Intracellular glucose concentration affects glucose sensing by transporter-like sensor Snf3 in yeast
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Intracellular glucose concentration affects glucose sensing by transporter-like sensor Snf3 in yeast

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Abstract. In this study, a quantitative assay was set up to directly measure the signalling activity of transporter-like sensor Snf3 at various extracellular sugar concentrations.

We determined apparent affinities of glucose to Snf3 under different growth conditions. In conditions with high levels of intracellular ligand, the sensing through Snf3 sensor was less effective.

Introduction
Gene expression in micro-organisms is regulated according to extracellular conditions such as nutrient concentrations. Transporter-like sensors, i.e. non-transporting sensor proteins with high sequence similarity to transporters, are involved in sensing of sugars as well as amino acids. In yeast Saccharomyces cerevisiae, two transporter-like sugar sensors, Snf3 and Rgt2, have been identified. It has previously been indirectly shown that Snf3 senses low glucose concentrations whereas Rgt2 senses high concentrations. A model has been suggested of the function of transporter-like sensors1 (Fig1) where the conformation of the sensor protein depends on the relative concentrations of ligand inside and outside the cells.

In this study, we directly measure the signalling activity of Snf3 at various extracellular sugar concentrations. To separate extra- and intracellular sugar pools, yeast strain deficient in glucose transport was used in all experiments (Fig 2).

Conclusions
The apparent average affinity of Snf3 to glucose was about 0.4 mM. The apparent affinity was measured on cells grown on glycerol as well as for cells grown on maltose. EIC50 was 1.7 mM for cells grown on maltose, which is about 4 times higher than on glycerol (Fig 4-5.). Since intracellular glucose concentration was high during growth on maltose (Fig 6), it indicates that accumulation of intracellular glucose affects ligand affinity of Snf3. The effect was more pronounced when the gene for hexokinase 2 (hkh2) was deleted, which significantly increased intracellular glucose and at the same time the Mth1 degradation as a result of Snf3 signalling was abolished. The results are in accordance with the previously suggested model for transporter-like sensors (Fig 1).

Method
Yeast strains based on EBY.vW10002 with deletion of 17 hexose transporters were used. Sugar sensing was measured by following the degradation of MTH1 protein, which occurs in response of activation of SNF3 (Fig3). Standard methods for Western blotting were used for the analysis. For estimating the average effective concentrations for signalling (EC50), MTH levels in various glucose concentrations were determined 10 min after sugar addition.

References
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