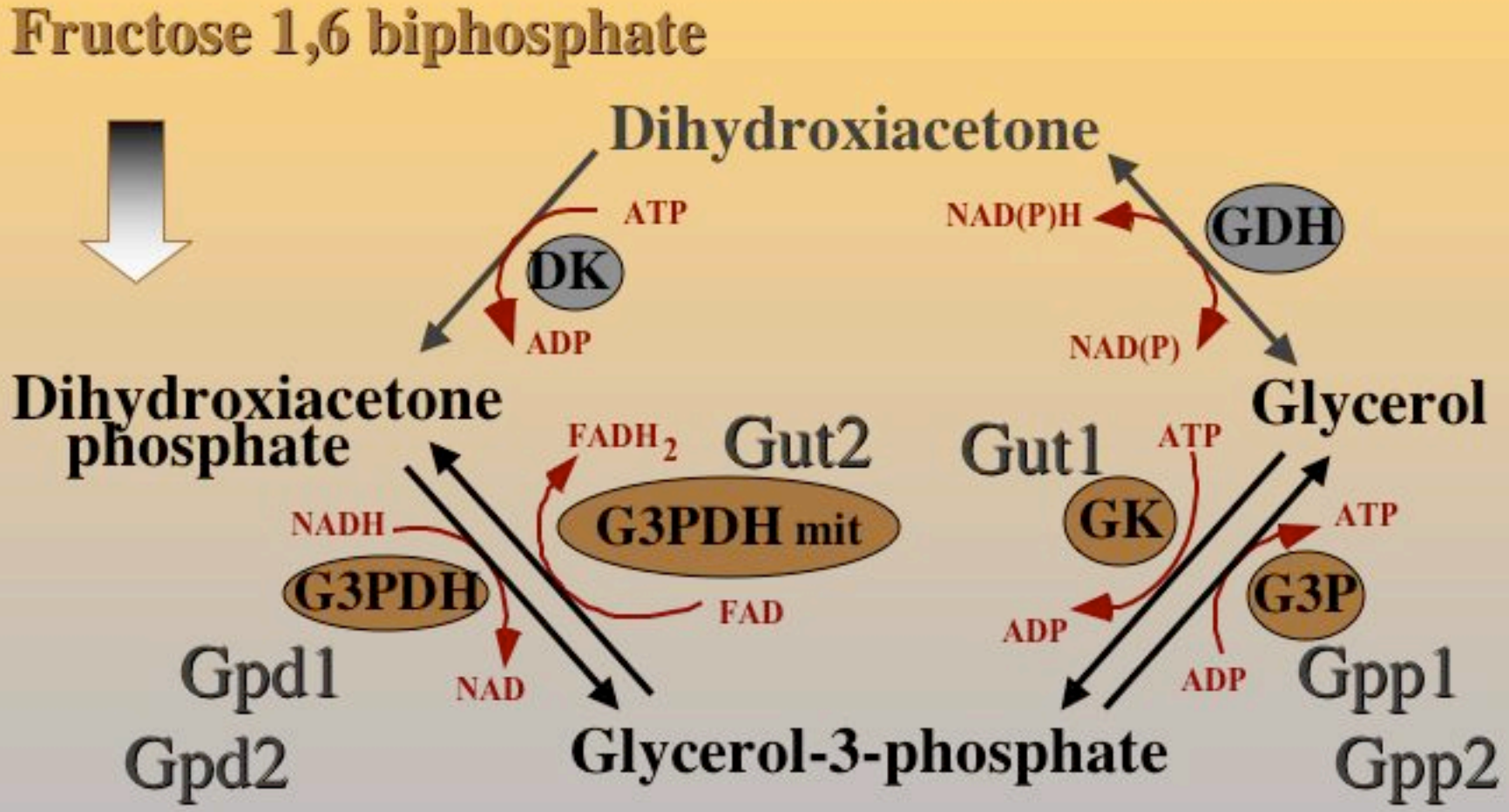
Glycerol metabolic pathway in *S. cerevisiae*



