Endocytosis and Vacuolar Degradation of the Yeast Cell Surface Glucose Sensors Rgt2 and Snf3*

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Background: In yeast, glucose is sensed by two cell surface glucose sensors. The glucose sensors are down-regulated by ubiquitination and degradation. The stability of the glucose sensors may be associated with their ability to sense glucose. Differential regulation of the abundance of glucose sensors enables yeast cells to respond rapidly to changing glucose levels.

Sensing and signaling the presence of extracellular glucose is crucial for the yeast Saccharomyces cerevisiae because of its fermentative metabolism, characterized by high glucose flux through glycolysis. The yeast senses glucose through the cell surface glucose sensors Rgt2 and Snf3, which serve as glucose receptors that generate the signal for induction of genes involved in glucose uptake and metabolism. Rgt2 and Snf3 are evolutionarily derived from glucose transporters but appear to have lost the ability to transport glucose into the cell; instead, they function as glucose receptors (20, 21). This view is strongly supported by the identification of a dominant mutation in the glucose sensor genes (RGT2-1 and SNF3-1), which is thought to convert the sensors into the glucose-bound and therefore glucose signaling forms (20). Indeed, Mth1 degradation and subsequent HXT gene expression occur constitutively in Rgt2-1 and Snf3-1 mutant cells (22). These observations have led to the view that glucose acts like a hormone to initiate receptor-mediated signaling, and glucose sensors function in a similar way to mammalian cell surface receptors (5, 23).

Most organisms have evolved numerous mechanisms for sensing and signaling the availability of glucose, the universal fuel for life, ensuring its optimal utilization (1, 2). Glucose is by far the preferred energy source of the budding yeast Saccharomyces cerevisiae, because regulation of cellular function by glucose dictates the fermentative lifestyle of the organism (3, 4). The propensity of the yeast to ferment rather than oxidize glucose demands high glycolytic flux, and therefore, yeast cells consume the available glucose vigorously by increasing glucose uptake through glucose transporters (HXTs) (3, 5).

Expression of the HXT genes is repressed in the absence of glucose by a multiprotein repressor complex, composed of the transcriptional repressor Mth1 and the glucose responsive transcription factor Mth1 (6–10). The signal that leads to proteasomal degradation of Mth1 is the presence of glucose in the cytoplasm; glucose activates the HXT promoters (11–13). Addition of glucose to glucose-depleted cells induces degradation of Mth1 (14–18) and concomitant derepression of HXT genes (11, 12).

The yeast cells possess multiple glucose transporters with different affinities for glucose, enabling them to grow well over a wide range of glucose concentrations, from a few micromolar to a few molar (3). They sense extracellular glucose levels through the two glucose sensors, which have different affinities for glucose. Rgt2 has a low affinity for glucose, and Snf3 has a high affinity for glucose (21). This difference is presumably due to differences in the amino acid residues of the sensors that associate from DNA and thus to subsequent phosphorylation of Rgt1 by PKA, leading to Rgt1 dissociation from PKA (20). This view is strongly supported by the identification of a dominant mutation in the glucose sensor genes (RGT2-1 and SNF3-1), which is thought to convert the sensors into the glucose-bound and therefore glucose signaling forms (20). Indeed, Mth1 degradation and subsequent HXT gene expression occur constitutively in Rgt2-1 and Snf3-1 mutant cells (22). These observations have led to the view that glucose acts like a hormone to initiate receptor-mediated signaling, and glucose sensors function in a similar way to mammalian cell surface receptors (5, 23).

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This article has been withdrawn by the authors. The actin immunoblot from the WT strain in Fig. 2C was reused as the actin immunoblot from the end3Δ strain in Fig. 2C and as the actin immunoblot in the right panel of Fig. 2D. The HA immunoblot in Fig. 3D was assembled from different immunoblots and was represented as being from the same immunoblot to place them with increasing size in a single panel.

