Expression of *GUP1* and *GUP2*, *Saccharomyces cerevisiae* glycerol active transport genes

Rui Oliveira and Cândida Lucas
Centro de Ciências do Ambiente / Departamento de Biologia da Universidade do Minho
Campus de Gualtar
4710-057 Braga
Portugal

Introduction

Two highly homologous genes related to a phenotype of salt stress tolerance were identified in *Saccharomyces cerevisiae*.
Upon salt stress, a strain lacking the capacity to sinthesize glycerol (*gpd1gpd2*) 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 of this strain. Based on these findings, yeast genomic fragments cut out from a 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 and 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. These genes were named *GUP1* and *GUP2* from glycerol uptake (Holst *et al*, 2000).

Characterization of glycerol transport activity

Previous experiments of glycerol transport on wild type strains have shown that the active transport activity was absent in glucose-grown cells, being this transport detectable in cells grown with non-fermentable carbon sources such as ethanol (fig. 2).
Glycerol kinase (fig. 1), encoded by *GUT1*, interfered on transport measurements, being the saturation kinetics present in *gup1* deleted strains attributed to a residual uptake pulled by an active catabolism of glycerol under derepressing growth conditions (fig. 2). Only the combination of *gup1* and *gut1* deletions eliminated the saturable glycerol uptake and further deletion of the *GUP1* homologue (*GUP2*, *YPL189w*) did not change the uptake results. The phenotype in ethanol-grown cells from radioactive glycerol uptake and extracellular alcalinization of cell suspensions upon addition of glycerol (not shown), suggested that *GUP1* is the gene responsible for the proton symport described before (Lages and Lucas, 1997).

Regulation of glycerol transport activity

As mentioned above, glycerol active transport was found to be under glucose repression. However, the mutant screening that lead to *GUP1* identification was performed in *gpd1gpd2* genetic background growing on media containing glucose as carbon and energy source supplemented with NaCl as osmotic stress agent and glycerol for compatible solute purposes. Therefore, the glycerol transport study was extended to cells grown in this medium (fig. 3).
Surprisingly, *gpd1gpd2* strain displayed a strong uptake which, once determined as a total kinetic study revealed a Vmax more than two times higher than wild type cells grown in ethanol (figs. 2 and 3). This transport activity displayed proton uptake, and accumulation capacity, and protonophore sensivity characteristic of symports (not shown). Furthermore, the Km value was very close to the one attributed to *GUP1* activity (1mM).
One of the possibilities to explain these results was to attribute to *GUP1* homologue the responsability for this active uptake. As shown in fig. 3, *gup2* deletion affects glycerol transport by abolishing any measurable transport in a *gpd1gup1* genetic background. 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.

Optimization of RT-PCR

The expression of *GUP1* and *GUP2* was determined by relative quantitative RT-PCR. Previously, PCR conditions were optimized in order to quantitatively detect mRNA differences among strains and growth conditions tested. Exponential phase of amplification was determined for *GUP1* (fig. 4 and 5), *GUP2* (fig. 6 and 7) and the internal standard 18S rRNA (figs. 8 and 9). A number of amplification cycles corresponding to mid-exponential phase was determined for each analysis. By stoping PCR at this point, the fluorescence intensity of the bands is proportional to the amount of target molecules at the begining of PCR. Once optimization has been established, the linearity of the method was tested using different amounts of DNA to perform PCR (fig. 10 and 11).

Relative quantification of mRNA´s

According to microarrays data available at the Saccharomyces Genome Database, we have observed a decrease in mRNA levels during the shift from fermentative to respiratory metabolism for both genes (figs. 12 and 13).
In wild type cells, highest levels of *GUP1* mRNA were observed by growth on glucose as sole carbon and energy source. In cells grown on glycerol, the transcription was the lowest of all, indicating a possible effect of extracellular glycerol as inhibitor of transcription. Unexpectedly, the double mutant *gpd1gpd2* unable to synthesize glycerol, exhibited lower levels of mRNA in glucose-grown cells than the wild type. On the other hand, glycerol apparently increases *GUP1* transcription, as can be seen by the difference in mRNA levels between salt stress with and without added glycerol in the double mutant (fig. 12).
Contrarily, for *GUP2*, transcription is the lowest in glucose-grown cells, pointing to a probable involvement in salt stress response and ehanol utilization. In *gpd1gpd2* mutant, mRNA levels are higher for glucose-grown cells in the presence of NaCl and glycerol, which correlates with the high transport activity measured in this condition.

