NRG1 is Required for Glucose Repression of the SUC2 and GAL Genes of Saccharomyces Cerevisiae

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Research article

NRG1 is required for glucose repression of the SUC2 and GAL genes of Saccharomyces cerevisiae

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Abstract

**Background:** Glucose repression of transcription in the yeast, Saccharomyces cerevisiae, has been shown to be controlled by several factors, including two repressors called Mig1 and Mig2. Past results suggest that other repressors may be involved in glucose repression.

**Results:** By a screen for factors that control transcription of the glucose-repressible SUC2 gene of S. cerevisiae, the NRG1 gene was identified. Analysis of an nrg1Δ mutant has demonstrated that mRNA levels are elevated at both the SUC2 and of the GAL genes of S. cerevisiae when cells are grown under normally glucose-repressing conditions. In addition, genetic interactions have been detected between nrg1Δ and other factors that control SUC2 transcription.

**Conclusions:** The analysis of nrg1Δ demonstrates that Nrg1 plays a role in glucose repression of the SUC2 and GAL genes of S. cerevisiae. Thus, three repressors, Nrg1, Mig1, and Mig2, are involved as the downstream targets of the glucose signaling in S. cerevisiae.

**Background**

For the yeast Saccharomyces cerevisiae, glucose is the preferred carbon source. When glucose is present in the growth media, transcription of a large number of genes encoding products involved in the metabolism of alternative carbon sources is repressed [for reviews, see [1,2,3]]. These genes include the GAL, SUC2, MAL and STA genes, required, respectively, for the utilization of galactose, sucrose/raffinose, maltose, and starch.

At many of these genes, glucose repression is mediated, at least in part, by the glucose-dependent repressor Mig1, a zinc-finger protein that binds in vitro to DNA consensus sites consisting of a GC-rich core and flanking AT sequences [4, 5]. Mig1 is thought to bind to several promoters, including GAL1, GAL4, SUC2 and MAL62, and to effect transcriptional repression by interacting with the co-repressor complex Ssn6-Tup1 [6,7,8]. Mig1’s activity is regulated by phosphorylation and subcellular localization: in high glucose, Mig1 protein is hypophosphorylated and in the nucleus, where it can repress transcription; upon withdrawal of glucose, Mig1 is rapidly phosphorylated and transported into the cytoplasm [9]. This regulated phosphorylation requires the function of the Snf1/Snf4 kinase complex [10].

Deletion of MIG1, however, only partially relieves glucose repression at promoters such as SUC2, whereas deletion of either SSN6 or TUP1 completely abolishes glucose repression. Moreover, the STA1 gene of S. cerevisiae var. diastaticus, which is also repressed by glucose, is unaffected by mig1Δ [11]. Therefore, other proteins in addition to Mig1 are required for glucose repression. One of these proteins is Mig2, which shares se-
sequence similarity with Mig1 in their zinc finger regions [12, 13]. Genetic analysis suggests that Mig2 plays a minor role relative to Mig1.

Recently, a previously uncharacterized gene, NRG1 (Negative regulator of glucose-repressed genes), was shown to be required for glucose repression of the STA1 gene in S. cerevisiae var. diastaticus [11]. These studies demonstrated that LexA-Nrg1 behaves as a repressor of a reporter construct and that this repression is dependent on glucose, Ssn6, and Tup1. In addition, Nrg1 and Ssn6 interact with each other in two-hybrid and GST pull-down assays, indicating that Nrg1 may repress via the same pathway as Mig1. Consistent with these results, Nrg1 appears to bind to two sites within the STA1 promoter.

The SUC2 gene of S. cerevisiae has been extensively studied with respect to its glucose repression [1, 2]. Glucose repression of SUC2 is mediated by Ssn6/Tup1 and SUC2 has two Mig1 binding sites in its regulatory region. Additionally, in high glucose its promoter is also occupied by positioned nucleosomes, which cause transcriptional repression themselves [14, 15]. Derepression in low glucose is correlated with a loss of both Mig1- and nucleosome-mediated repression, although the precise relationship between the two pathways is not clear.