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Degradation of Rgt2 and Snf3

**EXPERIMENTAL PROCEDURES**

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**TABLE 1**

Yeast strains used in this study

| Strain | Genotype | Source |
|--------|----------|--------|
| BY4741 | Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δ | Ref. 28 |
| YM6870 | Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δa rgt2::KanMX snf3::KanMX | Ref. 28 |
| JKF122 | Mata his3Δ1 leu2Δ0 ura3Δ0 doa4::KanMX | This study |
| JKF123 | Mata his3-1 leu2-0 ura3-0 RPS5 | Ref. 35 |
| JKF124 | Mata his3-1 leu2-0 ura3-0 rps5-1/snm1 | Ref. 35 |
| JKF125 | Mata his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 end3::KanMX | This study |
| JKF126 | Mata his3Δ1 leu2Δ0 lys2Δ0 pep4::KanMX | This study |
| JKF127 | Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δa lys2Δ0 pht1Δ/KANMX | Ref. 36 |
| JKF128 | Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δa lys2Δ0 pht1Δ/H9262 | Ref. 36 |

**TABLE 2**

Plasmids used in this study

| Plasmid | Description | Source |
|---------|-------------|--------|
| KFP69  | pAD80, 3 × HA-CYCI terminator, Leu2 | Ref. 10 |
| JKP253 | pAD80-P<sup>_rgt2</sup>-Rgt2-3 × HA | This study |
| JKP252 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–545)-3 × HA | This study |
| JKP299 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–620)-3 × HA | This study |
| JKP300 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–720)-3 × HA | This study |
| JKP301 | pAD80-P<sup>_rgt2</sup>-Rgt2 (K637A)-3 × HA | This study |
| JKP302 | pAD80-P<sup>_rgt2</sup>-Rgt2 (K637A)-3 × HA | This study |
| JKP303 | pAD80-P<sup>_rgt2</sup>-Rgt2 (K637A)-3 × HA | This study |
| JKP304 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–545)-3 × HA | This study |
| JKP305 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–620)-3 × HA | This study |
| JKP306 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–720)-3 × HA | This study |
| JKP307 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–545)-3 × HA | This study |
| JKP308 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–620)-3 × HA | This study |
| JKP309 | pAD80-P<sup>_rgt2</sup>-Rgt2 (1–720)-3 × HA | This study |
| JKP310 | pUG34-P MET25-GFP-Snf3-1 | This study |
| JKP311 | pUG34-P MET25-GFP-Snf3 | This study |
| JKP312 | pUG34-P MET25-GFP-Rgt2-1 | This study |
| JKP313 | pUG34-P MET25-GFP-Snf3-1 | This study |
| JKP314 | pUG34-P MET25-GFP-Rgt2-1 | This study |

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form the glucose-binding site. Thus, it has been proposed that Rgt2 functions as a low affinity glucose receptor that senses high concentrations of glucose, whereas Snf3 serves as a high affinity glucose receptor that senses low levels of glucose (20, 21). However, it remains unknown whether the abundance and function of cell surface levels of the glucose sensors are associated with their affinity for glucose and thus affect glucose signaling.

Here, we provide evidence that cell surface levels of glucose sensors are regulated by ubiquitination and degradation in the vacuole. Our results indicate that the stability of glucose sensors is correlated with their affinity for glucose and that the constitutively active, signaling forms of glucose sensor mutants are stable against degradation. These observations suggest that conformation of the glucose sensors is critical for their stability. We discuss the biological significance of this observation in the perspective of the fermentative metabolism of yeast, which is characterized by high glucose uptake and increased glycolytic activity.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains**—The S. cerevisiae strains used in this study are listed in Table 1. Cells were grown in YP (2% bacto-peptone, 1% yeast extract) and SC (synthetic yeast nitrogen base medium—The complete medium supplemented with 0.17% yeast nitrogen base and 0.5% ammonium sulfate) media supplemented with the appropriate amino acids and carbon sources.