Conclusions

Taking together the results of the transport assays and RT-PCR, we had detected *GUP1* and *GUP2* mRNA's for every growth conditions, suggesting post-transcriptional regulation of glycerol transport activity. Nevertheless, a match between mRNA levels and active transport activity was observed for *gpd1gpd2* double mutant cells grown on glucose in the presence of NaCl and glycerol, for which the highest values were determined (figs. 3 and 13). Hence, *GUP2* is apparently involved in response to salt stress when simultaneous impairment of glycerol synthesis occurs and provided its presence in the medium. As for *GUP1*, no correlation was detected between mRNA levels and transport activity, suggesting that the major steps of regulation of *GUP1* expression lie downstream transcription.
In silico analysis of the promoter region of both genes had shown the presence of a stress response element (STRE - AG_4) at position -292 only on *GUP2*. So, the results presented here provide good evidence for different mechanisms for regulation of expression and the involvement of *GUP2* in salt stress response in yeast.

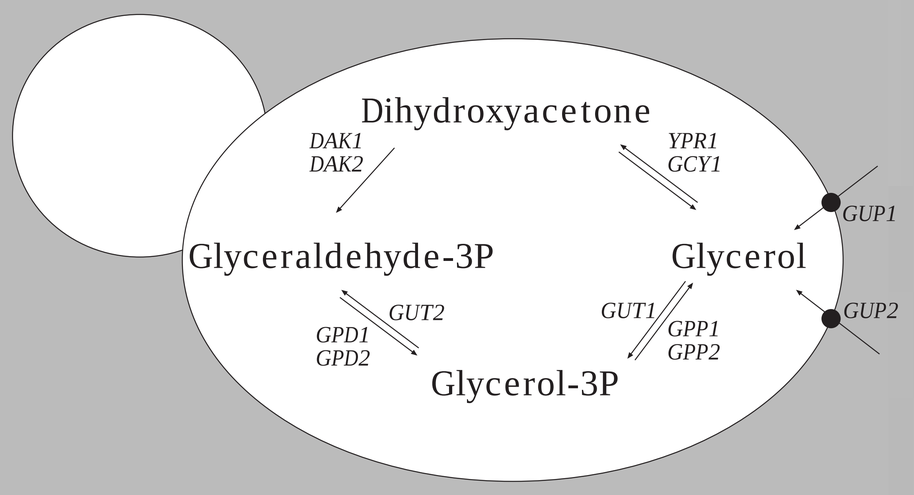

Figure 1. Metabolic pathways of glycerol.

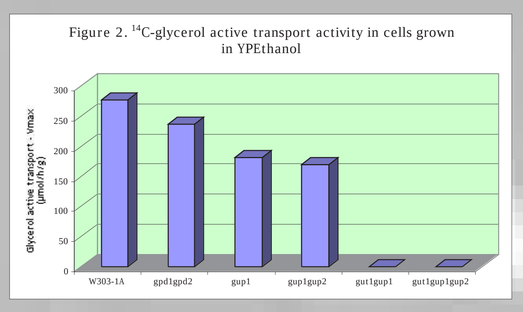

Figure 2. ^{14}C-glycerol active transport activity in cells grown in YPEthanol

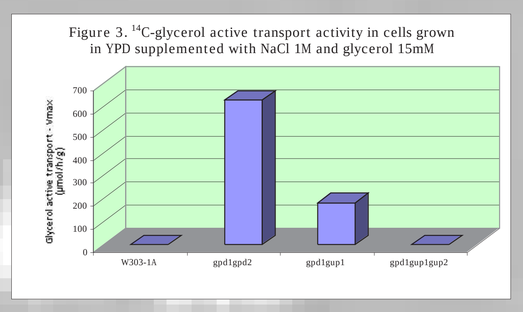

Figure 3. ^{14}C-glycerol active transport activity in cells grown in YPD supplemented with NaCl 1M and glycerol 15mM

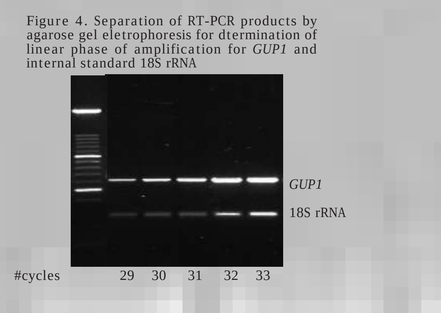

Figure 4. Separation of RT-PCR products by agarose gel eletrophoresis for dtermination of linear phase of amplification for *GUP1* and internal standard 18S rRNA

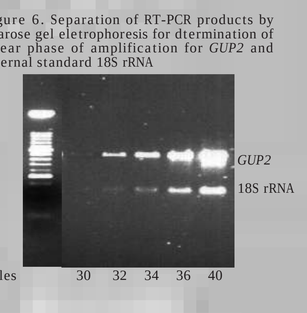

Figure 6. Separation of RT-PCR products by agarose gel eletrophoresis for dtermination of linear phase of amplification for *GUP2* and internal standard 18S rRNA

