

GLYCEROL METABOLISM AND TRANSPORT ACTIVITY REGULATION IN *SACCHAROMYCES CEREVISIAE*

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With the purpose of studying the correlation between glycerol metabolic pathway and glycerol active transport (1) in *S. cerevisiae*, an extensive study on glycerol transport was elaborated in all the available mutants from the genes of glycerol metabolic pathway having W303 as common genetic background: *gut1Δ*, *gut2Δ*, *gpp1Δ*, *gpp2Δ*, *gpd1Δ* and *gpd2Δ* and several double mutants. For this purpose we chose to diagnostic active transport determining uptake kinetic parameters (K_m and V_{max}) and maximum glycerol accumulation ratios, as well as the capacity of accumulated radiolabeled substrate to be extruded after accumulation in glucose- and ethanol-grown cells. All these mutant strains presented active uptake of the same order of magnitude of the wild type, except glycerol kinase deletion mutant, *gut1Δ*. Ethanol-grown cells of *gut1Δ* presented active uptake with identical K_m but lower V_{max} ($\pm 70\%$) than wild type strain. This indicated that, most probably, uptake V_{max} determinations in all the other strains might present some contribution of glycerol kinase activity. This hypothesis was reinforced with measurements of glycerol kinase activity in cell free extracts obtained under the same physiological conditions. Furthermore, *gut1Δ* and *gut2Δ* glucose-grown cells were also tested as to the activity of Fps1(2). Passive diffusion constant values were similar to the ones in wild type cells, but Fps1 activity could not be detected in *gut1Δ*.

The results suggest that glycerol kinase activity, from all the enzymes in glycerol pathway, is the only one to interfere with active uptake measurements, although it does not resume them. The same reasoning could not be applied to Fps1 activity detection, which is highly affected by the presence of glycerol kinase, questioning its role as a facilitator(2) and thus opening a clear way for the clarification, instead, of its role as a channel(3).

(1) Lages and Lucas (1997) *Biochim. Biophys. Acta* 1322: 8-18

(2) Sutherland *et al.* (1997) *J. Bacteriol.* 179: 7790-7795

(3) Tamas *et al.* (1999) *Mol. Microbiol.* 31: 1087-1104

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