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-

quence 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 C_2H_2 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 grwoth and 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-

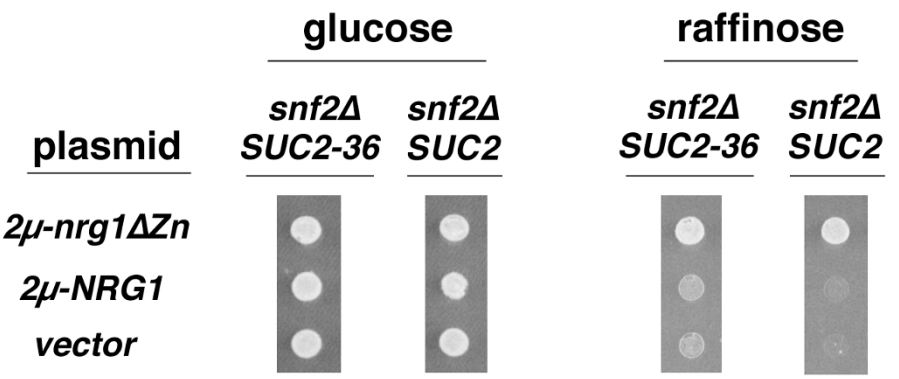

Figure 1
Overexpressing a truncated clone of *NRG1* suppresses *snf2Δ*. Yeast strains FY32 *(snf2Δ1::HIS3 SUC2)* and yHZ269 *(snf2Δ1::HIS3 SUC2-36)* were transformed with *nrg1ΔZn* or full-length *NRG1* cloned in pRS426, as well as vector alone. *Ura* $^{+}$ single colonies carrying each construct were 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

Strain	Genotype
FY32	*MATα his3Δ 200 snf2Δ1::HIS3 ura3-52*
FY1845	*MAT* **a** *his3Δ200 lys2-128δ snf2Δ1::HIS3 SUC2-36 ura3-52*
FY1846	*MAT* **a** */MATα his3Δ200/HIS3 LEU2/leu2Δ0 ura3Δ0/ura3Δ0*
FY1847	*MAT* **a** *his3Δ200 leu2Δ0 ura3Δ0 nrg1Δ1::URA3*
FY1848	*MATα his3Δ200 leu2Δ0 lys2Δ0 swp73Δ1::LEU2 ura3Δ0*
FY1849	*MAT* **a** *leu2Δ0 snf1Δ10*
FY1850	*MAT* **a** *his3Δ200 leu2Δ0 lys2Δ0 snf1Δ10 nrg1Δ1::URA3 ura3Δ0*
FY1851	*MAT* **a** *his3Δ200 leu2Δ0 met15Δ0 snf2Δ2::LEU2 ura3Δ0*
FY1852	*MAT* **a** *ade8 his3Δ200 leu2Δ0 met15Δ0 swi1Δ1::LEU2 ura3Δ0*
FY1853	*MAT* **a** *his3Δ200 leu2Δ0 lys2Δ0 swp73Δ1::LEU2 nrg1Δ1::URA3 ura3Δ0*
FY1854	*MAT* **a** *his3Δ200 leu2Δ0 snf2Δ2::LEU2 ura3Δ0 nrg1Δ1::URA3*
FY1855	*MAT* **a** *his3Δ200 leu2Δ0 swilM::LEU2 nrg1Δ1::URA3 ura3Δ0*
FY1856	*MATα his3Δ200 leu2Δ0 lys2-128δ ura3Δ0*
FY1857	*MATα his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::LEU2 ura3Δ0*
FY1858	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig2Δ1::HIS3 ura3Δ0 nrg1Δ1::URA3*
FY1859	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::LEU2 nrg1Δ1::URA3 ura3Δ0*
FY1860	*MAT* **a** *his3Δ200 leu2Δ0 met15Δ0 mig1-Δ2::LEU2 mig2Δ1::HIS3 nrg1Δ1::URA3 ura3Δ0*
FY1861	*MAT* **a** *his3Δ200 leu2Δ0 met15Δ0 mig1-Δ2::LEU2 mig2Δ1::HIS3 ura3Δ0*
FY1862	*MAT* **a** *his3Δ200 leu2Δ0 met15Δ0 mig2Δ1::HIS3 ura3Δ0*
FY1863	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::LEU2 ura3Δ0*
FY1864	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::URA3 snf2Δ2::LEU2 ura3Δ0*
FY1865	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::URA3 swi1Δ1::LEU2 ura3Δ0*
FY1866	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::URA3 swp73Δ1::LEU2 ura3Δ0*
FY1867	*MAT* **a** *his3Δ200 leu2Δ0 lys2-128δ mig1-Δ2::URA3 snf1Δ10 ura3Δ0*
FY1868	*MATα his3Δ200 leu2Δ0 lys2-128δ swi1Δ1::LEU2*