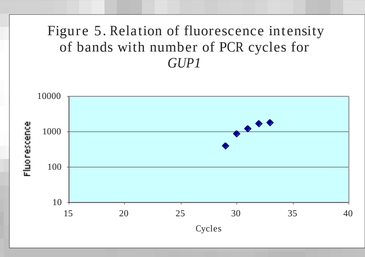

Figure 5. Relation of fluorescence intensity of bands with number of PCR cycles for *GUP1*

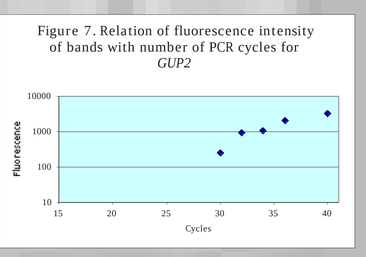

Figure 7. Relation of fluorescence intensity of bands with number of PCR cycles for *GUP2*

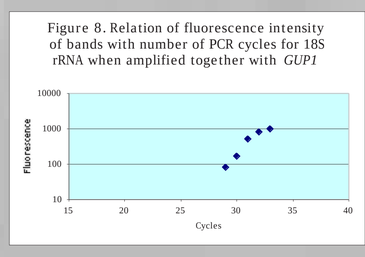

Figure 8. Relation of fluorescence intensity of bands with number of PCR cycles for 18S rRNA when amplified together with *GUP1*

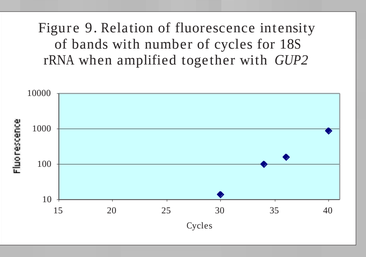

Figure 9. Relation of fluorescence intensity of bands with number of cycles for 18S rRNA when amplified together with *GUP2*

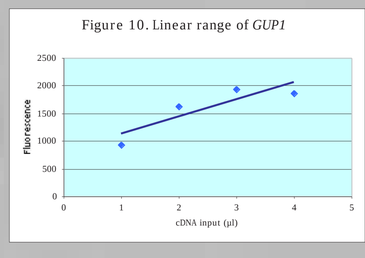

Figure 10. Linear range of *GUP1*

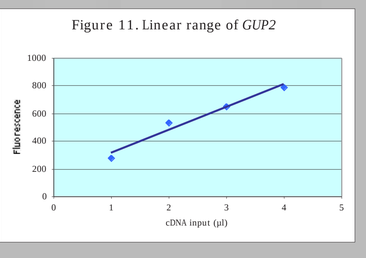

Figure 11. Linear range of *GUP2*

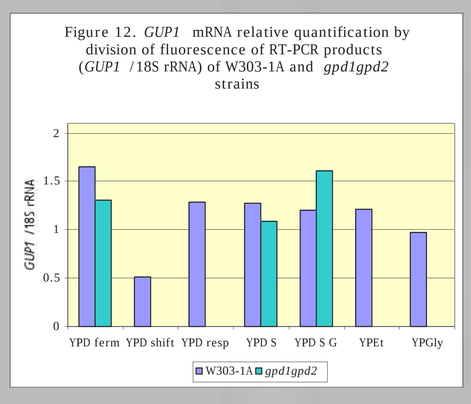

Figure 12. *GUP1* mRNA relative quantification by division of fluorescence of RT-PCR products (*GUP1* /18S rRNA) of W303-1A and *gpd1gpd2* strains

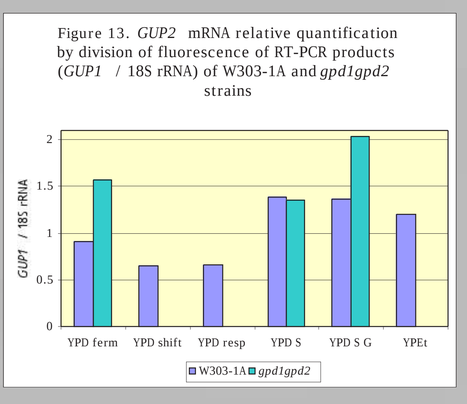

Figure 13. *GUP2* mRNA relative quantification by division of fluorescence of RT-PCR products (*GUP1* / 18S rRNA) of W303-1A and *gpd1gpd2* strains

Legend

YPD ferm - fermentative metabolism in YPD (OD_{600}<1)
YPD shift - shift to respiratory metabolism in YPD (OD_{600}=2)
YPD resp - respiratory metabolism in YPD (OD_{600}>3)
YPD S - YPD supplemented with NaCl 1M
YPD SG - YPD supplemented with NaCl 1M and glycerol 15mM
YPEt - YP with ethanol as carbon and energy source
YPGly - YP with glycerol as carbon and energy source

References

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Nelissen *et al*. 1997. *FEMS Microbiol Rev*. 21: 113-134
Lages and Lucas. 1997. *Biochim Biophys Acta*. 1322: 8-18