The role for Casein kinases in glucose sensing and signaling in yeast

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ABSTRACT

Background: Glucose is not only a nutrient, but also a signaling molecule controlling cell growth and development (1). In yeast, glucose induction of *HXT* (glucose transporter gene) expression is achieved via the Rgt2 and Snf3 glucose sensing receptor (GSR)-mediated signal transduction pathway. The plasma membrane-associated casein kinases Yck1 and Yck2 (Ycks) are involved in this pathway, but their exact role remains unclear.

Results: Here, we provide evidence that the YCks are required for the stability of the Rgt2 and Snf3 glucose receptors. Cell surface levels of Rgt2 are significantly decreased in a *yck1Dyck2^{ts}* mutant, but this is not due to endocytosis-mediated vacuolar degradation of the receptor. Similar observations are made in an *akr1D* mutant, where the Ycks are no longer associated with the plasma membrane. However, in an *akr1D* mutant, both the Ycks and the Rgt2 glucose receptor are colocalized to the cytoplasm, where Rgt2 is stable and functions as an effective receptor for glucose signaling. We also demonstrate that Rgt2 interacts with the Ycks and that this interaction occurs in a glucose-dependent manner.

Conclusions: Casein kinases interact and stabilize glucose sensing receptors.

INTRODUCTION

Accumulating evidence suggests that the yeast glucose receptor Rgt2 generates an intracellular signal in response to glucose that promotes glucose uptake and metabolism (2). More recently, we have shown that the stability of this receptor is regulated by casein kinases (3). To get more insights into this observation, we assessed the cell surface expression of Rgt2 using genetic, biochemical and cell biological approaches.

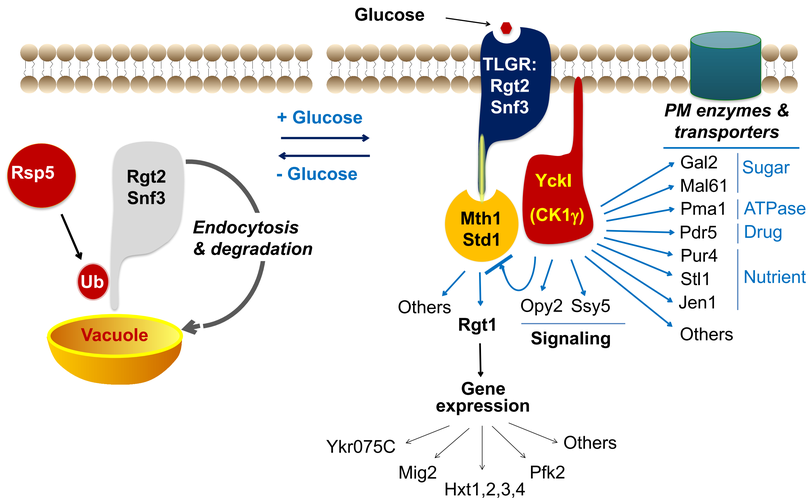

Fig. 1. TLGRs (Transporter-Like Glucose Receptors) play a key role in regulating glycolytic flux. TLGRs generate a signal in response to glucose for the induction of genes involved in glucose transport and metabolism, such as glucose transporter genes (*HXT*s).

METHODS

To understand how stability of the Rgt2 receptor is regulated by casein kinases, we investigated plasma membrane levels of the glucose receptors in wild type, yck, and akr1 mutant cells grown in different concentrations of glucose using a combination of genetic, biochemical and cell biological approaches.

RESULTS

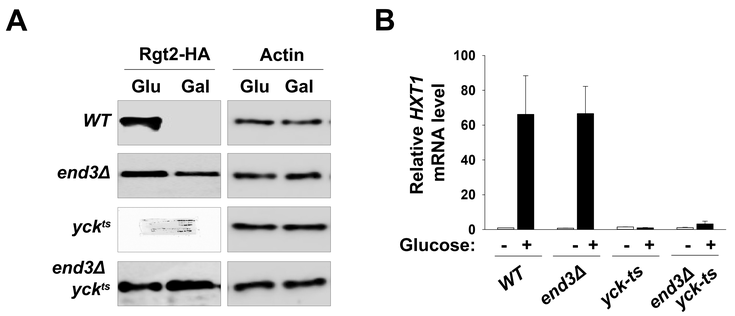

Fig. 2. Ycks are required for stability of Rgt2. Western blot (A) and RT-PCR analyses of Rgt2 expression in cells of the indicated genotypes.