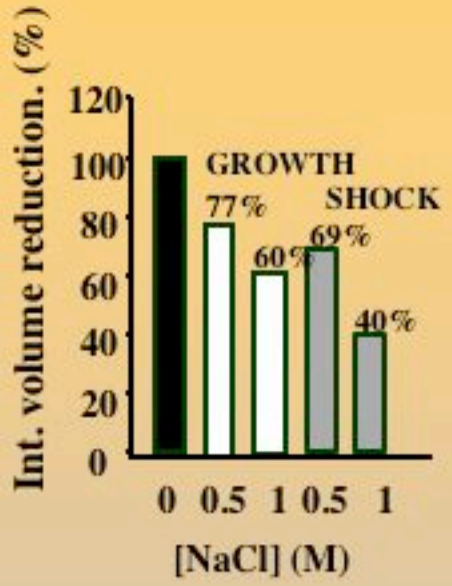
Glucose growing cells

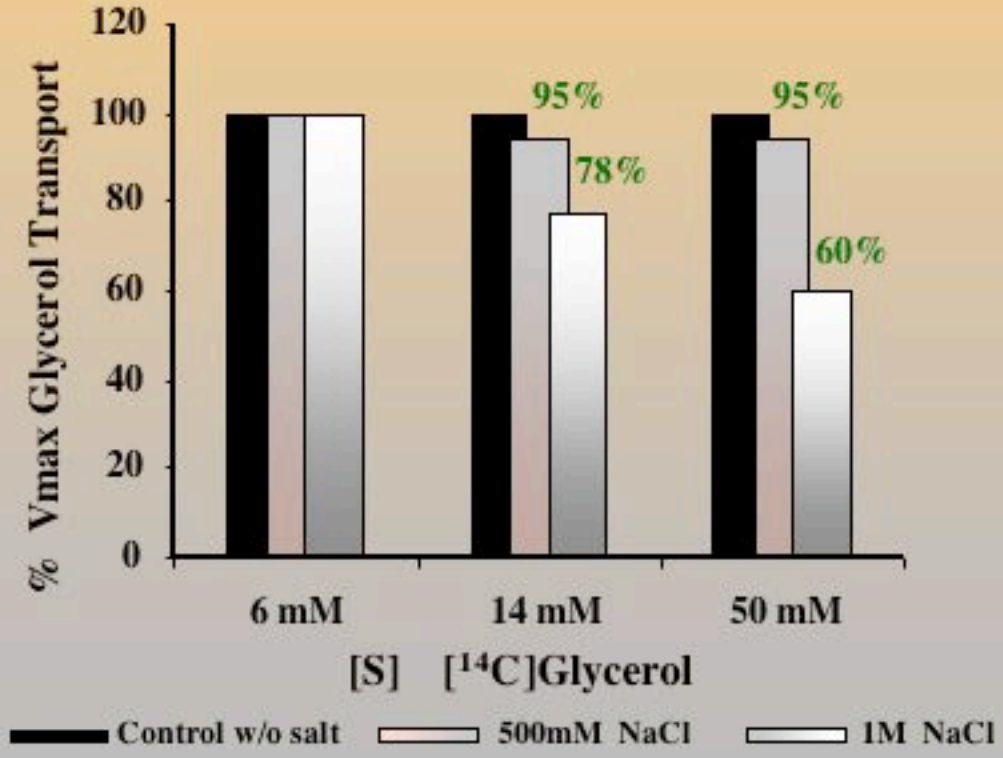
Exponential growth phase

Simple diffusion

No salt Kd 0.005 ± 0.001 l. h-1 g -1 dry wgt. (5) 100 %

1M NaCl Kd 0.002 ± 0.0004 l. h -1 g-1 dry wgt. (3) 40 %





Pre-stationary phase

Low affinity saturation kinetics
(Km ≈ 5mM) Fps1...?
and
Simple diffusion

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Ethanol grown cells

Incubation in	Transport of [^{14}C]glycerol		Transport of H^+ upon glycerol addition	
	Km (mM)	Vmax (μmol.h^{-1}g d.wt.$^{-1}$)	Km (mM)	Vmax (μmol.h^{-1}g d.wt.$^{-1}$)
No salt	1.14 ± 0.34	435.8 ± 21.6	1.82 ± 0.84	435.7 ± 162.7
1M NaCl	1.49 ± 0.78	428.9 ± 73.4	1.61 ± 0.30	550.2 ± 61.2

Lages and Lucas, 1997

It does not induce the tranporter:

- To grow in MM glucose with 1M NaCl
- To grow in MM glucose and transfer to MM glucose + 1M NaCl