**Plasmid Construction**—The plasmids used in this study are listed in Table 2. The plasmids were constructed by using standard molecular biology techniques as described below. Plasmids containing Rgt2-HA, Rgt2 (1–545)-HA, Rgt2 (1–620)-HA, Rgt2 (1–720)-HA, and Rgt2-1-HA under its endogenous promoter (1000 base pairs) were constructed in two steps. First, the promoter element was PCR-amplified from genomic DNA isolated from wild type yeast strain BY4741. The promoter element was cloned into pAD80 vector (KFP69, C-terminal 3×HA fusion vector). Next, the ORFs were cloned as a SacI-XbaI fragment cloned in the lenti vector (KFP69). Then SNF3 and SNF3-1 ORFs were cloned using EcoRI-BamHI fragments. Plasmids containing GFP-Snf3 and GFP-Snf3-1 were constructed by “gap repair” of BamHI-EcoRI linearized pUG34 vector (17).

**Yeast Membrane Preparation and Western Blotting**—Membrane-enriched fractions were essentially prepared as described previously (24). Briefly, after washing with phosphate buffered saline (PBS) and detergents, the samples were resuspended in ice-cold membrane isolation buffer (100 mM Tris-Cl, pH 8, 150 mM NaCl, 5 mM EDTA) containing 10 mM sodium azide, the cell pellet was resuspended in ice-cold membrane isolation buffer (100 mM Tris-Cl, pH 8, 150 mM NaCl, 5 mM EDTA) containing 10 mM sodium azide, protease, and phosphatase inhibitors and vortexed with acid-washed glass beads. After centrifugation, the supernatant was collected, and the membrane-enriched fraction was collected by centrifuging the samples at 30,000 rpm for 40 min at 4°C. The pellets were resuspended in the aforementioned buffer containing 5 mM urea and incubated for 30 min on ice and further centrifuged at 12,000 rpm for 40 min at 4°C. The proteins were precipitated with 20% TCA, neutralized with 20 μl of 1 M Tris base, and finally dissolved in 80 μl of SDS buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol).

For Western blotting, proteins were resolved by 10% SDS-PAGE and transferred to PVDF membrane (Millipore), and the membranes were incubated with appropriate antibodies (anti-HA, anti-Myc, anti-GFP, or anti-actin antibody; Santa Cruz) in TBST buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20), and proteins were detected by the ECL system (Pierce).
Quantitative RT-PCR—Total RNA was extracted by RNeasy mini kit (Qiagen) following manufacturer’s protocol, and 2 μg of total RNA was converted to cDNA by qScript cDNA super-mix (Quanta Biosciences). cDNA was analyzed by qRT-PCR using SsoFast Evagreen reagent (Bio-Rad) in CFX96 real time thermal cycler (Bio-Rad). ACT1 was used as an internal control to normalize expression of HXT1, RGT2, and SNF3 genes. Quantification data were the averages of three independent experiments with error bars representing standard deviation (S.D.). Statistical significance was defined by p values: *, p < 0.05, and **, p < 0.001 as compared with control.

Microscopy and Image Analysis—To visualize yeast cells expressing various GFP fusion proteins, cells were stained with FM4-64 (lipophilic styryl dye for selectively staining vacuolar membrane, 5 mg/ml stock in DMSO) and examined with Olympus Fluoview confocal microscope under 63× oil immersion objective lens using GFP and Texas Red filters. Images from confocal microscope were captured by Fluoview software (Olympus), and National Institutes of Health ImageJ v1.4r software was used to quantify fluorescence intensities from unmanipulated raw images. Regions of interest in the plasma or vacuolar membrane and an area outside the cell (background) were traced using the free-hand tool, and mean fluorescence intensities (both GFP and FM4-64) were measured. After background subtraction, the GFP signals in the plasma membrane were normalized to the FM4-64 signal of vacular membrane. At least 50 cells were counted, and the data represented were the averages with error bars representing S.D.

RESULTS

Glucose Starvation Induces Endosomal Degradation of Rgt2—To test the hypothesis that the cell surface levels of Rgt2 glucose sensor may be regulated by glucose concentration, we determined its expression at different glucose concentrations. Western blot analysis showed that the cell surface levels of Rgt2 were greater in high glucose-grown cells (2%) than in cells grown in low glucose medium (~0.1%) and are very low in cells grown in the absence of glucose (Gal) (Fig. 1A). However, RGT2 mRNA levels were not significantly different between yeast cells incubated with different concentrations of glucose (Fig. 1B), and the treatment of the protein synthesis inhibitor cycloheximide did not greatly affect Rgt2 turnover (Fig. 1C).