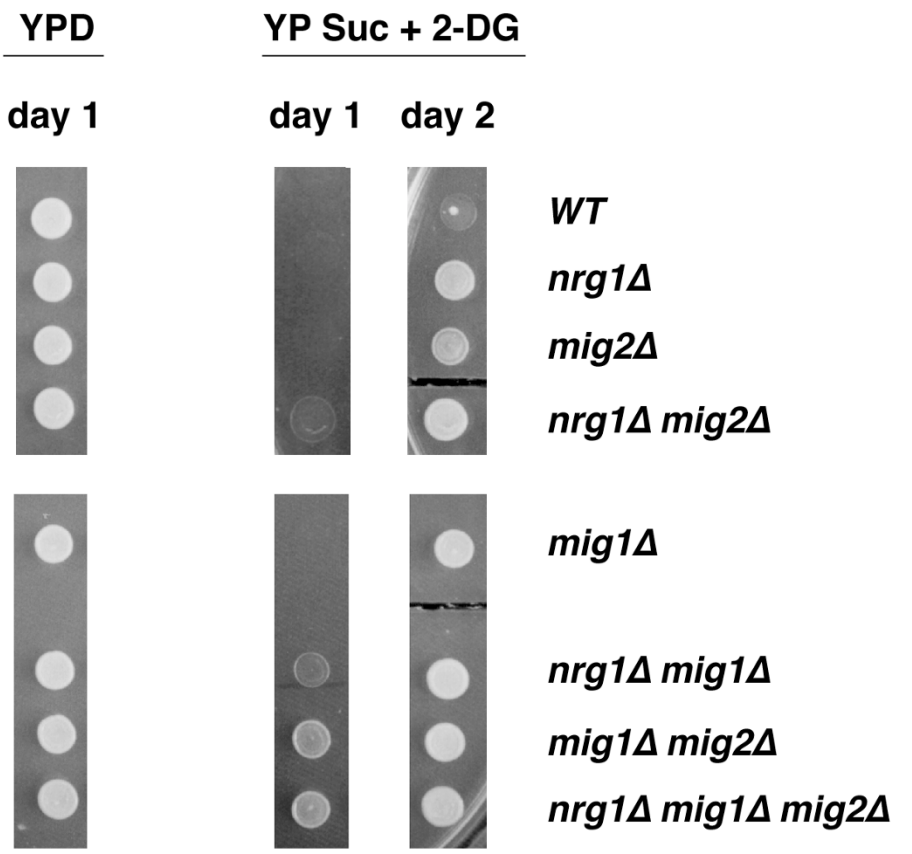

Figure 2
Deletion of *NRG1* partially abolishes glucose repression. *nrg1Δ* allows cells to grow on sucrose plates containing 2-deoxyglucose, and has additive effects with *mig1Δ* and *mig2Δ*. A single colony of each strain was inoculated into liquid YPD and grown to saturation (approx. 1×10^8 cells/ml). The cultures were then diluted 1:2 (upper panels) or 1:5 (lower panels) in sterile water, and spotted on YPD plates and YP sucrose plates with 200 μg/ml 2-deoxyglucose. Plates were photographed on after 1 and 2 days of incubation at 30°C.

