Two Different Signals Regulate Repression and Induction of Gene Expression by Glucose*

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In addition to being the universal carbon and energy source, glucose also regulates gene expression in many organisms. In the yeast *Saccharomyces cerevisiae* glucose regulates gene expression via two different pathways known as the glucose repression and glucose induction pathways. The signal for glucose induction of hexose transporter (*HXT*) genes is generated via two glucose-transporter like molecules, Snf3 and Rgt2. A strain lacking both sensors is unable to induce *HXT* gene expression and is defective in glucose uptake. The *snf3 rgt2* double mutant is also defective in glucose repression of transcription, raising the possibility that Snf3 and Rgt2 are also involved in generating the glucose repression signal. In this report, I show that induction and repression of gene expression by glucose in yeast is regulated by two independent signals. While the signal for induction of *HXT* gene expression is generated by Snf3 and Rgt2 glucose receptors, the repression signal requires the uptake and metabolism of glucose. In addition, the glucose induction of the *HXT* genes is required for repression of gene expression by glucose. Therefore the glucose repression defect of the *snf3 rgt2* strain is indirect and is due to the lack of glucose uptake in this double mutant.

In the yeast *Saccharomyces cerevisiae*, glucose has two major effects on gene transcription. First it represses expression of genes encoding enzymes for the metabolism of alternate sugars such as galactose and sucrose (1, 2). Second it induces the transcription of genes encoding glycolytic enzymes and glucose transporters required for efficient glucose metabolism (3, 4).

Although the repressive effect of glucose on gene expression has been known for decades, the primary signal required for glucose repression remains unknown. Recent data indicate that glucose repression requires the uptake and metabolism of glucose (5, 22). Derepression of glucose-repressed genes (such as *GAL1* and *SUC2*) in the absence of high concentrations of glucose requires the protein kinase Snf1 (6–8). Based on work carried out with the mammalian homologue of Snf1, the AMP-activated kinase (AMPK), it has been suggested that changes in AMP:ATP ratio generate the signal for glucose repression (9, 10, 11).

The signal for glucose induction of hexose transporter (*HXT*) gene expression is generated by two membrane receptors, Snf3 and Rgt2 (12, 13). Both proteins have similarities to glucose transporters and function as sensors of extracellular glucose. In the *snf3 rgt2* double mutant, induction of *HXT* gene expression by glucose is completely abolished. As a consequence of this defect the *snf3 rgt2* strain is defective for glucose uptake and displays impaired growth on glucose-containing media. Interestingly, the *snf3 rgt2* double mutant is also defective for glucose repression of *GAL1* and *SUC2* genes, indicating the requirement of these sensors for the glucose repression pathway (13, 14).

This report provides evidence that two different signals are responsible for repression and induction of transcription by glucose in yeast. It demonstrates that the generation of the signal for glucose repression of gene expression requires the uptake and the metabolism of glucose. Furthermore, it shows that the induction of *HXT* gene expression by glucose is essential for the generation of the glucose repression signal.

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—The yeast *S. cerevisiae* strains used in this study including the *hxt* null strain (RE700A) deleted for six of the *HXT* genes, have been described previously (12, 15). Yeast cells were grown on YNB (0.67% yeast nitrogen base (Difco) plus 0.5% ammonium sulfate) medium lacking the appropriate amino acids. To select for G418 resistance, YNB plates were supplemented with 200 μg/ml geneticin (Invitrogen).

**Construction of Plasmids**—The *GAL1::lacZ and SUC2::lacZ* reporter constructs and the *HXT1::lacZ* and *HXT2::lacZ* plasmids have been described in detail before (13, 16). The *URA3* marker of the plasmids pSF11 (containing the *ADH1* promoter in pRS426) and pSO432 (containing the *HXT1* gene under the control of *ADH1* promoter in pSF11) (13) was disrupted with the Kanamycin gene by using the PCR technique. The resultant constructs called pSO71 (*ADH1*-vector) and pSO74 (*ADH1-HXT1*) were created by gap repair. The Kanamycin gene was amplified using the primers OS105 and OS106 (containing in addition the *HXT1* gene), OS105 and OS106 (containing in addition the *HXT1* gene) and OS106 (containing in addition the *HXT1* gene) and OS106 (containing in addition the *HXT1* gene). The obtained constructs were checked by restriction and PCR analysis.