Because a number of yeast plasma membrane receptors and transporters are down-regulated by endocytosis and degradation in the vacuole (25, 26), we examined expression levels of Rgt2-HA in the end3Δ mutant defective in the internalization step of endocytosis and the pep4Δ mutant defective in vacuolar protease processing. Rgt2-HA levels in glucose-grown wild type cells were reduced by ~50% within 20 min after the cells were shifted to glucose-depleted (galactose) medium, but this reduction was not observed in the end3Δ and pep4Δ strains (Fig. 1D). Consistently, the amount of immunodetected Rgt2-HA was markedly increased within 30 min after addition of glucose to glucose-starved medium (Fig. 1E).

Confocal microscopy demonstrated that GFP-Rgt2 is present at the cell surface in glucose-grown cells and that ~80% of GFP-Rgt2 is removed from there when the yeast cells are shifted from glucose to galactose medium (Fig. 1F, WT). However, GFP-Rgt2 was constitutively detected at the cell surface of the end3Δ mutant (Fig. 1F) and the pep4Δ mutant (data not shown). It was also shown that substantial amounts of GFP-Rgt2 were localized to the vacuole in a glucose-independent manner, suggesting constitutive internalization and degradation of Rgt2 (Fig. 1F, FM4-64). Glucose and galactose only differ with respect to C-4, yet galactose does not activate the glucose sensors, suggesting that the glucose sensors display remarkable substrate specificity (27). Consistently, we found that Rgt2-HA levels are down-regulated in the cells grown on galactose, raffinose, or ethanol (Fig. 1G). These data indicate that Rgt2 is stable against degradation in the presence of high concentrations of glucose but endocytosed and degraded in the vacuole when glucose is absent or present only in small quantities.

Snf3 Expression Is Regulated at Both Transcriptional and Post-translational Levels—Given that glucose starvation induces endocytosis and degradation of Rgt2, we determined whether Snf3-HA, another glucose sensor, is also regulated by glucose concentration. Western blot analysis showed that the plasma membrane levels of Snf3-HA were higher in high glucose-grown cells (Glu) but low in cells grown on raffinose (Raf) (Fig. 1A). Snf3 mRNA levels were low in high glucose but endocytosed and degraded in the vacuole when glucose is absent or present only in small quantities. Snf3 Expression Is Regulated at Both Transcriptional and Post-translational Levels—Given that glucose starvation induces endocytosis and degradation of Rgt2, we determined whether Snf3-HA, another glucose sensor, is also regulated by glucose concentration. Western blot analysis showed that the plasma membrane levels of Snf3-HA were higher in high glucose-grown cells (Glu) but low in cells grown on raffinose (Raf) (Fig. 1A). Snf3 mRNA levels were low in high glucose but endocytosed and degraded in the vacuole when glucose is absent or present only in small quantities. Snf3 Expression Is Regulated at Both Transcriptional and Post-translational Levels—Given that glucose starvation induces endocytosis and degradation of Rgt2, we determined whether Snf3-HA, another glucose sensor, is also regulated by glucose concentration. Western blot analysis showed that the plasma membrane levels of Snf3-HA were higher in high glucose-grown cells (Glu) but low in cells grown on raffinose (Raf) (Fig. 1A). Snf3 mRNA levels were low in high glucose but endocytosed and degraded in the vacuole when glucose is absent or present only in small quantities.

Degradation of Rgt2 and Snf3

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results support the view that stability of the glucose sensors is associated with their ability to sense glucose.

**Rgt2 Degradation Is Ubiquitin-dependent**—Ubiquitination is a signal for endocytosis of plasma membrane proteins (29, 30). The Doa4 ubiquitin isopeptidase and the Rsp5 ubiquitin ligase are known to be involved in the ubiquitination of many plasma membrane receptors and transporters in yeast (31–34). To determine whether Rgt2 down-regulation is mediated by ubiquitination, we investigated glucose regulation of Rgt2 in the strain carrying the doa4Δ or rps5-1ts mutation (35). Rgt2-HA levels were constitutively high in both the doa4Δ mutant (Fig. 3A, left panels) and in the rps5-1ts mutant incubated at 37 °C.