DG). 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 previously 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, *nrg1Δ mig1Δ mig2Δ* (Figure 3A). These data demonstrate that Nrg1, Mig1, and Mig2 all contribute to the glucose repression of *SUC2*.

We also tested if an *nrg1Δ* affects glucose repression of the *GAL* genes as described in Materials and Methods. Both *nrg1Δ* and *mig1Δ* mutations cause a defect in the glucose repression of *GAL1* and *GAL10,* whereas *mig2Δ* 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 *nrg1Δ mig1Δ mig2Δ* 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 *nrg1Δ* and *mig1Δ* to suppress the Gal$^-$, Suc$^-$, and Raf$^-$ phenotypes of mutations in *SNF1* and *SWI/SNF* genes.

Our results (Figure 4) show that both *nrg1Δ* and *mig1Δ* suppress, albeit sometimes weakly, mutations in both *SNF1* and *SWI/SNF* genes. With respect to suppression of *snf1Δ, mig1Δ* is the stronger suppressor, with suppression detectable for the Gal" phenotype (Figure 4A). The observed suppression by *mig1Δ* is consistent with previous results [22]. The *nrg1Δ* mutation did not detectably suppress either the Suc$^-$ or Raf$^-$ phenotypes caused by *snf1Δ.* With respect to *swi/snf mutations,* we tested suppression of both *snf2Δ* and *swp73Δ* 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 *swp73Δ* by *mig1Δ* 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 *nrg1Δ* mutation suppresses the defects of a *snf1Δ* 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 *nrg1Δ* and *mig1Δ* 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 suggestions that they do not function in the same relative fashion at all glucose-repressible promoters. For example, while *mig1Δ* and *nrg1Δ* cause comparable defects at *GAL1-GAL10, nrg1Δ* 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 *nrg1Δ* 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-