**β-Galactosidase Activity Assays**—β-Galactosidase activity assays were performed with permeabilized yeast cells grown to mid-log phase as described previously (18). The mean activities are given in Miller units and are the averages of four to five assays of at least three independent yeast transformants. To assay the induction of *HXT1::lacZ* and *HXT2::lacZ* gene expression, cells were pregrown on YNB containing 5% glycerol plus 0.5% galactose lacking uracil and transferred to YNB medium containing 2% glucose, 5% glycerol, plus 0.5% galactose or 5% glycerol, plus 0.2% glucose and incubated overnight before β-galactosidase activity was assayed. The expression of the *GAL1::lacZ* and *SUC2::lacZ* constructs was assayed by pregrowing cells on YNB with 2% glucose lacking uracil. The next day cells containing *GAL1::lacZ* were transferred to YNB media containing either 2% galactose or 2% glucose plus 2% galactose. The cells containing the
are more than 100-fold derepressed (Fig. 1, B double mutant is defective in glucose induction of rgt2—Defective in Glucose Repression of Gene Expression
gene expression and in glucose repression of completely repressed in the presence of high concentrations of mediated by the transcriptional repressor Rgt1 (4). However, genes are repressed in the absence of glucose (on galactose) due to reduced glucose uptake and metabolism. To test this idea, the hxt Null Mutant, Defective in Glucose Uptake, Is Also defective for glucose induction and galactose. In a snf3 rgt2 double mutant, induction of the HXT1 gene expression by high levels of glucose is completely abolished (Fig. 1A) (13) because Snf3 and Rgt2 membrane proteins are required for generation of the high glucose induction signal. In addition to the glucose induction defect, the snf3 rgt2 mutant strain is also defective in glucose repression of GAL1 and SUC2 genes, encoding galactokinase and invertase, respectively (Fig. 1, B and C). While the expression of these two genes is normally completely repressed in the presence of high concentrations of glucose (1), in a snf3 rgt2 double mutant both GAL1 and SUC2 are more than 100-fold derepressed (Fig. 1, B and C).

The hxt Null Mutant, Defective in Glucose Uptake, Is Also Defective in Glucose Repression of Gene Expression—The snf3 rgt2 double mutant is defective in glucose induction of HXT gene expression and in glucose repression of GAL1 and SUC2 genes. It has been suggested that in contrast to glucose induction, the generation of the glucose repression signal requires the uptake and metabolism of glucose (5, 19, 22). Since the snf3 rgt2 double mutant grows very slowly on glucose, it is likely that the observed defect in glucose repression in this mutant is due to reduced glucose uptake and metabolism. To test this idea, I have measured the induction and repression of gene expression by glucose in an hxt null mutant that is deleted for six of the HXT genes (15). Like snf3 rgt2, the hxt null mutant is defective in uptake and metabolism of glucose and thereby grows only slowly on glucose. Induction of HXT1 by high levels of glucose and of HXT2 at low concentrations of glucose is normal in the hxt null mutant (Fig. 2). As expected both HXT genes are repressed in the absence of glucose (on galactose) mediated by the transcriptional repressor Rgt1 (4). However, HXT2 expression, which is glucose-repressed in wild type cells, is about 4-fold depressed by high concentrations of glucose (Fig. 2). I have also assayed the glucose repression of the GAL1 and SUC2 gene expression in the hxt null mutant and found that both genes are more than 400-fold derepressed compared with the wild type strain in the presence of high concentrations of glucose (Fig. 3).

