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Functional Domains in the Mig1 Repressor

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Mig1 is a zinc finger protein that mediates glucose repression in the yeast *Saccharomyces cerevisiae*. It is related to the mammalian Krox/Egr, Wilms' tumor, and Sp1 proteins and binds to a GC-rich motif that resembles the GC boxes recognized by these proteins. We have performed deletion mapping in order to identify functional domains in Mig1. We found that a small C-terminal domain comprising the last 24 amino acids mediates Mig1-dependent repression of a reporter gene. This effector domain contains several leucine-proline dipeptide repeats. We further found that inhibition of Mig1 activity in the absence of glucose is mediated by two internal elements in the Mig1 protein. A Mig1-VP16 hybrid activator was used to further investigate how Mig1 is regulated. Mig1-VP16 can activate transcription from promoters containing Mig1-binding sites and suppresses the inability of Snf1-deficient cells to grow on certain carbon sources. We found that a deletion of the *SNF1* gene increases the activity of Mig1-VP16 fivefold under derepressing conditions but not in the presence of glucose. This shows that the hybrid activator is under negative control by the Snf1 protein kinase. Deletion mapping within Mig1-VP16 revealed that regulation of its activity by Snf1 is conferred by the same internal elements in the Mig1 sequence that mediate inhibition of Mig1 activity in the absence of glucose.

In yeasts and other fungi, a large number of genes are turned off in the presence of glucose. This response is called glucose repression (9, 14, 28, 36). A key effector in glucose repression is a zinc finger protein known as Mig1 (26). Mig1 is a transcriptional repressor which belongs to the GC box-binding proteins, a subfamily of evolutionarily related zinc finger proteins that also includes the Sp1, Krox/Egr, and Wilms' tumor proteins. Like these mammalian proteins, Mig1 binds to a GC box, which is present in many genes that are repressed by glucose (20, 24).

Mutations in Mig1 show partial epistasis over mutations in Snf1 (or Cat1), a protein kinase which is required for expression of all glucose-repressed genes (6, 31). This suggests that one function of the Snf1 kinase is to prevent Mig1 from repressing its target genes in the absence of glucose. The mechanism by which Mig1 represses transcription is not clear, but it is thought that several yeast repressors, including Mig1, act by recruiting a general corepressor complex containing the Ssn6 (or Cyc8) and Tup1 proteins to their target promoters (16, 35, 38, 42). Recent evidence suggests that Tup1 is the active subunit within this complex (37, 38).

An important question is whether Mig1 contains distinct domains that mediate transcriptional repression and regulation by glucose. We have therefore performed an extensive deletion analysis of Mig1 in order to identify such domains within the protein. A large number of deletions were tested for their ability to repress a target promoter both in the presence and in the absence of glucose. We found that a small domain comprising the 24 C-terminal residues in Mig1 mediates repression. We also found that two internal elements in Mig1 mediate an inhibition of its activity in the absence of glucose. Further experiments with a Mig1-VP16 hybrid activator revealed that the activity of this activator is under negative control by the Snf1 protein kinase and that this regulation is conferred by the same two elements that mediate inhibition of Mig1 activity in the absence of glucose.

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MATERIALS AND METHODS

***lacZ* reporter plasmids.** The *lacZ* reporters used were based on pLGΔ-312 (11) and the pFL series of 2μm and *CEN6* plasmids (18). First, a 3,658-bp *Xma*I-*Age*I fragment of pLGΔ-312 carrying the *CYC1-lacZ* fusion was cloned into the unique *Xma*I site in plasmids pFL39 and pFL45. The resulting plasmids were called pJO20 (*TRP1 CEN6*) and pJO22 (*TRP1* 2μm), respectively. Plasmids pJO25 and pJO27 were then obtained from pJO20 and pJO22, respectively, by cloning a 500-bp *Hin*dIII-*Sal*I fragment of pWJ210 (26), which contains the *SUC2* upstream activation sequence (UAS) with its two Mig1-binding sites, between the unique *Hin*dIII and *Xho*I sites in these two plasmids.

The second type of reporter plasmids were made by cloning a 147-bp *Xho*I fragment of pJN127 (20) between the *Sal*I and *Xho*I sites of pJO20 and pJO22. This fragment contains the strong Mig1-binding site A from the *SUC2* promoter (26) cloned between two tandem repeats of a polylinker. Plasmids based on pJO22 (the 2μm vector) that had one (pJO81), two (pJO83), or three (pJO85) copies of the insert in the same orientation as in the *SUC2* promoter were isolated. The centromere plasmid pJO139 (based on pJO20) has three copies of the Mig1 site in the forward orientation.

Mig1 deletions. The Mig1 deletions were based on pMC120, which has a 2,160-bp *Sac*I fragment carrying the *MIG1* gene (25) cloned into the *Sac*I site of the *URA3 CEN4* plasmid pHR68 (25). Constructions involving unique restriction sites were made directly in pMC120. Other constructions were made in pUC119, after which the *Sac*I insert was moved back to pHR68. Constructions involving *Tfi*I were made in pJO39, a derivative of pUC119 in which the two *Tfi*I sites were removed by cutting and filling in with DNA polymerase I, and then moved back to pHR68.