Lages and Lucas, 1997

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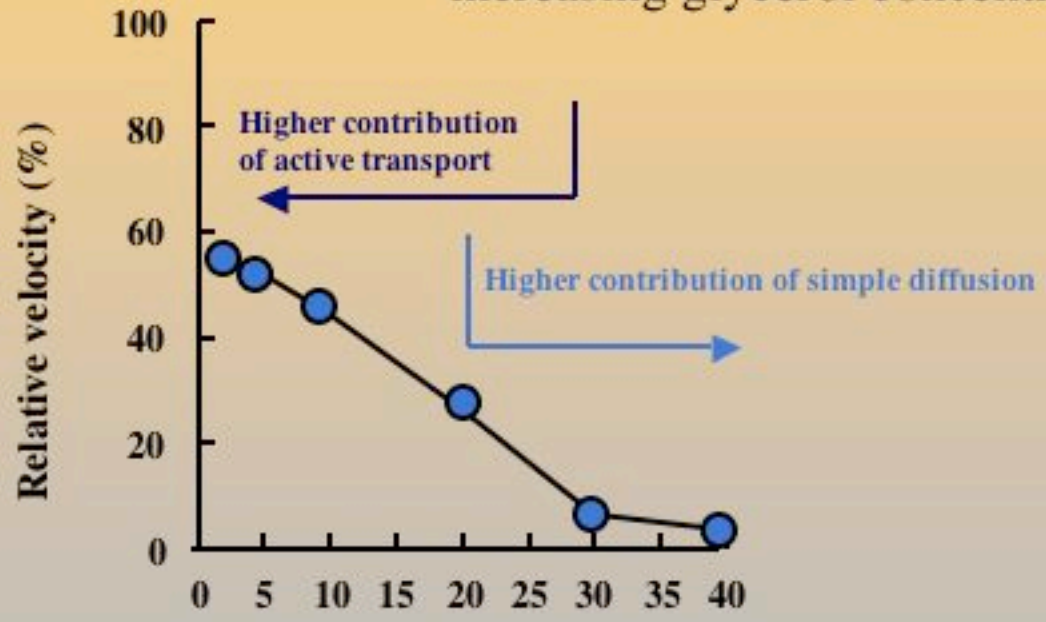
S. cerevisiae

wt W303

Effect of the prtonophore CCCP over the initial uptake rates of increasing glycerol concentrations



