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Three subunits of the RNA polymerase II mediator complex are involved in glucose repression

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ABSTRACT

Glucose triggers a complex response in yeast which includes induction and repression of a large number of genes. Glucose repression is in part mediated by the Mig1 repressor, a zinc finger protein that binds to the promoters of many glucose repressed genes. However, some genes that are required for gluconeogenic growth are also repressed by a Mig1-independent mechanism. We have isolated mutations in three genes that are involved in this Mig1-independent component of repression and cloned the genes by complementation. All three genes encode subunits of the recently discovered RNA polymerase II mediator complex. Two of them are yeast cyclin C and its associated kinase. Disruptions of the three genes have identical phenotypes with respect to glucose repression and show no synergism with each other. This suggests that these three subunits of the mediator complex function closely together in transmitting the transcriptional response to glucose.

INTRODUCTION

Glucose repression in the budding yeast *Saccharomyces cerevisiae* is a complex regulatory mechanism which prevents expression of a large number of genes in the presence of glucose (1–4). All glucose repressed genes are under positive control by the Snf1 (or Cat1) kinase, a yeast homolog of the mammalian AMP-activated protein kinase whose activity is inhibited by glucose (5). Epistasis data suggest that one function of the Snf1 kinase is to inhibit or counteract the activity of the Mig1 repressor, which binds to upstream repressing sequences in many glucose repressed genes (6–8). Thus, loss of Mig1 can suppress loss of Snf1, by causing a partial derepression of the *GAL* and *SUC* genes which allows the cells to grow on galactose and raffinose.

Mig1 is also involved in glucose repression of those genes that are required for gluconeogenic growth. Mig1-binding sites are present in the *FBP1* and *ICL1* promoters, and it was also recently shown that Mig1 represses the transcription of *CAT8* (9), which encodes a zinc cluster protein that functions as an activator for *FBP1*. The role of Mig1 in repressing these genes is thus similar to its role in repressing the *GAL* genes, where it represses both the *GAL4* gene and the targets of the Gal4 activator (7).

However, glucose repression of the gluconeogenic genes also seems to involve a second mechanism downstream of Snf1, since cells that lack both Snf1 and Mig1 still are unable to grow on gluconeogenic carbon sources. We have isolated mutations that affect this mechanism and cloned three of the genes involved. Two of them encode yeast cyclin C and its cyclin-dependent kinase (cdk), subunits of the RNA polymerase II mediator complex which are known to be involved in glucose repression of invertase and the meiotic genes (10–12). The third gene, *GIG1*, is identical to *SRB8* (13), which encodes another subunit of the mediator complex. Mutations in all three genes have identical phenotypes, and show no synergism with each other, indicating that these three subunits of the mediator complex function closely together in the same regulatory process.

MATERIALS AND METHODS

Yeast strains

All yeast strains were congenic to the *MAT***a** *SUC2 GAL ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* strain W303-1A (14). The *mig1-δ1::LEU2* allele has been described (6). The *SNF1 (CAT1)* gene (15,16) was disrupted by inserting the *HIS3* *Bam*HI fragment beteeen the *Afl*II and *Mlu*I sites, which removes most of the open reading frame. The *GIG1* gene was disrupted by cloning either the *URA3 Hin*dIII fragment or the *TRP1 Eco*RI–*Pst*I fragment between the internal *Bst*1107I and *Nco*I sites. *GIG2* was disrupted by cloning the *URA3 Hin*dIII fragment, the *LEU2* *Hpa*I–*Sal*I fragment, or the *TRP1 Eco*RI–*Pst*I fragment between the *Msc*I and *Sac*II sites of *GIG2*. The *GIG3* gene, finally, was disrupted by cloning the *URA3 Hin*dIII fragment or the *HIS3* *Bam*HI fragment between the *Bsa*BI and *Bgl*II sites of *GIG3*.

Plasmids

The vector pHR81 has been described elsewhere (17). Plasmid pGIG1 was recovered from a YCp50 library (18), pGIG2 from a pHR81 library (15) and pGIG3 from a pHR70 library (19). For overexpression, a 5.9 kb *Msc*I fragment of pGIG1 was subcloned into the *Sma*I site of pUC118 to create pDB32. The whole insert from pDB32 was then subcloned as an *Ecl136*II–*Bam*HI fragment into the *Bam*HI site of pHR81 to generate pDB56. For

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overexpression of *GIG2* we used the original pGIG2 plasmid. The *GIG3* gene, finally, was subcloned as a 1.6 kb *Acc65I* fragment into the *Ecl136II* site of pHR81 to generate pDB35.