Other Mig1 constructions. To construct the Mig1-VP16 fusion, pMC120 was cut with *Cla*I, filled in with the Klenow fragment of DNA polymerase I, and then ligated to a 388-bp *Pvu*II-*Bam*HI fragment of pSCTEV-GAL4(1–93)-VP16(413–490) encoding the VP16 activating domain (33). The resulting plasmid, pJO5, encodes a hybrid protein in which residues 1 to 351 of Mig1 are fused to residues 413 to 490 of VP16.

β-Galactosidase assay. The β-galactosidase assay was performed on cell lysates as previously described (22). However, instead of measuring the absorbance at 550 nm for each sample to compensate for debris scattering, the samples were first spun in a Microfuge. This removes all debris and reproducibly sets the absorbance at 550 nm to zero. All measurements were performed on at least three independent transformants.

Yeast strains. The yeast strains used are listed in Table 1. They are all congenic to the *MAT***a** *SUC2 GAL ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* strain W303-1A (34). The *mig1*-δ*1*::*LEU2* and *mig1*-δ*3* alleles have been described (25, 26). To facilitate transformation with *TRP1* plasmids, some strains have the stable *trp1*::*hisG* marker instead of the amber-suppressible *trp1-1*. This marker was introduced into the W303-1A background by transformation with the wild-type *TRP1* gene, followed by disruption of *TRP1* with the reusable *URA3*

TABLE 1. Yeast strains[a]

Strain	Relevant genotype	Reference
W303-1A	Wild-type background	34
H174	*mig1*-Δ*1*::*LEU2*	26
H366	*snf1*-Δ*1*::*HIS3*	This work
H368	*snf1*-Δ*1*::*HIS3 mig1*-Δ*1*::*LEU2*	This work
H443	*mig1*-Δ*1*::*LEU2 tup1*-Δ*1*::*HIS3*	This work
H497	*trp1*::*hisG mig1*-Δ*3*	This work

[a] All strains were congenic to W303-1A and therefore also contain the *MAT***a** *SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* markers.

construct of Alani et al. (1) and then selection for mitotic loss of *URA3* on 5-fluoro-orotic acid (4). The *SNF1* (*CAT1*) gene was disrupted by cloning the *HIS3 Bam*HI fragment between the *Afl*II and *Mlu*I sites, thus removing most of the open reading frame. The *TUP1* gene (41) was disrupted by cloning the *HIS3 Bam*HI fragment between the *Mlu*I and *Bgl*II sites in *TUP1*.

RESULTS

A small C-terminal proline-rich domain in Mig1 mediates repression in the presence of glucose. To map those regions in Mig1 that are required for glucose repression, we constructed a large number of in-frame deletions and frame shift mutations encoding Mig1 proteins with internal deletions and truncated C termini. These constructions were tested for their ability to repress a reporter gene in which β-galactosidase is expressed from a hybrid *SUC2-CYC1* promoter. The hybrid promoter contains the upstream regulatory region of the *SUC2* gene, including its two Mig1-binding sites, and the TATA box of the *CYC1* gene. Like the *SUC2* gene itself, this reporter is strongly repressed by glucose. The constructions were introduced on centromere plasmids into a Mig1-deficient yeast strain and tested for their ability to restore glucose repression of the reporter gene. Several independent transformants were assayed for each construction in order to obtain a reliable estimate of the level of expression.

We found that the wild-type *MIG1* gene mediates a 20-fold repression of the reporter in the presence of 2% glucose (Fig. 1). The deletions that were tested fell into two distinct groups. All constructions that retained the C-terminal 24 residues of Mig1 were able to repress the reporter. On the other hand, all constructions that lacked this region failed to repress transcription (with one exception, which is discussed below). We conclude that this small domain plays a crucial role in repression. In fact, it alone can mediate repression when fused directly to the DNA-binding domain (construction Mig1-Δ9), although not quite as efficiently as the wild-type protein. The C-terminal domain is thus necessary for repression and also sufficient together with the DNA-binding domain. We therefore designate this domain the effector domain of Mig1.

It should be noted that constructions which lack the effector domain are not completely inactive. Most of them cause a small (twofold) repression of the reporter. This effect is seen even with Mig1-Δ10, which contains only the DNA-binding domain. It is conceivable that this residual repression is due to competition between Mig1 and an activator protein for adjacent sites, since this would require only the DNA-binding domain of Mig1. Such competition was recently shown to play a role in repression of the *alcR* promoter by CREA, the Mig1 homolog in *Aspergillus nidulans* (21). The *SUC2* promoter is known to contain an activating region that partially overlaps the two Mig1-binding sites (30). However, we cannot rule out that the DNA-binding domain has some intrinsic repressing activity which does not involve such competition.

To test if the repression is Tup1 dependent, several constructions were also assayed in yeast strain H443 (Table 1), which lacks both *TUP1* and *MIG1*. We were unable to detect any significant repression in this genetic background (data not shown). This is consistent with the previous finding that loss of Tup1 causes complete derepression of the *SUC2* gene in *S. cerevisiae* (41).

