The Role of the Tail Domain of the Yeast Cell Surface Receptors Rgt2 and Snf3 in Glucose Signaling

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Abstract

Glucose is the fundamental fuel source for life. Thus, most organisms have evolved numerous mechanisms for sensing and signaling the availability of glucose, ensuring its optimal utilization. The yeast *Saccharomyces cerevisiae* senses extracellular glucose levels through the two paralogous glucose sensing receptors (GSRs) Rgt2 and Snf3, which appear to detect high and low levels of glucose, respectively. The difference in the roles of Rgt2 and Snf3 in glucose sensing is a consequence of their cell surface abundance rather than a result of the two paralogous proteins having different functions. Glucose binding to the glucose receptors initiates a signaling cascade that leads to expression of glucose transporter genes. The glucose sensing receptors are structurally similar to hexose transporters (Hxts), with 12 predicted transmembrane spanning segments, but they differ from all Hxts in their unusually long cytoplasmic C-terminal tails. Here, we provide evidence that the transmembrane domains of the glucose sensing receptors are responsible for recognition of glucose, whereas the tails of the receptors are involved in signaling. This is consistent with the previous observation that the tails are not required for generating the glucose signal but serve to enhance signaling. Based on these results, we discuss possible mechanisms of glucose sensing and signaling in the yeast.

Introduction

The yeast cells possess multiple glucose transporters with different affinities for glucose, enabling them to grow well over a broad range of glucose concentrations. Expression of major glucose transporters is regulated by the glucose sensing receptors (1). Rgt2 is required for induction of high glucose induced HXT genes, whereas Snf3 is required for induction of HXT gene expression by low glucose, identifying Rgt2 and Snf3 as glucose sensing receptors for high and low concentrations of glucose, respectively (2). It has been postulated that this difference is perhaps due to differences in the amino acid residues of the receptors that form the glucose binding pocket and that the different roles of Rgt2 and Snf3 in high and low glucose-induced gene expression are simply due to their different affinities for glucose (3). We have previously shown that the difference in the roles of Rgt2 and Snf3 in glucose sensing is a consequence of their cell surface abundance rather than a result of the two paralogous proteins having different functions (4). In this study, we investigate the functional roles of the TM and Tail domains in glucose sensing and signaling.

Methods

For yeast complementation growth assay, transformed cells were amplified on 2% galactose solid media and then suspended in sterilized di-water with a final concentration of OD600=0.200. Five 1/5 serial dilutions were created and spotted onto 2% glucose or 2% raffinose plates. The spotted colonies were incubated at 30°C for 48hrs.

For Western blotting, proteins were resolved by 10% SDS-PAGE and transferred to PVDF membrane (Millipore). Proteins were detected by the enhanced chemiluminescence (ECL) system.

