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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 level 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).

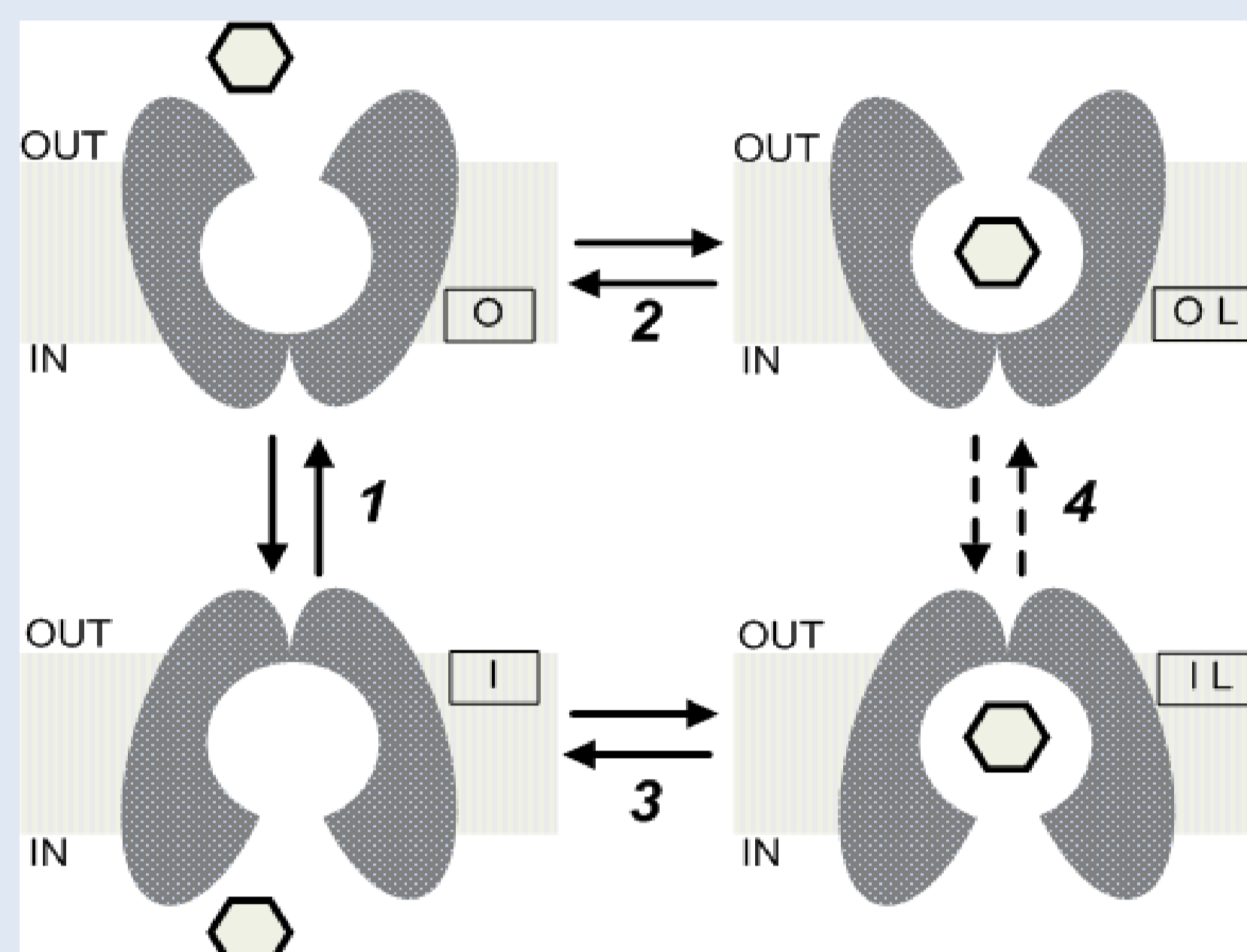


Fig 1. Model of ligand binding in transporter-like sensors. Outward-facing conformation (O) induces signal whereas inward-facing conformation (I) does not signal. The dotted arrows for reaction 4 indicate that for a non-transporting sensor working as proposed for Ssy1p¹ or Snf3 (present work), states OL and IL cannot be directly turned into one another. For a real transporter, on the other hand, reaction 4 must be efficient. The outward facing conformation of the sensor (states O and OL) is hypothesized to be signaling. O, outward facing, OL, outward facing, ligand bound; I, inward facing, IL, inward facing, ligand bound.