Structure of the Mig1 effector domain. The sequence of the effector domain is shown in Fig. 2A. The first 10 residues have a negative charge and a high probability of α-helix formation, but the C-terminal part of the domain contains several helix-breaking prolines. Interestingly, they are organized into leucine-proline dipeptide repeats (Fig. 2B). It is noteworthy that this sequence, RSLPLPFP, is very similar to the consensus motif for binding of SH3 domains, RXLPPLP (44). However, it differs from the latter in the spacing of the prolines. Thus, the core motif PXXP is strictly conserved in all SH3-binding peptides (44). These two prolines form a left-handed polyproline type II helix, with three residues per turn, which is crucial for SH3 binding (27). The fact that the prolines in Mig1 have a different spacing, PXPXP, suggests that they form a different kind of structure, which argues against an interaction with a classical SH3 domain. It should also be noted that neither Cyc8 nor Tup1, which are likely candidates for binding to the Mig1 effector domain, contains any SH3 domains.

Significantly, the C-terminal part of the effector domain including the leucine-proline repeats and the RSL motif is conserved in Mig1 from *Kluyveromyces lactis* (5), which suggests that these residues are functionally important (Fig. 2A). In contrast, the N-terminal half of the effector domain is not conserved. Similar motifs can also be found in CREA (7, 8), the Mig1 homolog in the more distantly related fungus *A. nidulans* (Fig. 2B). However, the effector domain has not yet been located in CREA, so it remains to be seen if these motifs are involved in repression. Repressing domains have also been mapped in two mammalian proteins that are related to Mig1, the Wilms' tumor protein (40) and Egr1 (10), but neither of them shows any obvious similarity to the Mig1 effector domain.

We also searched the SWISS protein database for sequence motifs resembling the Mig1 effector domain. Many matches were found, most of which involved proteins of no relevance to gene expression. This was expected, since the probability of finding random matches to a short sequence is high. However, one protein of special interest was found: the Rox1 repressor (2). As shown in Fig. 2B, Rox1 contains two motifs that resemble the Mig1 effector domain. Rox1 mediates heme repression of the hypoxic genes in *S. cerevisiae* (45), and it is thought that Mig1 and Rox1 both function by recruiting the Cyc8-Tup1 general corepressor complex to their target promoters (16, 37, 38). One might therefore expect to find similarities between the effector domains in Mig1 and Rox1. However, it remains to be shown if these motifs are involved in mediating repression by Rox1.

A frameshift mutation creates an artificial effector domain. As mentioned above, there was one case in which a deletion of the effector domain failed to abolish Mig1-dependent repression. This is Mig1-Δ15, in which a frameshift was generated by filling in the *Cla*I site. Mig1-Δ15 represses transcription but not as strongly as the wild-type protein (4-fold instead of 20-fold). A further deletion, Mig1-Δ16, was used to map the repressing activity to the C-terminal part of the protein (Fig. 1). Thus, there must be something unique about the C terminus of Mig1-Δ15 which causes it to repress transcription. An inspection of the sequences revealed that the *Cla*I frameshift creates a short C-terminal extension which by coincidence is highly similar to the wild-type effector domain in Mig1 (Fig. 2B). Thus, 5 of the last 11 amino acid residues are identical in the