Genetic screens have identified a large number of genes, named SNF (Sucrose Non-Fermenting) that are required for derepression of SUC2 transcription in the absence of glucose [16, 17, 18]. Genetic analyses and subsequent studies have traditionally divided SNF genes into two groups. One group encodes the protein kinase Snf1 and its associated regulator Snf4, required to antagonize the repression caused by Mig1 [10, 19]. The other group consists of members of the Swi/Snf complex required to counter the repressive effects of chromatin by remodeling nucleosomes in an ATP-dependent manner (for review see [20]. Suppressors of swi/snf mutations, such as spt6, do not suppress snf1Δ [21], and ssn6, a strong suppressor of snf1Δ only partially suppress swi/snf mutations [22].

NRG1 is predicted to encode a protein of 231 amino acids with two C2H2 zinc fingers in the carboxyl terminus. Sequence analysis revealed that the 2μ plasmid that confers suppression of snf2Δ encodes just the amino terminal region of Nrg1, lacking the zinc fingers. To test if the complete NRG1 gene causes the same high copy number phenotype, we subcloned the complete NRG1 gene into a 2μ plasmid and tested it for suppression of snf2Δ. Our results demonstrate that the complete NRG1 gene on a 2μ plasmid does not suppress snf2Δ (Figure 1).

In this work, we report the identification of Nrg1 in a genetic screen for new regulators of SUC2 transcription. We show that Nrg1 plays a role in the glucose repression of SUC2 and GAL genes in S. cerevisiae. Thus, at these genes, Mig1, Mig2 and Nrg1 are partially redundant for mediating repression by glucose. Consistent with our findings, recent results have demonstrated an interaction between Snf1 and Nrg1 [23]. We also present experiments that test the genetic interactions between mig1Δ, nrg1Δ and deletions of various genes encoding activators that function at the SUC2 promoter.

**Results**

*Isolation of a high-copy-number suppressor of snf2Δ*

The Swi/Snf complex is required for normal levels of expression of SUC2 when cells are grown in low glucose. To identify factors that might be functionally related to Swi/Snf, we screened for high-copy-number plasmids that could suppress a snf2Δ mutation (see Materials and Methods). To sensitize the screen, we used an allele of SUC2, SUC2-36, that allows an elevated level of SUC2 transcription in the absence of Swi/Snf [24]. The SUC2-36 mutation is a single base pair change, AT to GC at position -401 relative to the SUC2 ATG. SUC2-36 strains still have a Raf phenotype in a snf2Δ mutant.

To identify high-copy-number suppressor candidates, we used a 2μ circle library to transform the snf2Δ SUC2-36 strain FY1845 (Table 1) and screened 60,000 transformants for those with a Raf phenotype. Eighty-two candidates were identified, 25 of which contained the SNF2 gene. Among the remaining plasmids, most conferred a weak Raf phenotype. We focused on the candidate that conferred the strongest Raf phenotype. This plasmid contained a chromosome IV genomic fragment that spans from within the NRG1 gene (open reading frame YDR043C) through the HEM12 gene (YDR047W). Subcloning experiments identified the partial NRG1 clone as the sequence responsible for suppression of snf2Δ and demonstrated that this suppression occurred in both SUC2-36 and SUC2 + genetic backgrounds (Figure 1).

**NRG1 encodes a repressor of transcription**

To characterize further the role of Nrg1 with respect to SUC2 transcription, we constructed and analyzed an nrg1Δ mutant. The nrg1Δ mutant grows normally on media containing glucose, sucrose, or galactose, demonstrating that NRG1 is not essential for growth. For that nrg1Δ mutants can utilize several different carbon sources.

To test for the requirement for Nrg1 in glucose repression, we tested growth of an nrg1Δ mutant on YP sucrose media containing the glucose analog, 2-deoxyglucose (2-
Figure 1
Overexpressing a truncated clone of NRG1 suppresses snf2Δ. Yeast strains FY32 (snf2Δ1::HIS3 SUC2) and yHZ269 (snf2Δ1::HIS3 SUC2-36) were transformed with nrglΔZn or full-length NRG1 cloned in pRS426, as well as vector alone. Ura+ single colonies carrying each construct were resuspended in 200 µl sterile water, and spotted on SC-Ura plates containing glucose or raffinose as the carbon source. Plates were photographed on day 2.