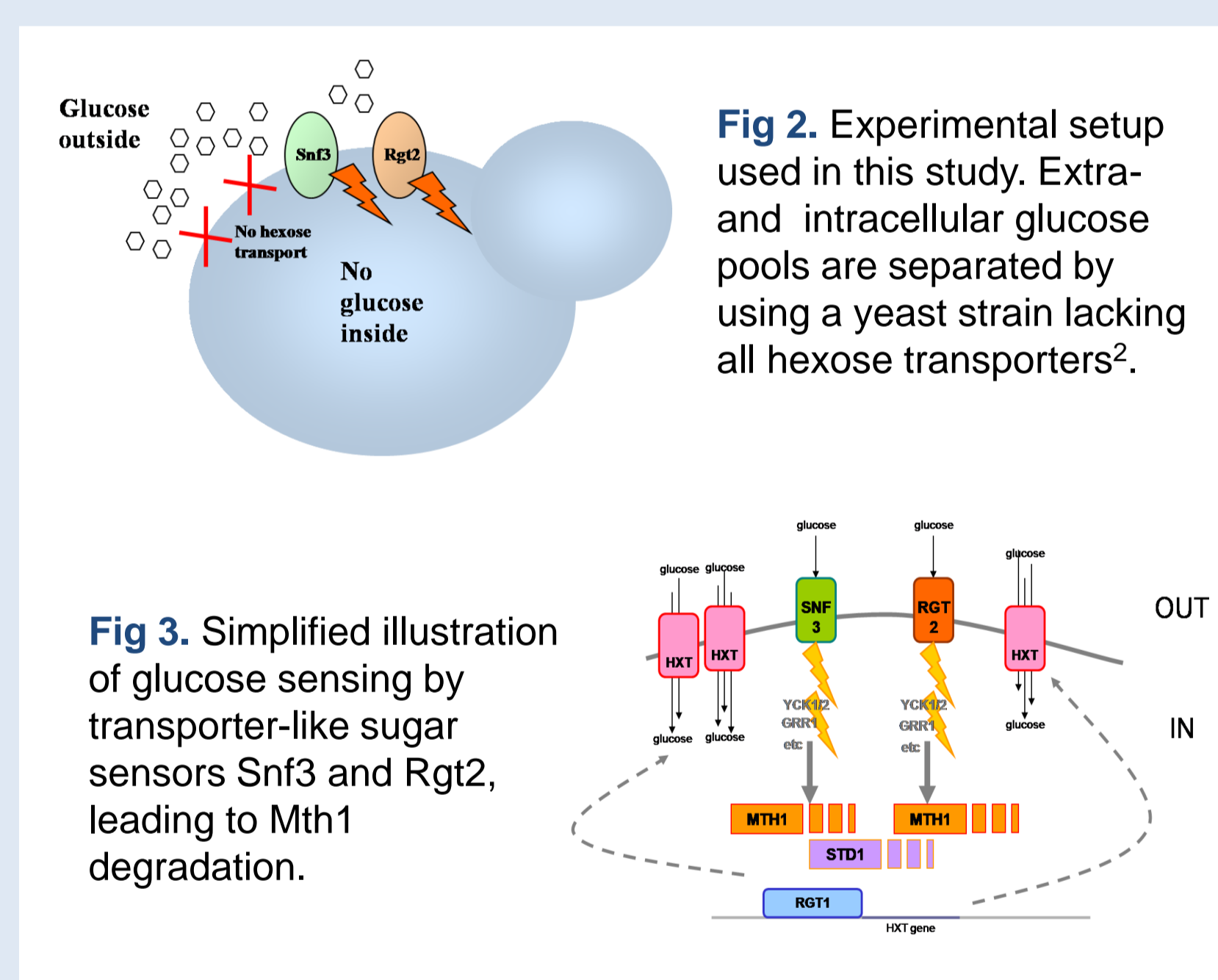


Fig 2. Experimental setup used in this study. Extra- and intracellular glucose pools are separated by using a yeast strain lacking all hexose transporters².

Fig 3. Simplified illustration of glucose sensing by transporter-like sugar sensors Snf3 and Rgt2, leading to Mth1 degradation.