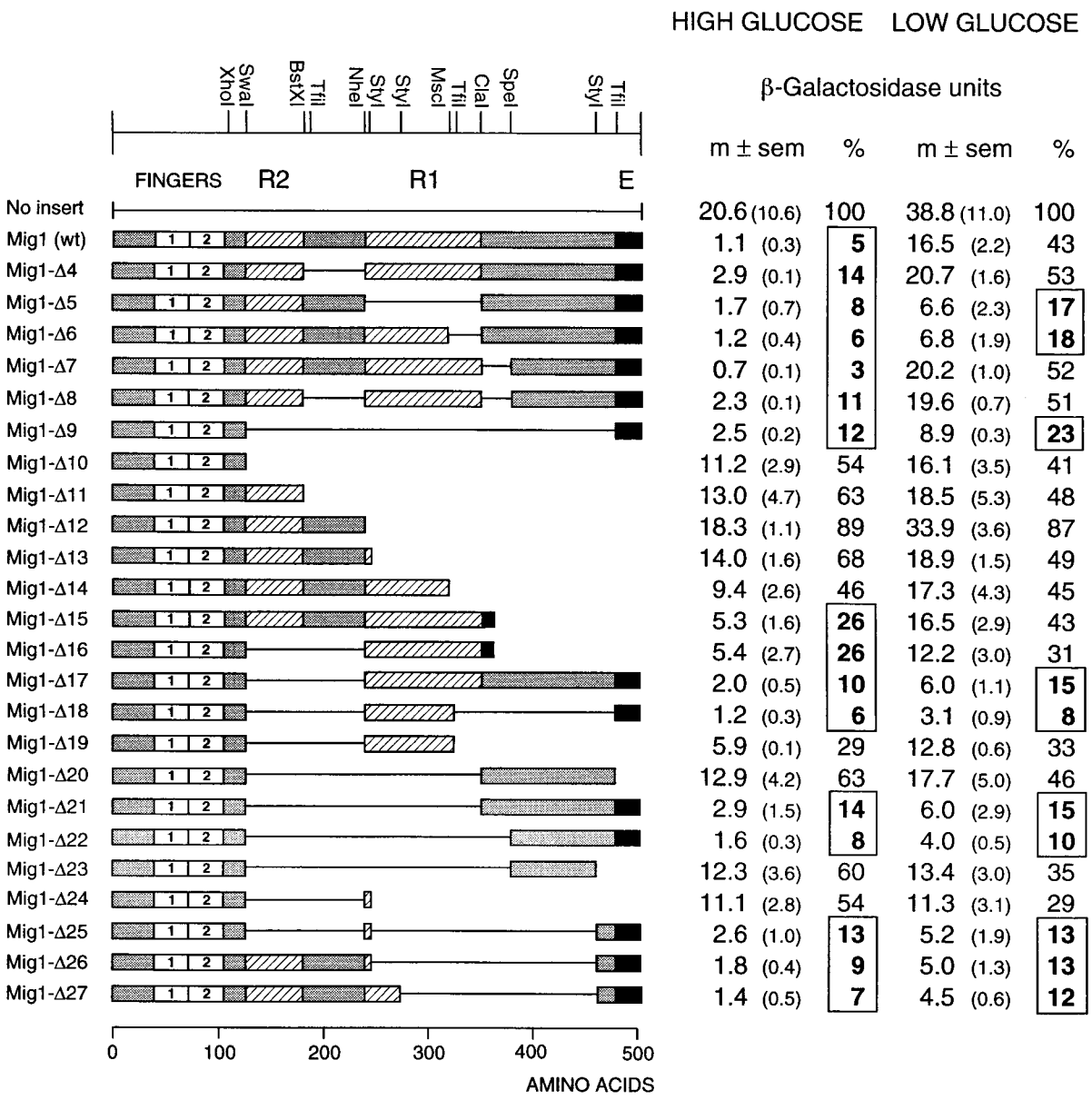

FIG. 1. Deletion mapping of the Mig1 repressor. The constructions that were tested are shown to the left. The effector domain (E) is shown as a solid box, and the two regulatory regions, R1 and R2, are shown as hatched boxes. The artificially generated effector-like domain in Mig1-Δ15 and Mig1-Δ16 is also shown as a solid box. The map at the top shows the restriction sites that were used to make specific deletions. Their distances (in nucleotides) from the first nucleotide in the *MIG1* open reading frame are as follows (first nucleotide in each site): *Xho*I, 283; *Swa*I, 364; *Bst*XI, 538; *Tfi*I, 554; *Nhe*I, 714; *Sty*I, 726; *Sty*I, 807; *Msc*I, 959; *Tfi*I, 978; *Cla*I, 1,050; *Spe*I, 1,137; *Sty*I, 1,380; and *Tfi*I, 1,449. Each deletion was put on a centromere plasmid and then transformed into the Mig1-deficient yeast strain H497 harboring the high-copy-number *SUC2-lacZ* reporter plasmid pJO27 (Table 2). To maintain selection for both plasmids, the cells were grown in synthetic medium lacking uracil and tryptophan. The ability of each construction to repress the *SUC2* promoter was then determined from β-galactosidase assays on extracts from cells grown under repressing conditions (high glucose) or derepressing conditions (low glucose). For each construction, the mean of at least three independent transformants is shown, with standard errors in parentheses. Also shown for each construction are β-galactosidase levels expressed as a percentage of the result obtained with no Mig1 construction present (empty vector). Values that reflect significant repression (fourfold or more) are enclosed in boxes.

two proteins. The similarity includes one of the leucine-proline repeats and also the arginine which is conserved in Mig1 from *K. lactis*. We conclude that the repressing activity of Mig1-Δ15 is most likely due to this fortuitous creation of a sequence motif resembling the wild-type effector domain.

Two regulatory elements inhibit Mig1 in the absence of glucose. We proceeded to assay our constructions under derepressing conditions. Our aim was to investigate if there are regulatory elements in Mig1 which inhibit its function in the absence of glucose. Such negative control could act in several ways, e.g., by affecting protein stability, nuclear transport, or DNA binding. Irrespective of the mechanism, we reasoned that a deletion of such negative regulatory elements in a protein that retains the effector domain might create a constitutive repressor that is also active in the absence of glucose. In these experiments, glucose-grown log-phase cells were shifted to 0.05% glucose as a sole carbon source for 3 h prior to the assay. This method has been shown to cause a significant derepression of the *SUC2* gene (29). Glucose at 0.05% does not trigger repression but helps to maintain cell viability.