Restoration of the Glucose Uptake Defect in the hxt Null Mutant Also Restores the Glucose Repression Defect—To test whether the defect in glucose repression is due to impaired glucose uptake and metabolism, the hxt null mutant was transformed with a construct containing the HXT1 gene expressed from the ADH1 promoter on a multicopy vector. The hxt null mutant is defective for growth on glucose, and this defect is more pronounced when the respiration inhibitor antimycin A is added to the media (Fig. 4). Overexpression of the HXT1 gene in this mutant restores the glucose transport defect and enables it to grow faster on glucose (Fig. 4). Restoration of the glucose uptake defect also restores the glucose repression defect of GAL1 and SUC2 expression almost to wild type levels (Fig. 5). This indicates that normal glucose uptake and metabolism is required to activate the glucose repression pathway and to inhibit GAL1 and SUC2 gene expression. However, glucose-induced transcription of HXT1 and HXT2 genes in the hxt null mutant overexpressing the HXT1 gene was unaffected, except that the glucose repression defect of the HXT2 gene was restored like that of GAL1 and SUC2 genes (data not shown).

Restoration of Glucose Transport in the snf3 rgt2 Double Mutant Restores Repression but Not Induction by Glucose—The glucose repression defect of the hxt null indicated that this defect is due to diminished glucose transport and metabolism. Therefore it was likely that the defect in glucose repression of the GAL1 and SUC2 gene expression in the snf3 rgt2 double mutant was due to impaired glucose uptake in this mutant. To test this idea, I have measured the induction and repression of gene expression by glucose in an hxt null mutant that is deleted for six of the HXT genes (15). Like snf3 rgt2, the hxt null mutant is defective in uptake and metabolism of glucose and thereby grows only slowly on glucose. Induction of HXT1 by high levels of glucose and of HXT2 at low concentrations of glucose is normal in the hxt null mutant (Fig. 2). As expected both HXT genes are repressed in the absence of glucose (on galactose) mediated by the transcriptional repressor Rgt1 (4). However,
repression of GAL1 and SUC2 genes in the snf3 rgt2 double mutant overexpressing the HXT1 gene was restored to wild type levels (Fig. 6). However, the snf3 rgt2 double mutant containing the ADH1-HXT1 construct was still defective in glucose induction of the HXT1 expression. This indicates that while the signal for glucose repression relies on the uptake and metabolism of glucose, the generation of the signal for induction of HXT gene expression is independent of glucose metabolism.

DISCUSSION

In the yeast S. cerevisiae, glucose has both positive and negative regulatory effects on gene expression. While expression of the genes encoding glucose transporters such as the HXT genes is 10- to 300-fold induced by glucose, transcription of genes required for metabolism of alternate carbon sources such as GAL1 and SUC2 genes is inhibited by 10- to 1000-fold (1, 2). The signal for induction of HXT gene expression by glucose is generated by two glucose sensors, Snf3 and Rgt2, and does not require the transport and metabolism of glucose (4, 12, 13). The nature of the primary signal mediating glucose repression of gene expression is unknown. Recent data indicate that the signal for glucose repression of gene expression is determined by the intracellular concentration of the glucose (5, 19).

It has been previously shown that the snf3 rgt2 double mutant, which is defective in induction of the HXT gene expression, is also defective in glucose repression of GAL1 and SUC2 gene transcription (13, 14). This raised the possibility that Snf3 and Rgt2 may be also involved in generation of the glucose repression signal. Alternatively, it was possible that the glucose repression defect of the snf3 rgt2 mutant was due to diminished glucose uptake and metabolism in this mutant. In this report I demonstrate that the glucose repression defect of the snf3 rgt2 mutant is due to the lack of HXT gene expression in this mutant (Fig. 7). Consistent with this idea, a strain deleted for six of the HXT genes (hxt null), unable to grow on glucose due to lack of glucose transport, is also defective in glucose repression GAL1 and SUC2 genes. Furthermore, restoration of the glucose transport defect in the hxt null mutant by overexpression of Hxt1 glucose transporter restores the glucose repression defect. Similarly, overexpression of HXT1 from the ADH1 promoter in the snf3 rgt2 double mutant restores the glucose repression but not the glucose induction defect of this mutant.