Table 1: Yeast Strains

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</tr>
<tr>
<td>FY1845</td>
<td>MATα his3Δ200 lys2-128Δsnf2Δ1::HIS3 SUC2-36 ura3-52</td>
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<tr>
<td>FY1846</td>
<td>MATα MATα his3Δ200/1::HIS3 LEU2/ura3Δ10</td>
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<tr>
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2-DG causes glucose repression but cannot be used as a carbon source by *S. cerevisiae*. Therefore, wild-type cells do not grow on YP sucrose plates that contain 2-DG, due to glucose repression of *SUC2*. However, strains defective for glucose repression can grow on this medium as they express *SUC2* even in the presence of 2-DG. We found that an *nrg1Δ* mutant was able to grow on YP sucrose plus 2-DG, suggesting that *nrg1Δ* mutants are indeed defective for glucose repression. To assess the role of Nrg1 relative to the two other factors known to be required for glucose repression, Mig1 and Mig2, we compared the mutant phenotypes caused by *nrg1Δ*, *mig1Δ*, and *mig2Δ*, as well as testing combinations of these deletions. We observed that the three single mutants grow with different strengths on YP sucrose 2-DG plates in the order *mig1Δ* > *nrg1Δ* > *mig2Δ* (Figure 2). The double and triple mutants had stronger phenotypes than the single mutants (Figure 2). These results strongly suggest that Nrg1, Mig1, and Mig2 are each required for glucose repression at the *SUC2* locus, with Mig1 playing the major role. We also tested growth of these strains on YP galactose + 2-DG plates and found that only the triple mutant was able to grow, albeit weakly, on this medium, perhaps because galactose is a poor carbon source (data not shown). This suggests that each of these three proteins contributed to glucose repression of the *GAL* genes.

**Glucose repression of transcription is defective in *nrg1Δ***

To test whether the *nrg1Δ* phenotype on 2-DG plates is caused by altered transcription, we performed Northern analyses to *SUC2* mRNA levels. Under repressing conditions (2% glucose), the level of *SUC2* mRNA was increased by two-to-four fold in an *nrg1Δ* strain compared to a wild-type control (Figure 3A). Consistent with previous published results, a *mig1Δ* mutant had a nine- to fourteen fold increase in *SUC2* mRNA levels while a *mig2Δ* mutant had no detectable defect in glucose repression of *SUC2* [4, 12]. We also analyzed the *SUC2*...
mRNA levels in double and triple mutant combinations. In general, multiple mutations caused greater derepression, up to 79-fold for the triple mutant, nrg1\(\Delta\) mig1\(\Delta\) mig2\(\Delta\) (Figure 3A). These data demonstrate that Nrg1, Mig1, and Mig2 all contribute to the glucose repression of SUC2.

We also tested if an nrg1\(\Delta\) affects glucose repression of the GAL genes as described in Materials and Methods. Both nrg1\(\Delta\) and mig1\(\Delta\) mutations cause a defect in the glucose repression of GAL1 and GAL10, whereas mig2\(\Delta\) alone had no effect (Figure 3B). As for SUC2, additive effects were observed in double and triple mutant strains, up to a 13-fold effect for the nrg1\(\Delta\) mig1\(\Delta\) mig2\(\Delta\) triple mutant (Figure 3B). These data indicate that all three proteins are involved in glucose repression of GAL1-GAL10, with Mig2 playing only a minor role.

**Deletion of MIG1 or NRG1 suppresses mutations in both SNF1 and SWI/SNF genes**

Activation of SUC2 transcription depends upon both the Snf1/Snf4 kinase complex and the Swi/Snf nucleosome remodeling complex. To address the relationship of Nrg1 to both complexes and to compare it to Mig1, we tested the abilities of nrg1\(\Delta\) and mig1\(\Delta\) to suppress the Gal', Suc', and Raf' phenotypes of mutations in SNF1 and SWI/SNF genes.