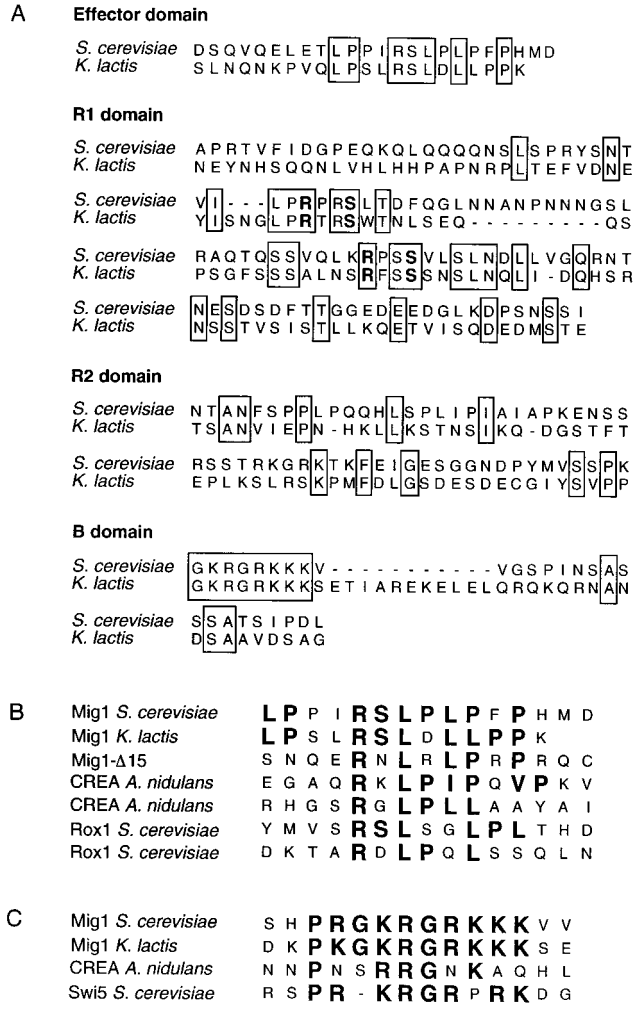

FIG. 2. (A) Sequences of the effector domain, the two regulatory domains, and the basic domain in the Mig1 protein. The sequences are aligned to the corresponding parts of Mig1 from *K. lactis* (5), with identical residues enclosed in boxes. Two conserved RXXS motifs that are possible protein kinase target sites are shown in boldface. (B) Comparison of the effector domain with other protein sequences. These include the homologous part of the Mig1 protein from *K. lactis* (5), the artificial effector domain created by the Mig1-Δ15 frameshift, and sequence motifs found in the CREA (7) and Rox1 (2) repressors. Conserved motifs are shown in boldface. (C) Comparison of the basic domain in Mig1 from *S. cerevisiae* and *K. lactis* (5) with the same region in CREA (7) and the nuclear localization signal in Swi5 (23). Similarities are shown in boldface.

The results are shown in Fig. 1. We found that the wild-type Mig1 protein does not repress transcription in 0.05% glucose except for the twofold effect which is also seen with the DNA-binding domain alone, nor do deletions Mig1-Δ4, Mig1-Δ7, or Mig1-Δ8 repress transcription. These constructions all retain most of the internal part of the Mig1 protein. However, some constructions do repress transcription in 0.05% glucose, indicating that they have become constitutive repressors. Thus, Mig1-Δ5 represses transcription five- to sixfold (two- to threefold better than the wild-type protein). The Mig1-Δ5 protein lacks amino acid residues 241 to 351. We conclude that this region confers a specific two- to threefold inhibition of Mig1 activity in the absence of glucose.

Interestingly, this region is also required for Snf1-dependent regulation of Mig1-VP16 (see below), and we therefore designate it regulatory domain R1 (Fig. 1). It should be noted that an even smaller deletion, Mig1-Δ6, had the same effect as Mig1-Δ5 when assayed for constitutive repression of the *SUC2* promoter (Fig. 1). This might be taken as evidence that the inhibitory element is located within the smaller deletion (residues 322 to 351). However, this deletion alone does not abolish negative control of Mig1-VP16 by Snf1 (see below). We therefore consider this interpretation less likely. It should be noted that different C-terminal sequences were fused to residue 321 in the two cases. Therefore, it is conceivable that the fusion of Mig1-derived sequences to residue 321 in Mig1-Δ6 causes steric interference with a negative regulatory element in the 241 to 321 region, which is not similarly affected when the activating domain of VP16 is fused to residue 321. However, other explanations are also possible.

A second inhibitory element could also be identified. Deletion Mig1-Δ17, which lacks residues 124 to 239, represses transcription sixfold on 0.05% glucose, threefold better than wild-type Mig1. However, Mig1-Δ4, which has a shorter deletion spanning only residues 183 to 239, does not show constitutive activity. We conclude that the region between residues 124 and 183 (region R2 in Fig. 1) confers a threefold inhibition of Mig1 activity in the absence of glucose. Further deletions were used to study how the R1 and R2 elements interact with each other. We found that a deletion of the R2 element in a construction that already lacks R1 does not affect the repressing activity (compare Mig1-Δ25 and Mig1-Δ26). Similarly, deletion of R1 in a construction that lacks R2 has no significant effect (compare Mig1-Δ17 and Mig1-Δ21). These results suggest that both R1 and R2 are required for inhibition of Mig1 activity in the absence of glucose.

The sequences of the R1 and R2 regions are shown in Fig. 2A. Also included is an alignment with the Mig1 protein from *K. lactis* (5) in order to identify functionally important residues that are likely to be conserved between the two species. It should first be noted that the R2 region is not strongly conserved in the two proteins. In fact, it is difficult to find a unique alignment in this part of Mig1. In contrast, the R1 region comprises a highly conserved region (Fig. 2). Interestingly, the conserved residues include two RXXS motifs. This is a common core site which is shared by several serine-specific protein kinases (17). One of these motifs is identical to a previously identified possible target site for the cyclic AMP-dependent protein kinase (26). The fact that the R1 domain is conserved between the two species is consistent with the notion that it plays an important role in regulating Mig1 activity.