In summary, these data indicate that repression and induction of gene expression by glucose is regulated by two different primary signals. While the glucose repression signal requires the uptake and metabolism of glucose, the glucose induction signal is generated in a receptor-mediated process. Furthermore, the glucose induction pathway is connected to the glucose repression pathway via the expression of glucose transporter (HXT) genes. The snf3 rgt2 mutant, unable to induce the expression of the HXT genes is also defective in glucose repression of GAL1 and SUC2 genes.

While Snf3 and Rgt2 sense extracellular glucose and generate the signal for induction of HXT gene expression, the nature of the intracellular signal and how it is transmitted from the cytoplasm into the nucleus is unknown. Recent data indicate that two homologues proteins, Mth1 and Std1, may be involved in the transmission of the signal from the cytoplasm into the nucleus. Generation of the primary signal for glucose repression requires the uptake and metabolism of glucose; however the protein(s) involved in sensing the glycolytic flux remain to be identified. The protein kinase Snf1 has been shown to be essential for derepression of glucose-repressed genes (6–8). In a snf1 mutant, expression of GAL1 and SUC2 genes is repressed even in the absence of glucose. The mammalian homologue of Snf1, the AMP-activated protein kinase (AMPK) has been shown to be regulated by the AMP:ATP ratio (9, 10). Therefore it has been proposed that changes in the AMP:ATP ratio provide the signal for glucose repression (9). Although it is known that switching yeast cells from high to low glucose causes increases in AMP levels; however, this increase appears to have no effect on Snf1 kinase activity in vitro (11).

Transcription of genes coding for lipogenic and glycolytic enzymes in liver and/or adipose tissue is up-regulated by

1 Sabire Ozcan, unpublished data.
glucose. To generate the signal for this up-regulation, glucose needs to be metabolized. The signal metabolite required for induction of gene expression in the liver appears to be glucose-6 phosphate (20, 21). In yeast, repression of transcription by glucose also requires the transport and metabolism of glucose. Although, mutations in Hxk2, the major glucose-

![Fig. 5. Restoration of the glucose uptake defect in the hxt null mutant also restores the glucose repression defect of GAL1 and SUC2 gene expression. The hxt null mutant containing either the ADH1 vector or the ADH1-HXT1 construct was assayed for glucose repression of GAL1 and SUC2 expression using the GAL1::lacZ and SUC2::lacZ constructs.](image)

![Fig. 6. Overexpression of Hxt1 restores only the glucose repression defect of GAL1 and SUC2 gene expression in a snf3 rgt2 mutant. The snf3 rgt2 mutant containing either the ADH1 vector or the ADH1-HXT1 construct was transformed with the HXT1::lacZ, GAL1::lacZ, or SUC2::lacZ promoter constructs and assayed for glucose induction of HXT1 or for glucose repression of GAL1 and SUC2 gene expression by measuring β-galactosidase activity.](image)

![Fig. 7. A model explaining the interdependence of glucose induction of HXT gene expression and glucose repression of GAL1 or SUC2 gene transcription. In wild type cells, glucose induces the expression of the HXT genes encoding glucose transporters. These transporters insert into the membrane and enable the efficient uptake and metabolism of glucose that is required for generation of the glucose repression signal to inhibit GAL1 and SUC2 gene expression in response to high concentrations of glucose. In a snf3 rgt2 mutant induction of HXT gene expression is abolished and thus the efficient uptake of extracellular glucose is impaired. As a consequence of the glucose uptake defect, the snf3 rgt2 double mutant does not generate the signal for glucose repression due to impaired glucose metabolism.]
phosphorylating enzyme in yeast, lead to defects in glucose repression, recent data indicate that glucose-6 phosphate is not the signal metabolite in yeast. The signal for glucose repression in yeast appears to be upstream of glucose-6-phosphate (5, 19).

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