Our results (Figure 4) show that both nrg1\(\Delta\) and mig1\(\Delta\) suppress, albeit sometimes weakly, mutations in both SNF1 and SWI/SNF genes. With respect to suppression of snf1\(\Delta\), mig1\(\Delta\) is the stronger suppressor, with suppression detectable for the Gal" phenotype (Figure 4A). The observed suppression by mig1\(\Delta\) is consistent with previous results [22]. The nrg1\(\Delta\) mutation did not detectably suppress either the Suc' or Raf' phenotypes caused by snf1\(\Delta\). With respect to swi/snf mutations, we tested suppression of both snf2\(\Delta\) and sup73\(\Delta\) and observed weak suppression of the Gal' and Suc phenotypes (Figure 4B). Suppression of the Raf' phenotype was not detectable. There appear to be some gene-specific interactions as suppression of sup73\(\Delta\) by mig1\(\Delta\) was stronger than the suppression observed for the other pairs tested.

**Discussion**

Our results demonstrate that Nrg1 plays a role in glucose repression of the SUC2 and GAL genes of *S. cerevisiae*. Consistent with a role in glucose repression, an nrg1\(\Delta\) mutation suppresses the defects of a snf1\(\Delta\) mutant. Recent results from an independent study have demonstrated an interaction between Snf1 and Nrg1 [23]. Our results also suggest that Nrg1 is partially redundant with two other factors required for glucose repression, Mig1 and Mig2. At SUC2 and GAL1-10, all three proteins appear to be involved in glucose repression, because double- and triple-deletion mutations have additive effects. Interestingly, both nrg1\(\Delta\) and mig1\(\Delta\) can also suppress the defects caused by mutations in genes encoding members of the Swi/Snf complex.

While Nrg1, Mig1, and Mig2 are partially redundant, current evidence suggests that they do not function in the same relative fashion at all glucose-repressible promoters. For example, while mig1\(\Delta\) and nrg1\(\Delta\) cause comparable defects at GAL1-GAL10, nrg1\(\Delta\) causes a weaker defect at SUC2. Mig2 appears to have only a minimal function at either promoter. In addition, Nrg1 is the major repressor at STA1, whose glucose-repression does not require Mig1 [11]. Therefore, some gene-specific specialization exists among these three glucose-dependent repressors.

A previous study of Nrg1 provided evidence that it interacts with Ssn6 and confers repression by recruitment of Ssn6/Tup1 [11]. We initially identified NRG1 in our studies by the isolation of a high-copy-number plasmid encoding a fragment of Nrg1, lacking the zinc-finger domain. Likely, the phenotype caused by this plasmid is caused by interference of repression by Ssn6/Tup1.

Our studies have not yet distinguished between a direct or indirect effect of Nrg1 on glucose repression at SUC2 and GAL1-GAL10. One possible indirect effect of Nrg1 could be by regulation of MIG1 transcription. However, Northern analysis showed that MIG1 mRNA levels are unaffected by an nrg1\(\Delta\) mutation (H. Zhou and F. Winston, unpublished data). We tested Nrg1 for binding to the SUC2 promoter and those experiments are briefly summarized here. We screened for DNA binding of Nrg1 to the SUC2 promoter region using a previously described GST-Nrg1 fusion protein [11] and a gel shift assay. Our results demonstrated specific DNA binding to two sites within the -1022 to -825 region 5' of SUC2 (H. Zhou and F. Winston, unpublished results). However, a deletion of this region does not alter SUC2 expression. Based on the similarity between the zinc fingers of Nrg1 and Mig1 and our binding studies, the binding site of Nrg1 may contain a GC-rich core. Another such site in the SUC2 promoter may occur at -570 with the sequence AGGCCCA. Although we did not detect a gel shift of a fragment containing this site, it is still possible that it is recognized and bound by Nrg1 in vivo. Furthermore, although an Nrg1 consensus binding [11] exists at -976 of SUC2, we were unable to detect binding to this site by GST-Nrg1. This region also did not compete the binding that we detected by GST-Nrg1. This discrepancy between our findings and previous results can be explained by the fact that Park et al [11] used 10-fold more GST-Nrg1 in their binding studies than we did. Finally, we did not de-
Deletion of NRGI causes defects in glucose repression. (A) A single colony of each strain was inoculated into YPD liquid with 2% glucose and grown to mid-log phase (approx. $1 \times 10^7$ cells/ml). The cells were harvested, and total RNA was isolated and analyzed by electrophoresis followed by hybridization with probes specific to SUC2 or SPT15. The intensities of each band were quantitated using phosphoimager and ImageQuant software. The amount of SUC2 mRNA in each strain was normalized to SPT15, and the result obtained for the wild-type strain was assigned the arbitrary unit of 1.0 and used to calculate the relative SUC2 mRNA levels in other strains. (B) Northern analysis of GAL1-10 mRNA in mutant strains. A single colony of each strain was inoculated into SD complete liquid with 2% glucose+2% galactose and grown to mid-log phase. The cells were harvested, and total RNA was isolated from each and analyzed by electrophoresis followed by hybridization with probes specific to GAL1, GAL10 or SPT15. Quantitation was carried out as for (A).
tect any binding of Nrg1 to the Mig1 binding sites. Thus, the DNA binding of Nrg1 to SUC2 remains to be resolved.