100% - in the absence of CCCP

Sutherland *et al.*, 1997

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S. cerevisiae

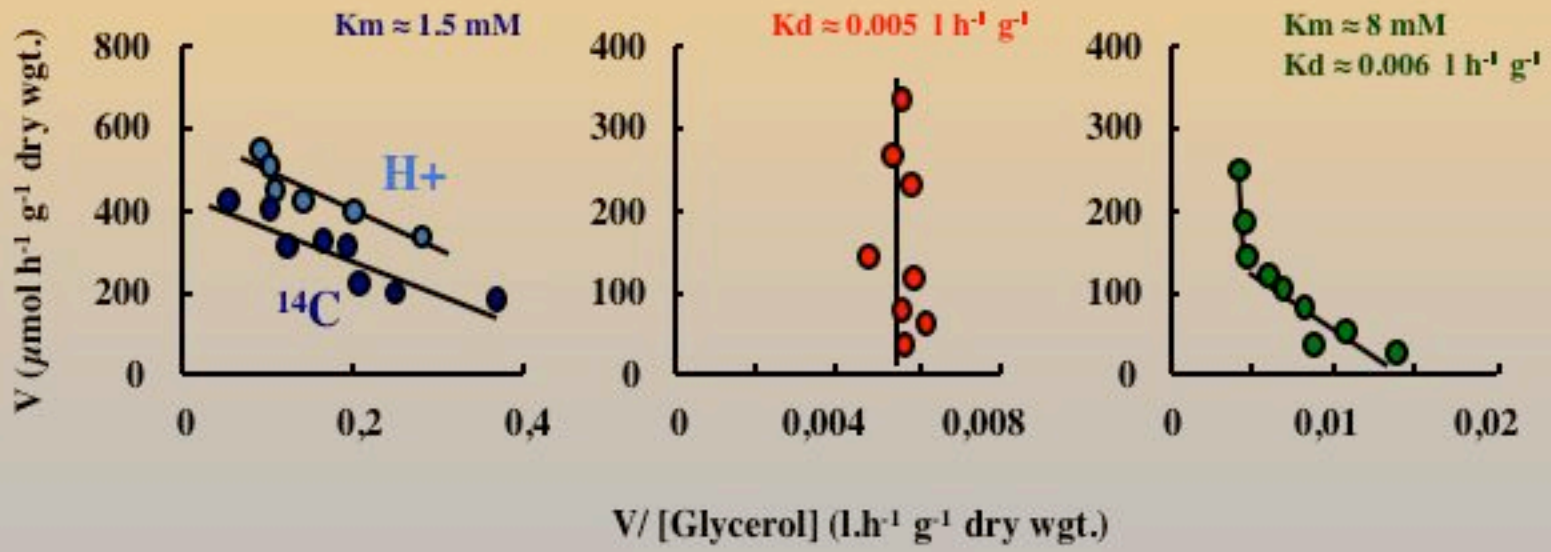
THE STATE OF THE ART

wt IGC 3507

Exponentially growing cells on ethanol

Exponentially growing cells on glucose

Glucose pre-stationary phase culture



Lages and Lucas, 1997

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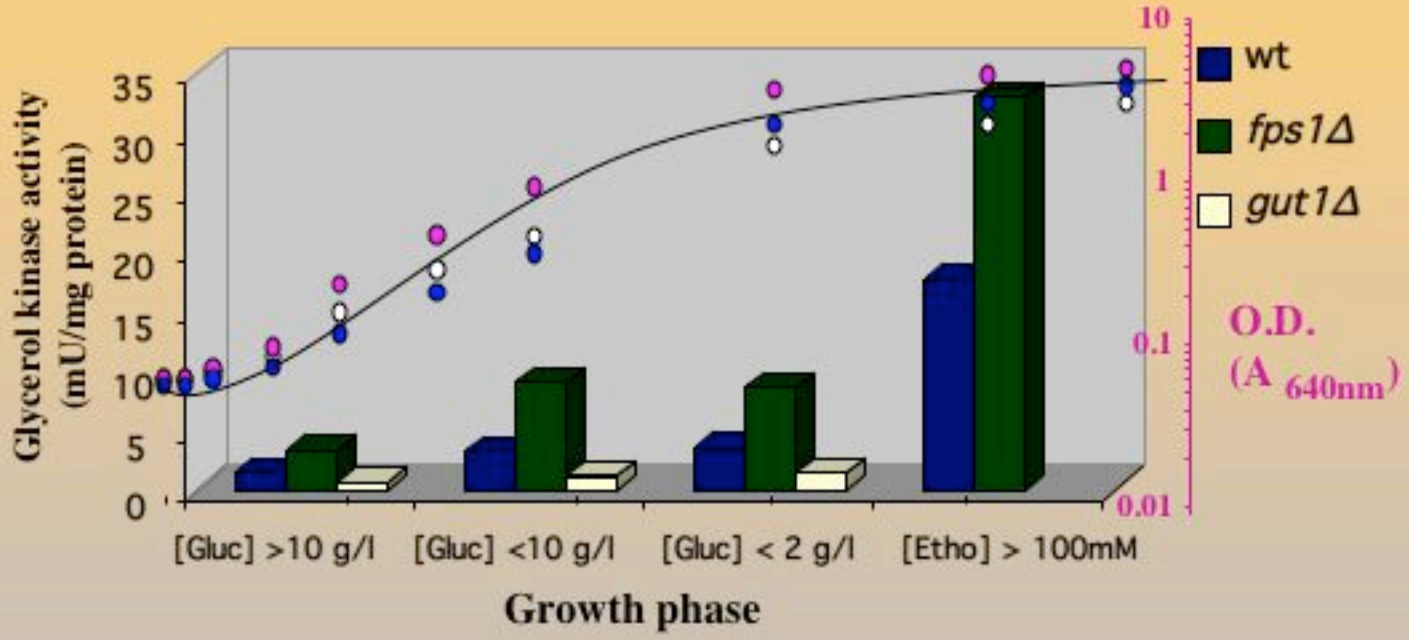
Glucose growing cells



Glycerol kinase activity	wt cells grown on ethanol	61 (mU/mg protein)
	wt cells grown on glycerol	68 (mU/mg protein)

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Comparing glycerol uptake Vmax

Ethanol grown cells

Strain	(operating)	Vmax (μmol h^{-1} g^{-1} d.wt.)	%
wt	(carrier + channel + glycerol kinase)	267±73 (19)	100
*gup*Δ	(channel + glycerol kinase)	181±12 (3)*	±67
*gup*Δ *fps1*Δ	(glycerol kinase)	137±10 (3)*	±51
*gut1*Δ	(carrier + channel)	165±25 (3)	±62
*gup*Δ *gut1*Δ	(channel)	No uptake*	0

GUP - Glycerol Uptake Permease putative gene

* Results obtained by B. Hölst - Carlsberg Laboratory, Denmark

Close collaboration with:

B. Hölst and M. Kielland-Brandt Carlsberg Laboratory
Copenhagen, Denmark

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