**FIGURE 1.** Rgt2 undergoes endocytosis and subsequent vacuolar degradation in glucose-starved cells. A, Western blot analysis of Rgt2-HA levels at the plasma membrane. Yeast cells (WT) expressing Rgt2-HA were grown in SC-2% glucose medium till mid log phase (A600 nm = 1.2–1.5), and equal amounts of cells were shifted to SC medium containing different concentrations of glucose (0–2%) for 30 min. Membrane fractions were analyzed using anti-HA antibody. B, qRT-PCR analysis of mRNA expression of RGT2 (mRNA) in yeast cells grown as described for Fig. 1A and densitometric quantification of the intensity of each band on the blot in A (Protein). C, yeast cells (WT) expressing Rgt2-HA were grown in SC-2% glucose (+) medium till mid log phase and shifted to 2% galactose (−) medium with or without cycloheximide (CHX, 50 μg/ml) for times as indicated. Membrane fractions were immunoblotted with anti-HA antibody (top panels), and the intensity of each band on the blot was quantified by densitometric scanning (bottom panels). D, yeast cells (WT, end3Δ, and pep4Δ) expressing Rgt2-HA were grown without cycloheximide as described for C. Yeast cells were harvested at different time points as indicated, membrane fractions were immunoblotted with anti-HA antibody (left panel), and the intensity of each band on the blot was quantified by densitometric scanning (bottom panels). E, yeast cells (WT, end3Δ, and pep4Δ) expressing Rgt2-HA were grown in SC-2% glucose medium (+) till mid log phase and shifted to 2% galactose (−) for 30 min and again shifted to SC-2% glucose medium for 30 min. Membrane fractions were immunoblotted with anti-HA antibody. F, GFP-Rgt2 was expressed from the MET25 promoter in wild type and end3Δ strains. Yeast cells expressing GFP-Rgt2 were grown in SC-2% glucose (2% glucose condition) set to 100%. The data represented were averages of at least 50 cell counts with error bars representing S.D. G, yeast cells (WT) expressing Rgt2-HA were grown in SC-2% glucose (Glu) medium till mid log phase and shifted to SC medium containing either 2% galactose (Gal), 2% raffinose (Raf), or 2% ethanol (EtOH) and incubated for 30 min. Membrane fractions were immunoblotted with anti-HA antibody (top panel). qRT-PCR analysis of mRNA expression of RGT2 (mRNA) and densitometric quantification of the intensity of each band on the blot (Protein) (bottom panel). Actin was served as a loading control in A, C, D, E, and G.
Degradation of Rgt2 and Snf3

(Fig. 3A, right panels), compared with those in wild type cells. Consistently, GFP-Rgt2 was shown to remain stable at the plasma membrane in those mutants (Fig. 3B). To identify the ubiquitination sites in Rgt2, we constructed a series of deletion mutants of Rgt2 and used them to map the regions that are important for its stability (Fig. 3C). Rgt2 degradation is abolished by the deletion of the entire C-terminal cytoplasmic domain (residues 1–545) or significantly inhibited by the deletion of the last 143 amino acids (residues 1–620) (Fig. 3D).

However, the deletion of the last 13 amino acids of Rgt2 (residues 1–720) did not affect its stability, implicating that the 100 amino acids between residues 620 and 720 that contain the two lysine residues, Lys637 and Lys657, may be necessary for Rgt2 ubiquitination. Indeed, substitution of the two lysine residues by alanine (K637A and K657A) markedly increased Rgt2 stability in glucose-starved cells, suggesting that the two lysine residues may serve as major ubiquitination sites (Fig. 3E).

Endocytosis-mediated degradation of Snf3 is dampened by glucose regulation of the expression of the SNF3 gene, suggesting that Snf3 levels are mainly regulated by transcriptional con-
For this reason, ubiquitination of Snf3 was not thoroughly examined in this study.