**Conclusions**

In conclusion, these studies have identified Nrg1 as a third repressor required for glucose repression at SUC2 and the GAL genes. Based on the similarity between the zinc fingers of Nrg1 and Mig1, the phenotypes of nrg1Δ and mig1Δ, and the reported interaction between Nrg1 and Ssn6 [11], Nrg1 likely functions by binding to the target promoters and recruiting the Ssn6-Tup1 complex. The relative and possible cooperative roles of each of these repressors in recruiting Ssn6-Tup1 remains to be determined.

**Materials and methods**

**Yeast strains**

All S. cerevisiae strains are listed in Table 1 and are in the S288C genetic background [25, 26]. Deletion of MIG1 was achieved by transforming strain yHZ416 with the HindIII digest of pJN22 (for mig1Δ::LEU2) or pJN41 (for mig1Δ::URA3) [4], and selecting for Leu+ or Ura+ transformants, respectively. PCR-directed gene replacement [27] was used to construct deletions of NRG1 and...
MIG2. PCR reactions were carried out using as templates pRS vectors carrying the desired markers [25, 28]. For NRG1, the oligos used were HZ034, 5’- TCG ACC AGC ATA TTA CTA CCC TTA GCA AAC TTT CAG GCA CTG TGC GGT ATT TCA CAC CG 3’; and HZ035, 5’- GTA GTA CTG CTA ATG AGA AAA ACA CGG GTA TAC TGA GAG TGC AC 3’. The PCR fragment was used to transform a haploid strain directly. All gene replacements were verified by PCR, Southern analyses, and tetrad analyses.

Media

The media used in this study were previously described [29]. Glucose, galactose, sucrose or raffinose was added to 2% final weight per volume. For solid media containing sucrose or galactose, we do not yet have an explanation for this phenomenon. To overcome this growth defect, uracil was added to YP plates to a final concentration of 1 µg/ml.

Subcloning of NRG1 constructs

The 1.8 kb SacI-Sall fragment of the original library clone, containing only the 5’ half of Nrg1 without the zinc fingers, was cloned into the SacI-Sall sites of pRS426 to generate pHZ56. To clone the complete NRG1 ORF, HZ032 and HZ033 were used to PCR from genomic DNA the complete wild-type NRG1 from -1119 to +719. The PCR fragment was digested with Sad and cloned into the SacI-Smal sites of pRS426 to generate pHZ52.

Northern analysis

Cell cultures were grown in liquid media as indicated to mid-log phase (1-2 x 10^7 cells/ml), and total RNA was prepared as previously described [27,30]. RNA was separated by electrophoresis on 1% agarose-formaldehyde gels, transferred to membrane and blotted with specific radio-labeled probes. The probes were: for SUC2, the 1.3 kb BamHI-HindIII fragment of pRB59 [31]; for GAL1-10, the 2 kb EcoRI-EcoRI fragment of BNN45 [32] and for SPT15, the 0.8 kb SpeI-HindIII fragment of plP45 (I. Pinto, personal communication). All probes were labeled by random priming.

Acknowledgments

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