Mutant screen

A total of 44 single colonies from two *snf1 mig1* double disrupted yeast strains of opposite mating type were plated on YPE, YPL, YPG and YPA plates (1% yeast extract, 2% pepetone, 3% of either ethanol, lactate, glycerol or acetate). A large number of spontaneous mutations occur that can grow on these plates. A single mutant was picked from each plate, to ensure that every mutant had an independent origin.

RESULTS

Isolation of mutations

Cells that lack the Snf1 (Cat1) protein kinase are unable to grow on all carbon sources except glucose. Disruption of the *MIG1* gene in *snf1* deficient cells restores their ability to use galactose and sucrose, but they are still unable to grow on gluconeogenic carbon sources, such as lactate, acetate and ethanol. However, mutations that permit *snf1* cells to grow on these carbon sources are much more easily obtained if *MIG1* already is disrupted. This suggests that the genes required for gluconeogenic growth are under dual negative control by Mig1 and another mechanism.

We used the synergism with *mig1* in a screen for mutations affecting this putative second mechanism. Thus, spontaneous mutations were picked that would permit *snf1 mig1* double disrupted cells to grow on rich media containing gluconeogenic carbon sources. The mutations were tested for dominance by crossing them to a *snf1 mig1* strain of opposite mating type. Of 44 isolated mutations, eight were dominant, and 36 recessive.

Characterization of the dominant mutations

Surprisingly, six of the eight dominant mutations turned out to be ochre suppressors. Thus, in addition to a partial suppression of the *snf1* phenotype, they could also suppress the *ade2-1* and *can1-100* ochre mutations which are present in the W303-1A background (14), but not the *trp1-1* amber mutation. It should further be noted that the six ochre suppressors all were isolated on acetate. No such mutations were obtained on ethanol, glycerol or lactate. The reason why ochre suppressor mutations are able to partially suppress a deletion of the *SNF1* gene remains to be elucidated. However, one possible mechanism is that a protein which functions downstream of Snf1 has an ochre stop codon, the suppression of which generates a carboxy-terminal extension with a dominant negative effect on glucose repression. Another possibility would be that a *SNF1*-related pseudogene exists which contains an ochre stop codon. However, pseudogenes are rare in yeast, and there is no evidence of another *SNF1*-related gene in the yeast genome.

Characterization of the recessive mutations

Of the 44 recessive mutations, 29 had a phenotype which included an unexpected inability to grow on galactose (see below). These mutations fell into four complementation groups, designated *gig1, gig2, gig3* and *gig4,* for glucose inhibition of gluconeogenic growth. A total of 14 mutations were assigned to *gig1*, 11 to *gig2*, three to *gig3* and one to *gig4*. The initial characterization of the *gig* mutant strains revealed that they can grow on rich media containing glycerol, lactate, actetate or ethanol. However, under more stringent conditions, i.e. on synthetic media, the mutants can only use lactate. The reason for this difference is not clear, but it suggests that the requirements for growth on lactate are less stringent than for the other gluconeogenic carbon sources. It should be noted, however, that additional mutations which permit growth also on glycerol, ethanol or acetate occur at a high frequency in these strains.

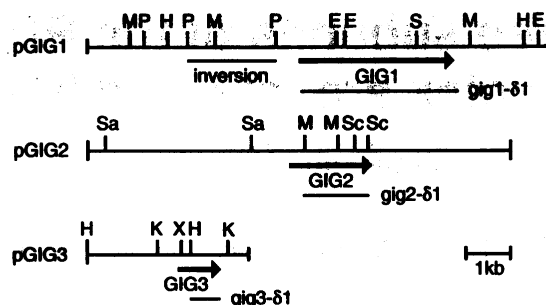

Figure 1. Restriction maps of the cloned genes. Open reading frames are shown as arrows below the maps. The extent of the deletions that were used in the gene disruptions are also shown. Abbreviations: E, *EcoRI*; H, *HindIII*; K, *KpnI*; M, *MscI*, S, *SalI*, Sa, *SacI*; Sc, *SacII*; X, *XcmI*.