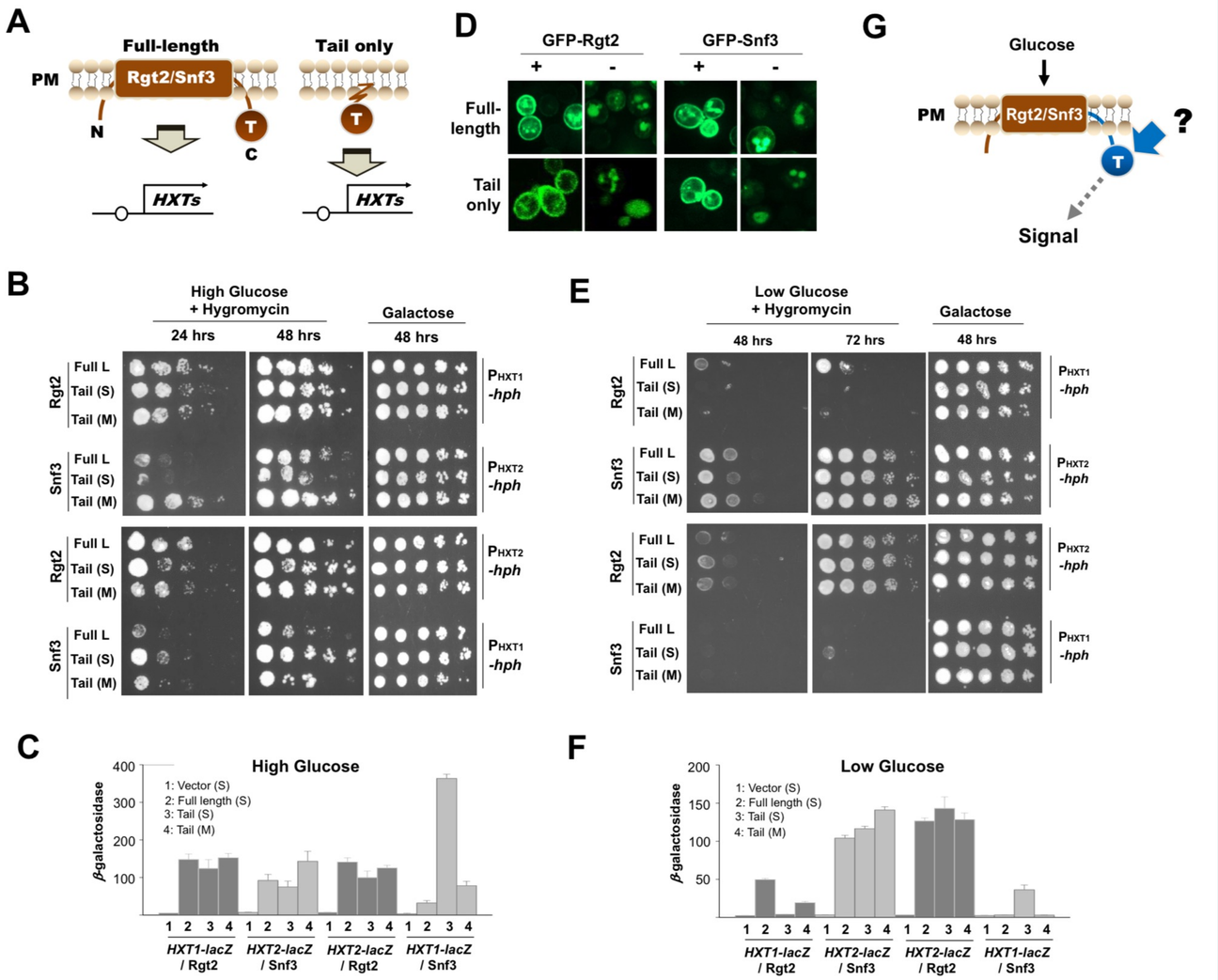

Figure 1. A novel role for the C-terminal cytoplasmic domains of the cell surface receptors Rgt2 and Snf3 in glucose sensing and signaling. (A) Rgt2 and Snf3 are glucose sensing receptors that detect the presence of extracellular glucose. In this study, we test whether the CTD can signal the presence of glucose when tethered to the plasma membrane. (B) The HXT1-hph and HXT2-hph reporter strains expressing indicated GFP-Rgt2 or GFP-Snf3 proteins were spotted on 2% glucose (as a high glucose condition) plates supplemented with hygromycin. The first spot of each row represents a count of 5 × 107 cell/ml, which is diluted 1:10 for each spot thereafter. Full L, full-length; Tail (S), tail domain expressed from a single copy plasmid (pRS316); Tail (M) tail domain expressed from a multi copy plasmid (pRS426). (C) Expression of HXT1-lacZ and HXT2-lacZ genes. β-Galactosidase activity was assayed in permeabilized cells and expressed in Miller Units. Values are means for at least three independent experiments. (D) Confocal microscopy of expression and localization of GFP-Rgt2 and GFP-Snf3 proteins. (E) The HXT1-hph and HXT2-hph reporter strains expressing indicated GFP-Rgt2 or GFP-Snf3 proteins were spotted on 2% raffinose (as a low glucose condition) plates supplemented with hygromycin, as described in (B). (F) Expression of HXT1-lacZ and HXT2-lacZ genes. β-Galactosidase activity was assayed, as described in (C). (G) The Snf3 tail domain, when tethered to the PM, can signal the presence of extracellular glucose. However, its underlying mechanisms remain unknown.

Results and Conclusion

Results

1. The glucose sensing receptors Rgt2 and Snf3 signals the presence of glucose.
2. Expression of the PM (plasma membrane)-tethered tail domain of Snf3 leads to activation of the glucose transporter genes HXT1 and HXT2.
3. The C-terminal tail domain (CTD) of the Snf3 receptor, but not that of the Rgt2 receptor, can signal the presence of extracellular glucose when artificially anchored to the plasma membrane.

Conclusion

When tethered to the cell surface, the intracellular cytoplasmic C-terminal domain of the glucose sensing receptor Snf3 can signal the presence of extracellular glucose, demonstrating novel role for the CTD in glucose sensing and signaling.

Future Work

1. Understand the exact role of the intracellular cytoplasmic C-terminal domain of Rgt2 and Snf3 in glucose sensing and signaling.
2. Determine whether the CTDs regulate the stability of the TM domains of the glucose sensing receptors.
3. Identification of the CTD-interacting proteins that transmit the glucose signal to the nucleus.

References

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