REGULATION OF GLYCEROL TRANSPORT GENES *GUP1* AND *GUP2* IN *S. CEREVISIAE*

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Two highly homologous genes related to a phenotype of salt stress tolerance were identified in *Saccharomyces cerevisiae*[1]. These were named *GUP1* and *GUP2* from Glycerol Uptake. To evaluate the extent of the involvement of these genes in glycerol active transport[2], an extensive work of glycerol transport characterisation was performed in mutants defective on either gene, together with different combinations of deletions in the genes encoding for enzymes from glycerol metabolic pathway. These strains were cultivated in ethanol, glucose and glucose with 1M NaCl. In ethanol grown cells the activity of glycerol proton symport could be attributed to the activity of Gup1, while on glucose grown cells, as expected[2], none of the strains exhibited glycerol uptake. On the other hand, the double mutant *gpd1*Δ*gpd2*Δ*,* as well as the other deletion combinations defective on either or both *GPD* genes, once cultivated on glucose in the presence of salt, revealed a surprisingly high transport activity. The deletion of *GUP1* reduced this activity to approximately 50%, which suggested that the remaining uptake could be eventually due to *GUP2.* The hypothesis of an artefact created by glycerol kinase driven glycerol uptake has been discarded measuring enzyme specific activity, which was very low. In view of these results, we performed some transport assays, which results suggest that Gup2 might be identical to Gup1, *i.e.*, act as a proton symport, thus justifying the impossibility of making a physiological based distinction.

The expression of these two genes was determined by RT-PCR on wt cells. Results have shown that both genes are active on ethanol grown cells, being *GUP1* is the major intervenient. On the other hand, on glucose grown cells, the expression of *GUP2* is absent, indicating that this gene is under glucose repression, but *GUP1* is expressed. This result is in opposition to what had been determined physiologically[2]. Nevertheless, the level of expression is very low, eventually justifying why no transport activity was detected in these cells. Moreover, the presence of salt stress during growth on glucose did not lead to transport activity detection, but both *GUP* genes present significant levels of expression in these cells. These results suggest complex expression and activity regulation processes, which are presently under study in cells deleted in several different combinations of *GUP* and *GPD* genes.

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2. Lages, F., Lucas, C (1997) *Bioch.Biophys Acta,* 1322: 8-18.