Regulation of glycerol transport genes GUP1 and GUP2 in Saccharomyces cerevisiae

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Two highly homologous genes related to a phenotype of salt stress tolerance were identified in Saccharomyces cerevisiae. These genes were named GUP1 and GUP2 from glycerol uptake (Holst et al., 2000).

Upon salt stress, at a certain level of NaCl, cells became unable to osmoregulate their growth and morphology. A transport screen was performed in the two strains, which suggested that the primary function of these two genes was to mediate the uptake of glycerol, as shown by the inability of the mutant to grow on glycerol under conditions of NaCl stress.

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 mutated in the ORF YGL084c (which is a homologue of YGL051c, YPL159w) was identified by BLAST searches with 57% sequence identity and 77% similarity at amino acid level.

The model presented above suggests GUP1 to be under glucose repression. However, the mutant screening that led to GUP1 identification was performed in media containing glycerol as a carbon and energy source supplemented with NaCl and glucose. Therefore, the glycerol transport study was extended to cells grown in the medium and, as a control, in the absence of glucose.

To elucidate the role of glycerol transporters with respect to sodium accumulation, intracellular levels of several solutes were determined by HPLC in cells grown under salt stress with and without externally added glycerol. Besides glycerol, trehalose and acetate acid were found and no other compound was detected (Figure 5). Trehalose levels increase when salt stress is present whereas acetate acid levels decrease (Figure 6). Furthermore, acetate acid levels were not affected when the glycerol transport activity was increased by the GUP1 deletion in the absence of glucose.

The results presented above suggest that GUP1 is under glucose repression, and that this repression is abolished by the GUP1 deletion in the absence of glucose. The model proposed is based on these observations: (1) high transport activity in the GUP1 deletion in the absence of glucose, (2) high transport activity in the GUP1 deletion in the absence of glucose and externally added glycerol, and (3) high transport activity in the GUP1 deletion in the absence of glucose and externally added acetate acid.

The following model is proposed based on available data that includes three distinct environmental conditions:

1. Cells under glucose repression (GUP1 deletion in the presence of glucose): GUP1 is expressed under these conditions, as shown by Northern blotting and by RT-PCR.
2. Cells under glucose repression (GUP1 deletion in the absence of glucose): GUP1 is not expressed, as shown by Northern blotting and by RT-PCR.
3. Cells under glucose repression (GUP1 deletion in the absence of glucose and externally added glycerol): GUP1 is expressed, as shown by Northern blotting and by RT-PCR.

Relative quantification of GUP1 mRNA by RT-PCR showed that GUP1 is expressed under all conditions tested (including glucose/glycerol cells) (Figure 5). Northern blotting expression in comparison to what was determined with the physiological approach. Nevertheless, a marked difference in mRNA levels was detected, indicating that GUP1 might be expressed under these conditions.

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