A Mig1-VP16 hybrid activator is inhibited by Snf1 in the absence of glucose. Epistasis data suggest that Mig1 functions downstream of the Snf1 kinase and that one important function for the latter is to inhibit or counteract the repressing activity of Mig1 (28). An obvious question is therefore whether the negative regulatory elements that we have found in Mig1 are targets (direct or indirect) for regulation by Snf1. A simple experiment would be to check if the inhibitory effects of the R1 and R2 elements disappear in a Snf1-deficient strain. Unfortunately, expression of the reporter is very low in a *snf1* mutant strain even in the absence of the wild-type *MIG1* gene because Mig1-independent mechanisms also contribute to glucose repression (14, 28). It is therefore impossible to assay our Mig1 constructions in a *snf1* strain.

To circumvent this problem, we decided to make a hybrid protein in which the effector domain of Mig1 was replaced by the activating domain of the viral protein VP16, known to function as a strong activator in *S. cerevisiae* (33). We reasoned that if Mig1 is regulated by the Snf1 kinase, this regulation might also apply to such a hybrid activator, provided that the latter retains the regulatory domains in Mig1. The activity of

TABLE 2. Plasmids[a]

Plasmid	Markers	Reference
pHR68	*URA3 CEN4*	25
pMC120	*URA3 CEN4 MIG1*	This work
pJO5	*URA3 CEN4 MIG1-VP16*	This work
pJO25	*TRP1 CEN6 SUC2-lacZ*	This work
pJO27	*TRP1* 2μm *SUC2-lacZ*	This work
pJO81	*TRP1* 2μm (Mig1 site)$_1$-*lacZ*	This work
pJO83	*TRP1* 2μm (Mig1 site)$_2$-*lacZ*	This work
pJO85	*TRP1* 2μm (Mig1 site)$_3$-*lacZ*	This work
pJO87	*TRP1* 2μm (no UAS)-*lacZ*	This work
pJO139	*TRP1 CEN6* (Mig1 site)$_3$-*lacZ*	This work
pJO154	*TRP1 CEN6* (no UAS)-*lacZ*	This work

[a] Plasmids pJO81, pJO83, pJO85, and pJO139 contain one, two, or three synthetic Mig1-binding sites, as indicated. Plasmids pJO87 and pJO154 contain no Mig1-binding sites.

the Mig1-VP16 protein was assayed with a series of plasmids in which synthetic Mig1-binding sites were inserted in front of the *CYC1-lacZ* fusion (Table 2) to check that it functions as an activator. These synthetic reporters were used instead of the *SUC2* promoter because the latter contains upstream activating elements that activate transcription in the absence of Mig1-VP16. We found that Mig1-VP16 can activate transcription from the synthetic promoters and that the activity increases synergistically with the number of Mig1 sites. Thus, activation was 5-fold with a single Mig1 site, 20-fold with two Mig1 sites, and 40-fold with three Mig1 sites (Table 3).

We next examined whether the Mig1-VP16 fusion is regulated by the Snf1 kinase. The ability of Mig1-VP16 to activate transcription from a synthetic promoter with three Mig1-binding sites was assayed in wild-type and Snf1-deficient cells under both derepressing conditions (3% raffinose or 2% galactose) and repressing conditions (8% glucose). The experiments were performed in Mig1-deficient cells because the wild-type Mig1 repressor interferes with the function of Mig1-VP16 (see Fig. 4, below). The results are shown in Table 4. Interestingly, we found that the ability of Mig1-VP16 to activate transcription is inhibited fivefold by Snf1. Moreover, this inhibition is only seen under derepressing conditions, when the Snf1 kinase is known to be active (43).

Mapping of regulatory domains within Mig1-VP16. We proceeded to map the regions involved in mediating Snf1-dependent inhibition of Mig1-VP16 (Fig. 3 and Table 4). We found that a deletion of the region between the DNA-binding domain and the VP16 domain (Mig1-VP16-Δ2) eliminates negative control by Snf1, creating a constitutive activator. This suggests that Snf1-dependent inhibition of Mig1-VP16 activity is mediated by an internal part of Mig1.

The inhibitory elements in Mig1-VP16 were further mapped by using more deletions. We found that a deletion of the R1 domain (Mig1-VP16-Δ5) completely eliminates negative control by Snf1, creating a constitutive activator with even higher activity than the original Mig1-VP16 (Table 4). This shows that Snf1-dependent inhibition of Mig1-VP16 requires the R1 domain but also suggests that the latter has some residual effect on Mig1-VP16 even in the absence of Snf1. A shorter deletion (Mig1-VP16-Δ4), which removes the C-terminal part of R1, caused only a partial escape from Snf1 control. This is in contrast to the results shown in Fig. 1, in which the corresponding Mig1-Δ6 deletion was as effective as a deletion of the entire R1 domain (Mig1-Δ5). As discussed above, this difference could be due to the fact that different C-terminal sequences are fused to the remaining part of R1 in the two constructions.