Surprisingly, while growth on gluconeogenic carbon sources is improved, growth on galactose is instead inhibited in the *snf1 mig1 gig* triple deficient cells. It should be noted that while *snf1* cells are unable to use galactose, loss of *MIG1* restores their ability to grow on galactose, due to a partial derepression of the *GAL* genes. The *gig* mutations thus counteract this effect and restore the original *snf1* phenotype. It should further be noted that this effect is unique for galactose. The *gig* mutations did not significantly affect the ability of the *snf1 mig1* cells to grow on other fermentable carbon sources.

To investigate the phenotypes of the *gig* mutations in a wild-type background, the *mig1* and *snf1* disruptions were removed by genetic crosses. The resulting *gig* mutant spores were then tested for growth on different carbon sources. We found that all four mutant strains grow somewhat slower than wild-type cells on glucose. The *gig1, gig2* and *gig3* mutants also share an ability to grow on raffinose in the presence of 2-deoxyglucose, a phenotype which was confirmed in disrupted strains (see below). In contrast, the *gig4* mutant appeared somewhat more sensitive to 2-deoxyglucose. It should also be noted that the gal$^-$ phenotype of the *snf1 mig1 gig4* cells was less pronounced than for the other *gig* genes. These findings suggest that the Gig4 protein may be functionally less closely associated with the other three Gig proteins.

Cloning of the *gig1, gig2* and *gig3* genes

We took advantage of the gal$^-$ phenotype of the *snf1 mig1 gig* cells to clone the genes. Three different genomic yeast libraries were transformed into the *snf1 mig1 gig* triple mutant strains and screened for plasmids that could restore growth on galactose. In this way, the *gig1, gig2* and *gig3* genes were cloned (Fig. 1). The complementing activity in each plasmid was mapped by deletion analysis, and sequence data were obtained from the inserts.

The sequence revealed that the *GIG1* gene is identical to YCR081W, an open reading frame on chromosome III (20),

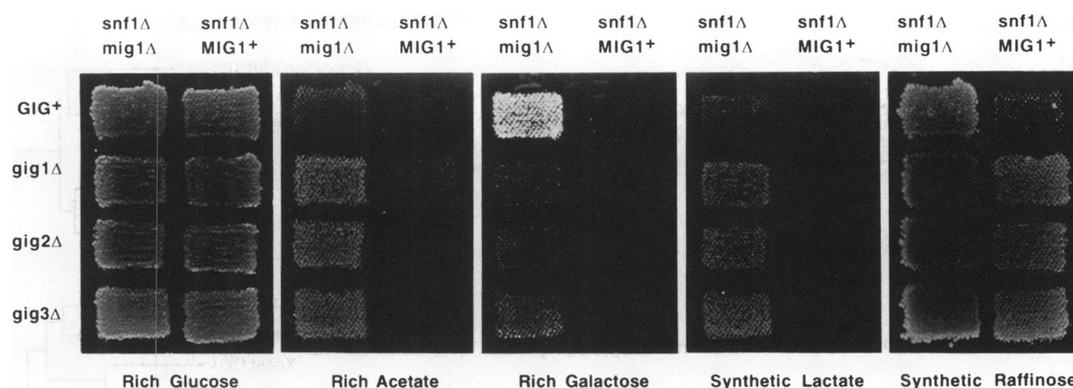

Figure 2. Growth of *snf1 gig* double disrupted and *snf1 mig1 gig* triple disrupted cells on various carbon sources. The cells were grown on rich glucose (YPD) and then replicated to different media, as indicated in the figure.

which was recently found to encode Srb8, a subunit of the RNA polymerase II mediator complex (13). The encoded protein is large (1226 amino acid residues) and hydrophilic. Its sequence does not show any obvious similarity to other protein sequences in the data bases. Nor does it contain any internal repeats, except for the fact that the motif LLXILITYG occurs twice in the sequence. Our clone differs in one respect from the published sequence of chromosome III (20). Thus, we found that a *Pst*I fragment is inverted as compared to the published sequence. This does not affect the predicted protein sequence, as the inversion is outside the open reading frame. However, it does change the protein encoded by the 5′ adjacent YCR080W open reading frame.

The *GIG2* and *GIG3* genes are identical to *SRB10/SSN8* and *UME5/SRB11/SSN3,* (10–12), which encode cyclin C and a cyclin C-dependent kinase. These two proteins were also recently shown to be subunits of the RNA polymerase II mediator complex (11,13). It should be noted that one of our *gig2* complementing plasmids encodes a truncated kinase in which the 56 C-terminal residues are missing. This shows that that the C-terminal region is not strictly required for activity. However, the complementing activity of this plasmid was clearly reduced as compared to the wild-type gene.

