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

ABSTRACT

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.

INTRODUCTION

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. 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. 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. 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. This regulated phosphorylation requires the function of the Snf1/Snf4 kinase complex. 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. Therefore, other proteins in addition to Mig1 are required for glucose repression. One of these proteins is Mig2, which shares sequence similarity with Mig1 in their zinc finger regions. 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. 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. 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. 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. 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. 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. Suppressors of swi/snf mutations, such as spt6, do not suppress snf1Δ, and ssn6, a strong suppressor of snf1Δ, only partially suppress swi/snf mutations. 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. 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.

CONCLUSION

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, 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.