A deletion of the R2 domain (Mig1-VP16-Δ1) resulted in a partial escape from Snf1 control. Thus, Snf1 still has a twofold effect on the activity of Mig1-VP16-Δ1 (Table 4). This confirms that the R2 domain is also involved in negative control of Mig1, although it seems to be less important than R1. We conclude that inhibition of Mig1-VP16 activity by Snf1 resembles glucose regulation of Mig1 in that it is mediated by the R1 and R2 regions. Moreover, it seems that R1 plays a more prominent role in this regulation, since a deletion of this element alone is sufficient to eliminate negative control by Snf1. These results are consistent with the notion that Snf1 mediates negative control of Mig1 activity in the absence of glucose.

Galactose and raffinose are usually regarded as nonrepressing, but they are both fermentable carbon sources like glucose. We therefore proceeded to assay the Mig1-VP16 plasmids on a gluconeogenic carbon source (glycerol-lactate), which should produce a more completely derepressed state. This experiment could be performed only in the *SNF1* strain, since *snf1* mutant cells fail to grow on glycerol-lactate even in the absence of Mig1. The results are shown in Table 4. We found that the synthetic promoter has a weak basal activity in the absence of Mig1-VP16 which is higher than on the other carbon sources. This basal activity is mediated by the Mig1-binding sites, since a reporter without these sites was silent (Table 4). Significantly, Mig1-VP16 did not further increase expression, which suggests that it is completely inactive on glycerol-lactate. Thus, negative control of Mig1-VP16 on glycerol-lactate seems to be more stringent than on galactose or raffinose. However, this negative control can still be relieved by deleting either of the two inhibitory domains. Thus, Mig1-VP16-Δ5, which lacks the R1 domain, can activate transcription sevenfold on glycerol-lactate, and Mig1-VP16-Δ1, which lacks R2, activates transcription fourfold (Fig. 2B). This is consistent with the results obtained on galactose and raffinose.

Finally, it should be noted that the level of expression was significantly lower on 8% glucose than under derepressing conditions for all constructions tested. This is surprising, since one would expect Mig1-VP16 to be fully active on glucose, on which Snf1 is thought to be inactive. However, it should be emphasized that Mig1-VP16 still activates transcription more than 20-fold on glucose. Significantly, the basal level of reporter gene expression in the absence of Mig1-VP16 seems to be lower on glucose. This could in part explain why the Mig1-VP16-induced expression also is lower on glucose. Most importantly, no further increase in expression is seen in the absence of Snf1 (Table 4). Thus, while the level of expression on glucose is lower than expected, the absence of a Snf1-dependent effect is consistent with the notion that Snf1 is inactive in the presence of glucose (43).

TABLE 3. Mig1-VP16 activates transcription from tandem Mig1 sites[a]

Reporter plasmid	No. of Mig1 sites	β-Galactosidase activity (U) with centromere plasmid insert:		
		None	*MIG1*	*MIG1-VP16*
pJO87	0	0.13	0.13	0.11
pJO81	1	0.11	0.11	0.52
pJO83	2	0.13	0.10	1.97
pJO85	3	0.13	0.10	4.26

[a] The values shown are β-galactosidase units in extracts from log-phase H497 cells containing the reporter plasmid indicated and either pHR68, pMC120, or pJO5 (Table 2). To maintain selection for the plasmids, the cells were grown in synthetic 2% glucose medium lacking uracil and tryptophan.

TABLE 4. Ability of Mig1-VP16 constructs to activate transcription under derepressing and repressing conditions in *snf1*Δ and *SNF1* cells[a]

Reporter plasmid (no. of Mig1-binding sites)	Construction tested (insert in pHR68)	Mean β-galactosidase activity, U (SE)						
		Raffinose		Galactose		Glucose		Glycerol-lactate (*SNF1*)
		SNF1	*snf1*Δ	*SNF1*	*snf1*Δ	*SNF1*	*snf1*Δ	
pJO139 (3)	None	0.5 (0.0)	0.6 (0.0)	0.6 (0.1)	1.0 (0.1)	0.2 (0.0)	0.3 (0.0)	2.7 (0.1)
	Mig1-VP16	12.0 (0.8)	59.8 (2.4)	11.1 (0.9)	49.4 (2.5)	6.2 (0.3)	6.8 (0.2)	3.5 (0.2)
	Mig1-VP16-Δ1	16.6 (1.4)	27.6 (0.6)	17.8 (1.7)	31.1 (0.5)	1.7 (0.4)	1.7 (0.2)	15.5 (0.9)
	Mig1-VP16-Δ2	69.9 (4.7)	63.1 (0.7)	45.1 (2.4)	31.5 (2.4)	1.7 (0.1)	2.0 (0.3)	21.7 (1.2)
	Mig1-VP16-Δ3	0.6 (0.1)	0.8 (0.0)	0.9 (0.1)	1.1 (0.0)	0.2 (0.0)	0.3 (0.0)	3.2 (0.3)
	Mig1-VP16-Δ4	35.2 (3.6)	74.9 (2.0)	44.9 (6.4)	62.9 (5.1)	6.1 (0.5)	8.9 (0.5)	6.0 (0.3)
	Mig1-VP16-Δ5	90.9 (5.3)	86.7 (2.2)	96.7 (6.1)	88.0 (14.3)	9.4 (1.4)	9.3 (2.1)	25.3 (4.0)
pJO154 (0)	None	0.1 (0.0)	0.3 (0.1)	0.0 (0.0)	0.1 (0.0)	0.2 (0.0)	0.2 (0.0)	0.0 (0.0)
	Mig1-VP16	0.2 (0.1)	0.2 (0.0)	0.1 (0.1)	0.1 (0.0)	0.2 (0.0)	0.2 (0.0)	0.2 (0.0)