Functional analysis of the cloned genes

To investigate their function, we made one-step disruptions of all three genes in wild-type, *snf1*, *mig1* and *snf1 mig1* strains. The resulting strains were examined for a number of phenotypes (Fig. 2). We found that the *mig1 snf1 gig* triple disrupted strains behave identically to the original *mig1 snf1 gig* mutant strains. Thus, they can grow on all rich media containing gluconeogenic carbon sources, and on synthetic media containing lactate, but not on ethanol, glycerol or acetete.

Notably, these effects are seen only in the absence of *MIG1*. We conclude that *MIG1* and the *GIG* genes act in parallel, and that both mechanisms must be disrupted to permit gluconeogenic growth. However, the disruptions also have effects in *snf1* cells which are independent of the absence of *MIG1*. Thus, *snf1 gig* double disrupted cells are able grow on raffinose, indicating a partial derepression of the *SUC2* gene. It should be noted that a disruption of *MIG1* alone similarly permits *snf1* cells to grow on raffinose.

In *SNF1 MIG1* wild-type cells, all three disruptions cause a reduced growth. This effect is most clearly seen on gluconeogenic carbon sources and on galactose, but growth on glucose and raffinose is also affected. However, we also found that all three disrupted strains differ from wild-type cells in that they can grow on raffinose in the presence of 2-deoxyglucose. This shows that glucose repression of the *SUC2* gene, whose expression is required for use of raffinose, is impaired in these cells.

We proceeded to test the effect of double and triple disruptions of the three genes in the same cell. We found that such strains behave identically to single disrupted strains with respect to all phenotypes tested (data not shown). This is in contrast to the strong synergistic interactions which are seen with *mig1* disruptions, and suggests that all three genes function closely together in the same regulatory pathway.

We also tested if any of the three genes could complement disruptions of the other two genes when overexpressed. Such partial cross-complementation is frequently seen between genes that are functionally related to each other. However, we found that none of the genes can complement the other two when present on a high copy number plasmid.

DISCUSSION

In an attempt to analyze how the gluconeogenic genes are repressed by glucose, we isolated mutations that will permit *snf1 mig1* double disrupted cells to grow on gluconeogenic carbon sources. Most of the mutations fell into three complementation groups, *gig1*, *gig2* and *gig3*. Cloning of the genes showed that *GIG2* and *GIG3* are identical to *UME5/SSN3/SRB10* and *SSN8/SRB11*, recently discovered subunits of the RNA polymerase II mediator complex which encode cyclin C and its associated kinase (10–12). The third gene, *GIG1*, is identical to *SRB8*, another subunit of the mediator complex (13).

The appearance of the cyclin C-cdk pair in our screen is not surprising since the *ssn3* and *ssn8* mutations also were obtained as suppressors of *snf1* (on raffinose), and show synergism with *mig1* (21,22). However, it underlines a basic similarity between the *GAL* and *SUC* genes and the gluconeogenic genes. Loss of Mig1 causes a partial derepression of the former, but has little effect on the latter. This has prompted suggestions that they could be repressed by a different mechanism. Nevertheless, it was recently shown that Mig1 represses *CAT8* which encodes a Gal4-related activator of these genes (9). This is strikingly similar to how Mig1 controls the *GAL* genes (7). Our finding that the mediator complex is involved in Mig1-independent repression of

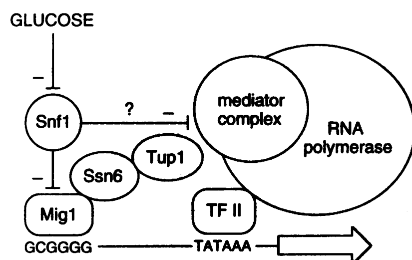

Figure 3. Model which shows some of the proteins that are involved in glucose repression.

gluconeogenic growth further emphasizes the similarity to other glucose repressed genes such as *SUC2*. It seems that the gluconeogenic genes differ mainly in their greater sensitivity to repression. Thus, while loss of either Mig1 or cyclin C permits *snf1* cells to grow on raffinose, both must be eliminated to permit growth on lactate. Conceivably, a third mechanism which is specific for the gluconeogenic genes could explain this difference. This would also be consistent with our finding that further suppressor mutations can be obtained that permit *snf1 mig1 gig* triple disrupted cells to grow on synthetic ethanol media.