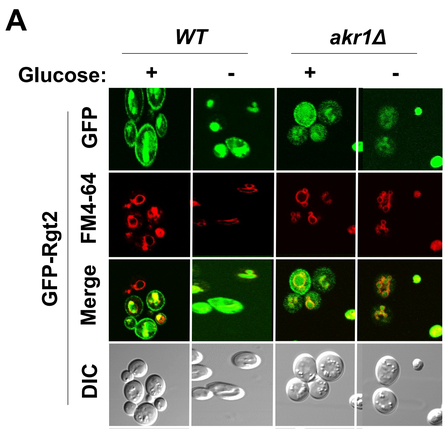

Fig. 3. (A) *WT* and *akr1* strains expressing GFP-Rgt2 were grown in glucose (2%) or galactose (2%) as described above and analyzed by confocal microscopy. Yeast cells were stained with FM4-64 to mark the vacuolar membrane and observed under the Zeiss LSM 510 META confocal laser scanning microscope. DIC and GFP fluorescence images are shown. (B) *WT* and *yck1yck2^{ts}* strains expressing Rgt2-HA were grown in glucose or galactose. Whole cell lysates prepared from *WT*, *akr1*, and *yck1yck2^{ts}* strains were immunoprecipitated with agarose-conjugated anti-HA antibody, and the precipitates were analyzed by Western blotting with anti-HA antibody (IP).
* Both the Ycks and Rgt2 are mis-localized to the cytoplasm of the *akr1* mutant, where Rgt2 functions as an effective receptor for glucose signaling, suggesting that the cytoplasmic Rgt2 is stabilized by the cytoplasmic Ycks

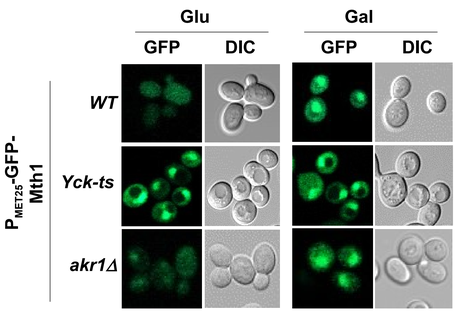

Fig. 4. Yeast cells of the indicated genotype expressing GFP-Mth1 were grown as described above. Subcellular localization of GFP-Mth1 was analyzed by confocal microscopy. Mth1 is a co-repressor of the Rgt1 HXT repressor and required for the binding of Rgt1 to the HXT promoters. Glucose induces degradation of Mth1, leading to induction of HXT expression.

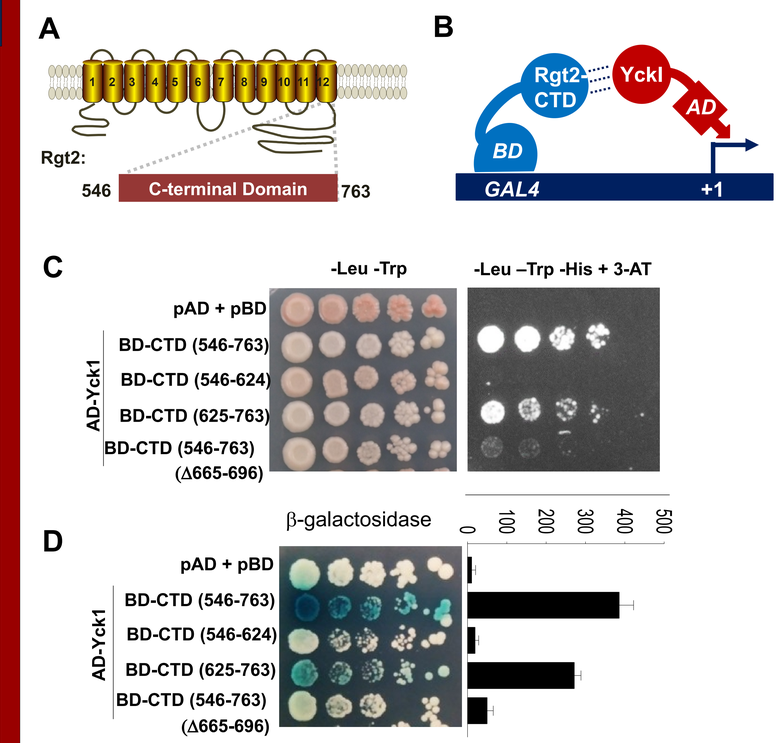

Fig. 5. Rgt2 interacts with Yck1 through its C-terminal domain (A) in the yeast two hybrid system (B). Interactions between Yck1 and Rgt2 were scored for expression of the GAL-HIS3 (-His+3-AT) (C) and GAL-lacZ reporters (D).

RESULTS AND CONCLUSIONS

Both Rgt2 and Yck1 are mis-localized to the cytoplasm of the *akr1* mutant, where Rgt2 remains stable and active as a functional receptor. These results suggest that glucose binding to Rgt2 enables it to interact with the Ycks, and that this interaction not only increases the stability of Rgt2 but also serves to activate downstream signaling events.

FUTURE WORK

- Understand how the Rgt2 interaction with the Ycks is regulated.
- Determine whether the Ycks regulate plasma membrane localization of Rgt2.
- Identify the exact role of the Ycks in Rgt2/Snf3-mediated glucose sensing and signaling.

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