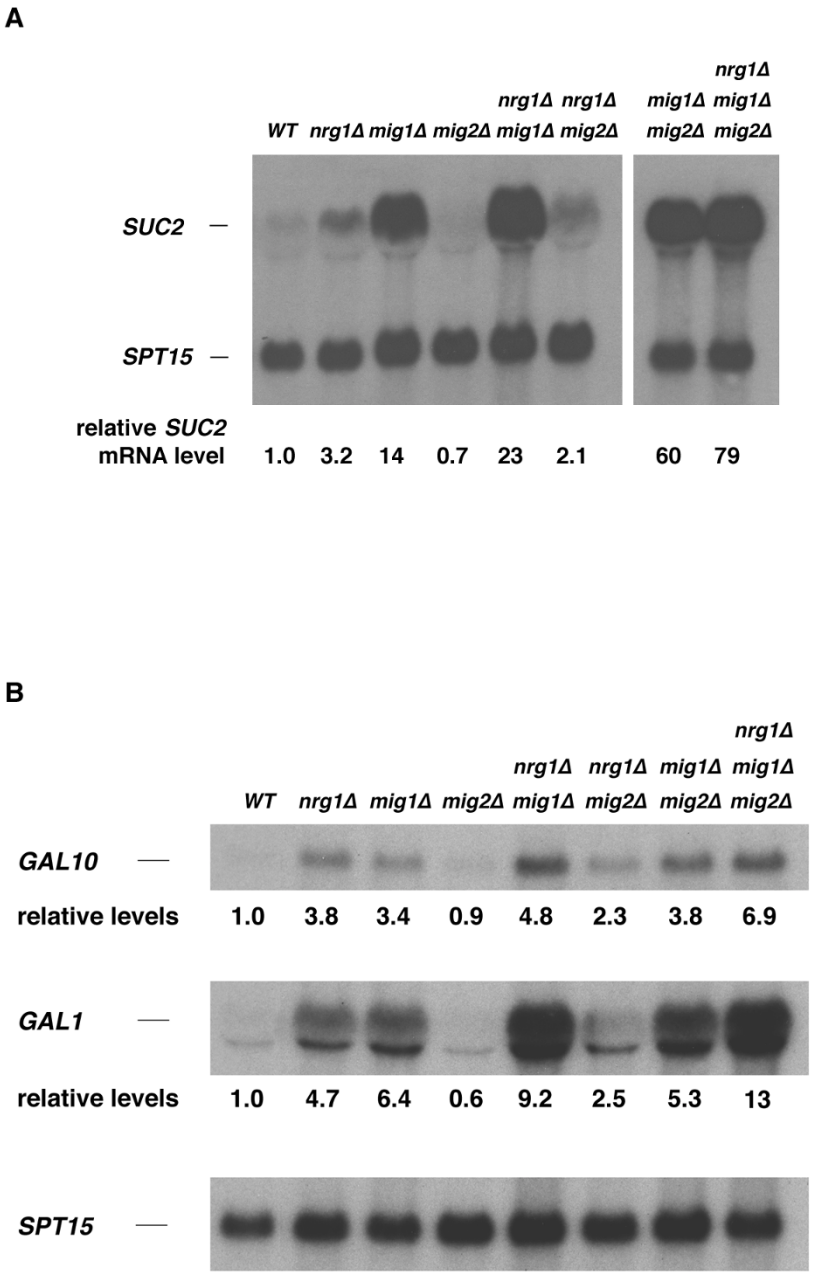

Figure 3
Deletion of *NRG1* causes defects in glucose repression. (A) A single colony of each strain was inoculated into YPD liquid with 2% glucose and grown to mid-log phase (approx. 1×10^7 cells/ml). The cells were harvested, and total RNA was isolated and analyzed by electrophoresis followed by hybridization with probes specific to *SUC2* or *SPT15*. The intensities of each band was 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).