It is thought that Mig1 represses transcription by recruiting a general co-repressor complex in which the Tup1 protein is the active subunit (23–25). The fact that at least three subunits of the mediator complex now have been shown to be involved in glucose repression raises the question if they could function downstream in this pathway, as targets for Tup1. This would be consistent with the finding that at least one of these three proteins is involved in α2-mediated repression, which is also Tup1-dependent (26). However, it should be noted that loss of Tup1 has a much more severe phenotype (27) than loss of either of the three mediator subunits, which has only minor effects in wild-type cells. This argues against the latter being the main targets for Tup1. Conceivably, one of the essential subunits of the mediator complex, such as Srb4, Srb6 or Srb7, could be more directly involved in mediating repression, with loss of this function being linked to loss of viability. It is also notable that we did not obtain any mutations in *SRB9*, despite the fact that more than half of all recessive *srb* alleles mapped to this locus (13). This suggests that the non-essential subunits of the mediator complex may differ with respect to their involvement in repression and/or activation.

Another intriguing observation is the strong synergism between mutations in the three *GIG* genes and in *MIG1*. Strong synergism is frequently seen between mutations in parallel pathways. It raises the possibility that Mig1 and the three mediator subunits could function in two distinct pathways downstream of Snf1, with the mediator complex being a direct or indirect target of the Snf1 kinase (Fig. 3). This would be consistent with our finding that overexpression of Mig1 can partially suppress the effects of disruptions in the three *GIG* genes (data not shown) which shows that the latter are not strictly required for repression by Mig1. However, the fact that loss of Tup1 causes a complete derepression of many glucose repressed genes also suggests that if two parallel pathways exist, then both of them require Tup1. Further experiments are needed to resolve these questions.

Interestingly, the cyclin C-dependent kinase is most similar to the yeast Kin28 kinase and to the mammalian cdk7, both of which

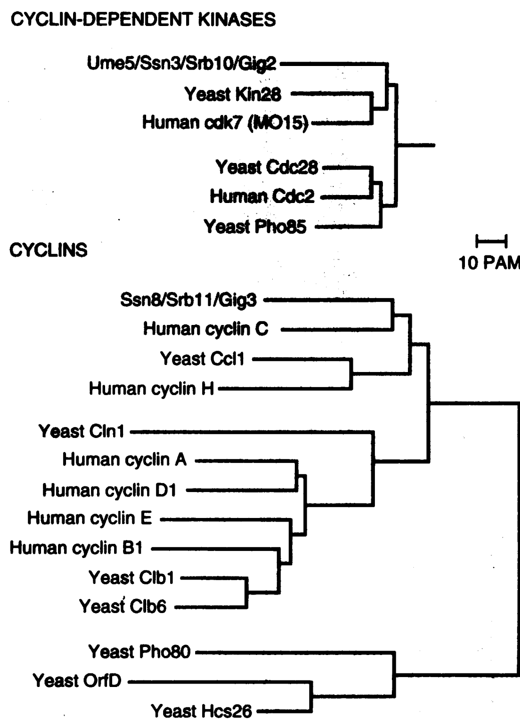

Figure 4. Dendrograms which show the position of cyclin C and its cdk within the cyclin and cdk protein families, respectively. The dendrograms were calculated from corrected distance data using the neighbor-joining method (32), as previously described (33).

are subunits of TFIIH (28,29). Moreover, cyclin C is most closely related to cyclin H (30), the regulatory subunit of cdk7 (Fig. 4). This suggests that a single ancestral cyclin–cdk pair was duplicated to produce the two pairs that are associated with RNA polymerase II. It raises the possibility that these two cyclin–cdk pairs could have similar or related functions *in vivo*. The cdk7 kinase has been implicated in phosphorylation of the C-terminal domain in RNA polymerase II, but can also activate other cyclin-dependent kinases *in vitro*. It has therefore been proposed to have a dual role in transcription and in cell cycle control. However, recent experiments with its yeast homologue Kin28 failed to show that that this kinase has cdk-activating activity (31). Further studies of cyclin C and its associated kinase may help to resolve these matters and establish to what extent the two cyclin–cdk pairs that are associated with RNA polymerase II also have other functions in the cell.

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