**Constitutively Active Glucose Sensors Are Stable against Degradation**

There are dominant mutations in the glucose sensor genes (RGT2-1 and SNF3-1) that lock the sensor proteins into a glucose-bound conformation and cause constitutive, glucose-independent expression of HXT genes (21) (Fig. 4A). We examined the stability of the active forms of the glucose sensors by Western blotting and found that, compared with wild type glucose sensors, both Rgt2-1 and Snf3-1 sensors remain stable regardless of glucose concentration (Fig. 4, B and C). It was also noted that low levels of Snf3-1-HA in glucose-grown cells (Fig. 4C, High) may be due to glucose repression of SNF3 gene expression (Fig. 2).

We also examined whether the degradation-resistant glucose sensor mutants (Rgt2-1 and Snf3-1) can generate a signal even in the absence of glucose that leads to constitutive expression of HXT genes. Rgt2 is required for high glucose induction of HXT1 expression, and Snf3 is required for low glucose induction of HXT2 expression (21). Accordingly, we expressed Rgt2-1 and Snf3-1 in HXT1-NAT and HXT2-NAT reporter strains, respectively, in which the NAT (nourseothricin) resistance gene is expressed under the control of the HXT promoters (36). Colony assays showed that expression of Rgt2-1-HA or GFP-Rgt2-1 sensor allows the yeast cells to grow equally well in medium containing different concentrations of glucose (Fig. 4D).

Snf3-1 is resistant to degradation, but its expression is repressed by glucose (Fig. 4C). Therefore, we observed that glucose repression of SNF3 gene expression leads to the poor growth phenotype of the HXT2-NAT reporter strain expressing Snf3-1-HA (Fig. 4E, Snf3-1-HA, Glu) and that, by contrast, expression of GFP-Snf3-1, whose expression is not regulated by glucose, enables the reporter strain to grow on glucose (Fig. 4E, GFP-Snf3-1, Glu). These results reinforce the view that Snf3 expression is regulated at both transcriptional and post-translational levels (Fig. 2).

Consistently, confocal microscopy demonstrated that Rgt2-1 and Snf3-1 glucose sensors, compared with wild type Rgt2 and Snf3 sensors, accumulate at the plasma membrane, regardless of glucose concentration (Fig. 4F). These results suggested that conformation of the glucose sensors may be critical for their stability.

**Signaling Defective Rgt2 Mutant Is Constitutively Targeted for Vacuolar Degradation**

To corroborate our hypothesis that glucose sensors may be stable in their glucose-bound, signaling state, we examined the stability of signaling defective glucose sensors against degradation. The yeast galactose transporter Gal2 can recognize both galactose and glucose, and Phe504 of Gal2, which corresponds to Trp529 of Rgt2, is critical for sub-
strate recognition (37). We replaced Trp at position 529 with aromatic amino acids Phe and Tyr using site-directed mutagenesis and determined the stability of the resulting Rgt2 mutants Rgt2W529F and Rgt2W529Y in high glucose-grown cells. The results showed that, in contrast to wild type Rgt2, the mutant sensors, Rgt2W529Y in particular, was endocytosed and degraded even in the presence of glucose (Fig. 5A), leading to inhibition of the glucose induction of HXT1 gene expression (Fig. 5B). Thus, Rgt2W529Y was not able to complement the growth defect of the rgt2snf3 double mutant in glucose medium (Fig. 5C). High glucose-induced proteasomal degradation of Mth1 is triggered by glucose activation of the Rgt2 sensor (14, 16). Western blot analysis showed that glucose-dependent Mth1 degradation occurs in cells expressing the wild type Rgt2 sensor but not the Rgt2W529Y sensor (Fig. 5D). These observations support the view that the stability of the glucose sensors may be determined by their ability to sense glucose.