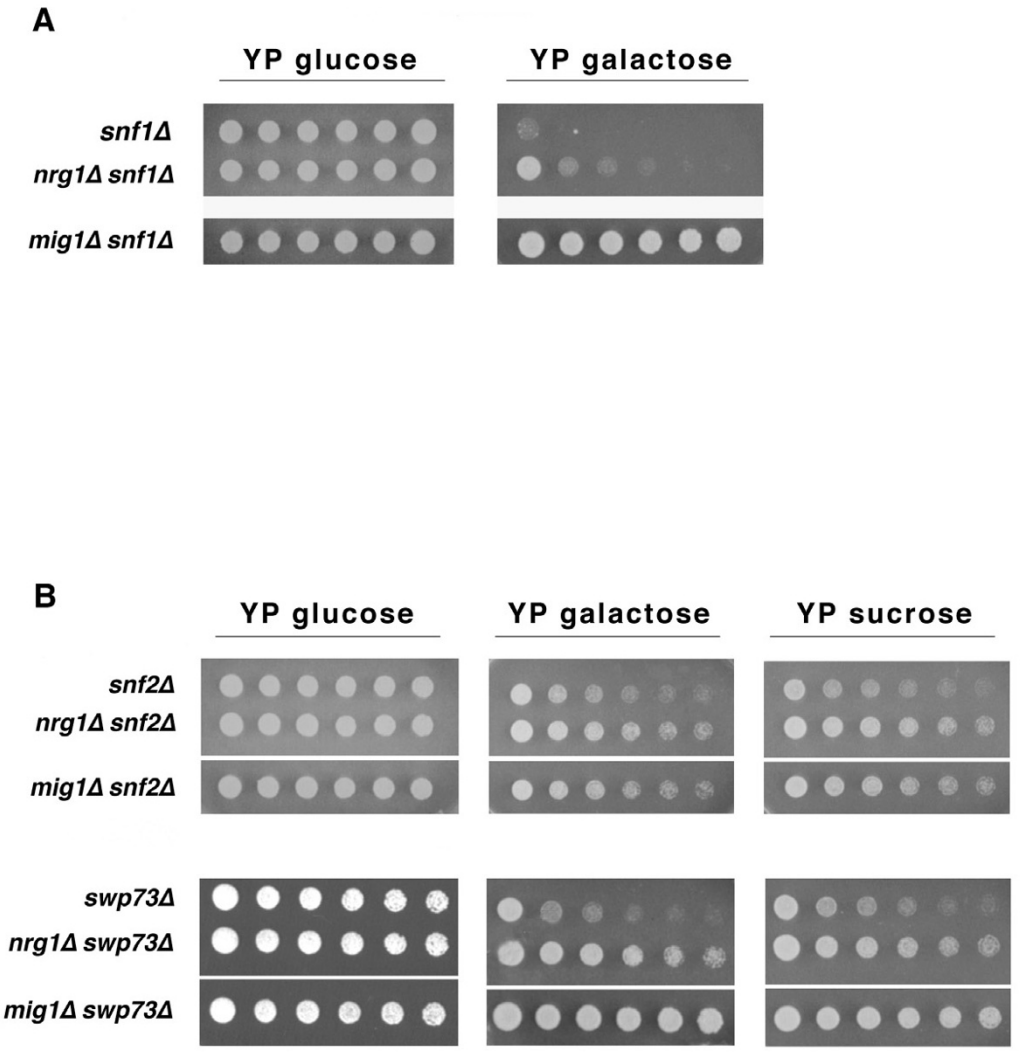

Figure 4
Mutations in *SNF1* and *SNF/SWI* can be suppressed by both *nrg1Δ* and *migΔ*. A single colony of each strain was inoculated into YPD liquid and grown over-night to saturation and adjusted in water to 1×10^8 cells/ml. The cultures were then diluted 1:2 in sterile water and spotted on YPD, YP galactose and YP sucrose plates, with uracil added to each plate to 80 μM. The first spot of each row represents a cell count of 5×10^7 cells/ml, which is diluted 1:4 for the second spot and 1:2 for each spot thereafter. YPD and YP sucrose plates were photographed after incubation at 30°C for 2 days, and YP galactose plates were photographed after 5 days.

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 *Hind*III digest of pJN22 (for *mig1-Δ2::LEU2)* or pJN41 (for *mig1-Δ2::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 TTC 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 CGT CAA AGA TTG TAC TGA GAG TGC AC 3'. For *MIG2,* the oligos were HZ045, 5' TGA CCT CGA GAA CAA ACA AAA TAA AAA TAA AAA AAG AGA CTG TGC GGT ATT TCA CAC CG 3'; and HZ046, 5' TTA GAG GAA AAA TGG TGA GAT AAA AAG GGG CCG TAA AGG AGA TTG 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 a carbon source other than glucose or glycerol, antimycin A was also added to a concentration of 1 μg/ml. To test for glucose repression of *SUC2* and *GAL* genes, 2-deoxyglucose was added to YP sucrose-antimycin A and YP galactose-antimycin A plates to a final concentration of 200 μg/ml [4]. We discovered during the course of this study that a *ura3Δ0* strain had half the amount of *GAL 1-10* mRNA of a *URA3* strain when grown in SD media containing 2% glucose and 2% galactose. A *ura3Δ0* strain also grew more slowly than a *URA3* strain on minimal 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 80 μM.

Subcloning of NRG1 constructs

The 1.8 kb *SacI-SalI* fragment of the original library clone, containing only the 5' half of NrG1 without the zinc fingers, was cloned into the *SacI-SalI* sites of pRS426 to create 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-SmaI* sites of pRS426 to generate pHZ52.

Northern analysis

Cell cultures were grown in liquid media as indicated to mid-log phase (1-2 × 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 *Bam*HI-*Hind*III fragment of pRB59 [31]; for *GAL1-10,* the 2 kb *Eco*RI-*Eco*RI fragment of BNN45 [32] and for *SPT15,* the 0.8 kb *Spe*I-*Hind*III fragment of pIP45 (I. Pinto, personal communication). All probes were labeled by random priming.

Acknowledgments

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