

Fps1p CHANNEL IS THE MEDIATOR OF THE MAJOR PART OF GLYCEROL PASSIVE DIFFUSION IN *Saccharomyces cerevisiae*: ARTEFACTS AND RE-DEFINITIONS

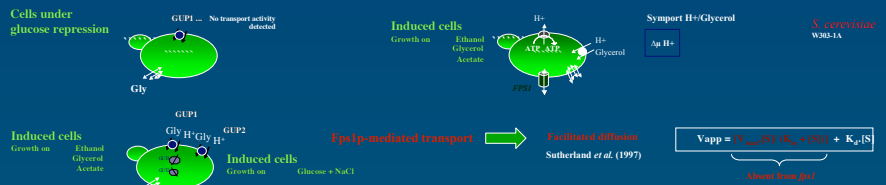
Rui Oliveira, Fernanda Iages, Magda Silva-Graça and Cândida Lucas

CB-UM - Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, PORTUGAL



ABSTRACT

Glycerol has been shown to cross *Saccharomyces cerevisiae* plasma membrane through (1) a H^+ /symport detected in cells grown on non-fermentable carbon sources, (2) the constitutively expressed Fps1p channel and (3) by passive diffusion. Fps1p channel has been named a *facilitator*, for mediating glycerol low affinity transport of the facilitated diffusion type. We present experimental evidence that this kinetics is an artefact created by glycerol kinase activity. Instead, the channel is shown to mediate the major part of glycerol passive diffusion. This is not incompatible with Fps1p major role *in vivo*, which has been previously shown to be the control of glycerol export under osmotic stress or in reaction to turgor changes. Furthermore, *yfl054c* and *fps1* mutants were equally affected by exogenously added ethanol, being the corresponding passive diffusion stimulated. For the first time, to our knowledge, a phenotype attributed to the functioning of *YFL054c* gene is presented. Glycerol passive diffusion is thus apparently channel mediated. This is discussed according to glycerol chemical properties, which contradict the widely spread concept of glycerol *liposoluble nature*. Considering the multiple roles that glycerol intracellular levels play, most probably as a central key to metabolism control and how tight its pathway regulation appears to be, it is consistent that glycerol *in* and *out* fluxes are also closely controlled by the cell.



S. cerevisiae

MC 3587

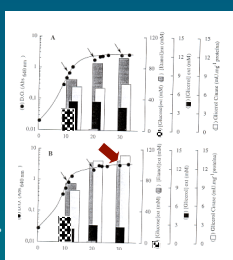
YEM + 1% ethanol

No uptake detected

YEPA

Uptake detected in post-diauxic phase

Very high amounts of Gut1p



S. cerevisiae

MC 3587

MM 2% Gluc / YEPA

MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl

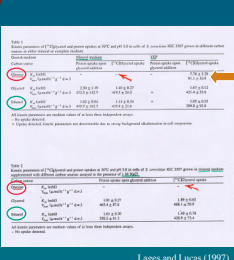
MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl

MM 2% Gluc + 1M NaCl



Lages and Lucas (1997)

S. cerevisiae

W303-1A

Exponentially growing cells

Diauxic shift cells

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

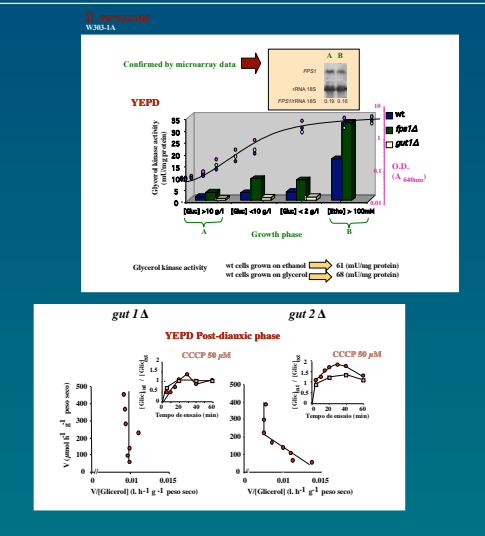
YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

YEM post-diauxic phase

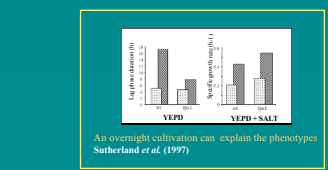
YEM post-diauxic phase



- Active transport keeps being detected as previously described
- Facilitated diffusion appears only during diauxic-shift cells in YEPA
- fps1* mutants present kinetic parameters identical to wt's
- gut1* mutants loose the facilitated diffusion-like transport during diauxic-shift cells in YEPA

$$V_{app} = \frac{V_{max}[S]}{K_m + [S]} + K_p[S]$$

$$V_{app} = \frac{V_{max}[S]}{K_m + [S]} + K_p[S]$$



- Due to glycerol chemical properties passage through lipids is most improbable
- Fps1p is instead apparently responsible for most of what has been called so far *passive diffusion*
- Fps1p homolog also mediates first order kinetics glycerol uptake according to molecular similarity to *FPS1*, the role *in vivo* being still unknown