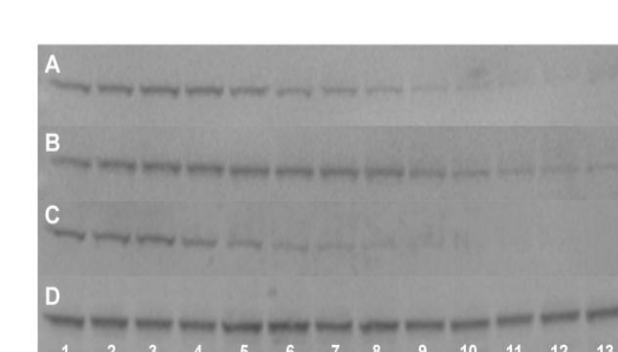
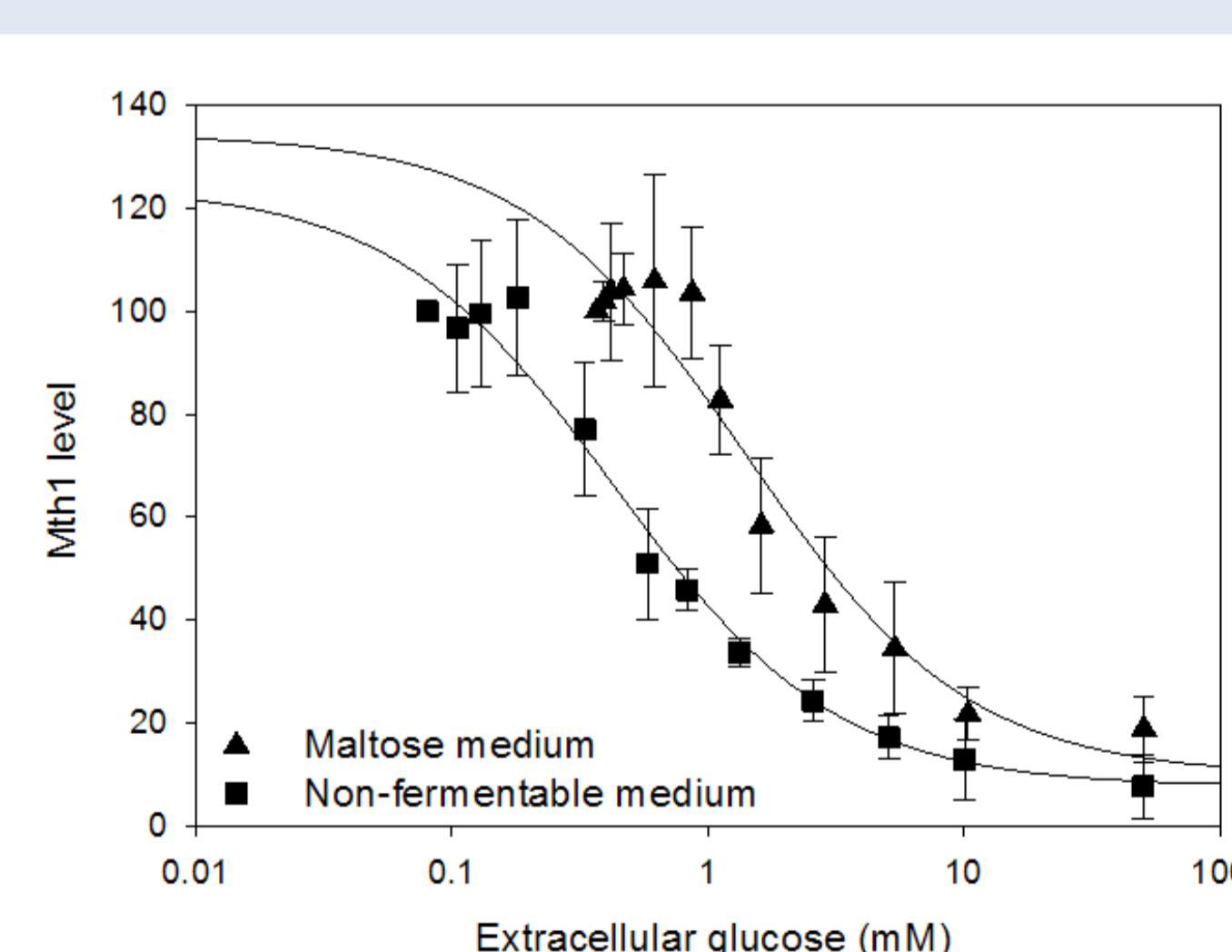


Fig 5. EC₅₀ measurements with concentration of glucose increasing from left to right. A. WT glycerol, B. WT maltose, C. dhxk2 glycerol, D. dhxk2 maltose.

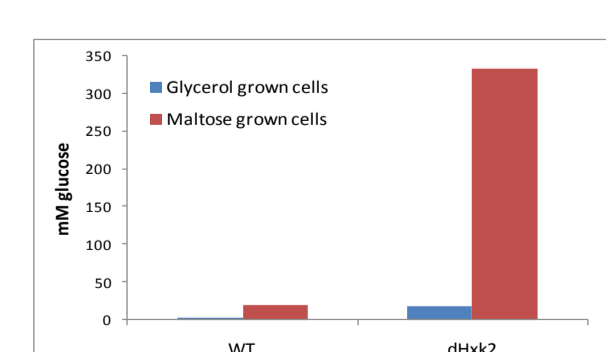


Fig 6. Intracellular glucose concentrations in yeast cells grown in glycerol or maltose medium. Measurements were made after methanol quenching.

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. EC₅₀ 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 (hvk2) 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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Acknowledgments

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