**DISCUSSION**

Many yeast nutrient receptors and transporters, such as Zrt1 (38), Ctr1 (35), Fth1 (39), Smf1 (40), Fur4 (24), and Gap1 (31), are regulated in a homeostatic fashion. They are induced in the absence of their ligands but internalized and targeted for degradation in the vacuole when their ligands become available in excess (25, 26). Hence, endocytic degradation of these plasma membrane proteins functions as a homeostatic regulatory loop.
Degradation of Rgt2 and Snf3

FIGURE 5. Signaling defective Rgt2 glucose sensor is constitutively endocytosed. A, yeast cells (WT and end3Δ) expressing the indicated Rgt2-HA proteins were grown as described for Fig. 1F, and membrane fractions were immunoblotted with anti-HA antibody (top panels). The intensity of each band on the blot was quantified by densitometric scanning (bottom panel; *, p < 0.05; **, p < 0.001). B, yeast cells (rgt2Δsnf3Δ) expressing the indicated Rgt2-HA proteins were grown as described for Fig. 3C, and the mRNA levels of HXT1 were quantified by qRT-PCR. The values shown are means ± S.D. (*, p < 0.05; **, p < 0.001). C, yeast cells (rgt2Δsnf3Δ) expressing the indicated Rgt2-HA proteins were spotted on 2% glucose plate supplemented with antimycin A (1 µg/ml) (2% Glu AA) or SC-2% galactose plate (2% Gal) and photographed as described for Fig. 4D. D, yeast cells (rgt2Δsnf3Δ) coexpressing Mth1-Myc and the indicated Rgt2-HA proteins were grown as described for Fig. 1F, and cell lysates were immunoblotted with anti-Myc antibody (top left panels, Mth1-Myc). Actin was served as a loading control (top right panels, actin). Quantification data of Mth1-Myc protein by densitometry are shown (bottom panel; *, p < 0.05; **, p < 0.001).

7254 JOURNAL OF BIOLOGICAL CHEMISTRY
Degradation of Rgt2 and Snf3

The yeast cells cope with changes in glucose availability by expressing at least six members of the hexose transporter family with different affinities for glucose (45–48). They express only those glucose transporters most appropriate for the amounts of glucose available in the environment (49). The glucose sensors have different roles in glucose signaling: the low affinity glucose sensor Rgt2 is responsible for expression of the low affinity glucose transporter Hxt1; the high affinity glucose sensor Snf3 regulates the expression of the high affinity glucose transporters Hxt2, Hxt3, and Hxt4 (21). This is consistent with our findings that Rgt2 is stable in high glucose grown cells, whereas Snf3, in cells grown on low glucose, reinforces the view that the stability of the glucose sensors is correlated with their affinity for glucose. Moreover, the glucose sensors are localized to the vacuole regardless of the presence of glucose (Figs. 1F and 2E). These observations suggest that the glucose sensors may be inherently unstable but stabilized by glucose.

Our findings provide a conceptual framework to explain the regulation of glucose sensing activity at the yeast cell surface that directly affects the ability of the organism to adapt to fluctuating glucose levels. Glucose starvation induces endocytosis and degradation of Rgt2, and thus Rgt2 is stable in cells grown on high glucose. By contrast, Snf3 accumulates at the cell surface of the cells grown on low glucose, mostly due to the regulation of Snf3 expression by both feedforward and feedback mechanisms. Snf3 protein is internalized and degraded not only in high glucose-grown cells but also in glucose-depleted cells, whereas expression of the SNF3 gene is repressed by high glucose concentrations but is derepressed when glucose is absent (Fig. 2). We have previously shown that that Mig1 and Mig2 repressors mediate glucose repression of SNF3 gene expression (17, 28). Therefore, glucose-induced Snf3 degradation is reinforced by glucose repression of SNF3 gene expression, but glucose depletion-induced Snf3 degradation is dampened by derepression of SNF3 gene expression. As a result, substantial amounts of Snf3 are present at the cell surface of glucose-depleted cells (Fig. 2A). This should serve to provide for a rapid reestablishment of induction of HXT gene expression when glucose is available in the medium.

Consequently, one of the glucose sensors, or both, may be present at the plasma membrane at a given glucose concentration. Snf3 may be the predominant sensor in low levels of glucose and Rgt2, in high glucose conditions. Both Rgt2 and Snf3 may coexist in an intermediate between high and low levels of glucose (Fig. 6). Moreover, yeast cells may keep glucose sensing activity constant at the plasma membrane over a wide range of glucose levels, enabling them to respond rapidly to changes in glucose levels and thereby to coordinate their metabolism.

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