[a] Different constructions were introduced into yeast strains H174 (*SNF1*) and H368 (*snf1*Δ) and tested for their ability to activate transcription from reporter plasmids in which the *CYC1-lacZ* fusion is preceded by zero or three tandem Mig1-binding sites (Table 2 and Fig. 3). The cells were grown in synthetic medium containing 3% raffinose, 2% galactose, 3% glycerol-lactate, or 8% glucose. To maintain selection for the plasmids, uracil and tryptophan were omitted from the medium. The cells were harvested in mid-log phase, and extracts were prepared and assayed for β-galactosidase activity. The values shown are the means for at least three independent transformants, with standard errors in parentheses.

A small basic domain is required for Mig1-VP16 activity. The deletion analysis also revealed that a small basic domain which is located adjacent to the second zinc finger is required for Mig1-VP16 activity. Thus, we found that while Mig1-VP16-Δ2 is a constitutive activator, a further deletion (Mig1-VP16-Δ3), which removes the DNA between the *Xho*I and *Swa*I sites, is completely inactive (Fig. 3 and Table 4). The sequence encoded by this region is located adjacent to the second zinc finger and includes a stretch of basic residues that are identically conserved in Mig1 from *S. cerevisiae* and *K. lactis* (Fig. 2A). We therefore designate this the B (for basic) domain. Several residues within the B domain are conserved in CREA, the Mig1 homolog in *A. nidulans*, which shows only very limited similarity to Mig1 outside the DNA-binding domain (7). Interestingly, the B domain is also similar both in sequence and in its location to the nuclear localization signal in Swi5 (23), another yeast zinc finger protein (Fig. 2C). It is therefore possible that the B domain may be involved in nuclear transport. Alternatively, it could be involved in DNA recognition, possibly by interacting with the AT box, which is required for high-affinity DNA binding by Mig1 (20). A third possible explanation for our results would be that the activating and DNA-binding domains interfere with each other when juxtaposed too closely. However, this would not explain why the sequence of the B domain is so highly conserved.

Partial suppression of *snf1* by Mig1-VP16. Cells that lack the Snf1 kinase are unable to use all carbon sources except glucose. A deletion of *MIG1* in Snf1-deficient cells restores their ability to grow on fermentable carbon sources such as galactose and raffinose. This suggests that one function of the Snf1 kinase is to prevent Mig1 from repressing the *GAL* and *SUC* genes. In contrast, *snf1 mig1* doubly deficient cells are still unable to grow on gluconeogenic carbon sources such as glycerol, lactate, and ethanol. However, there is evidence that

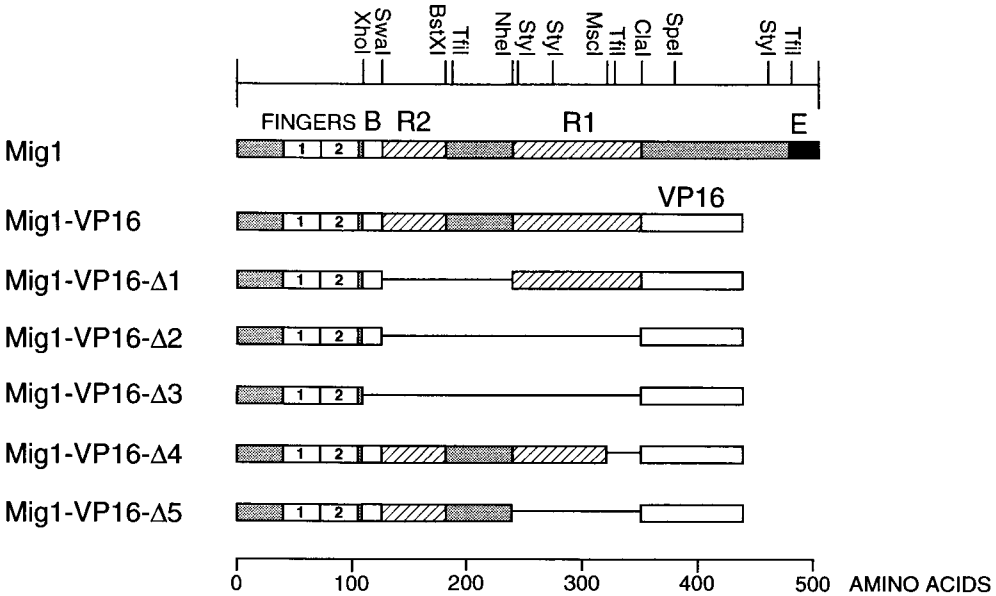

FIG. 3. Constructions tested for Mig1-VP16 activity (see Table 4). The R1 and R2 regulatory elements are shown as hatched boxes, and sequences required for Mig1-VP16 activity are shown as open boxes. The latter include the two zinc fingers, the B domain, and the VP16 activating domain.

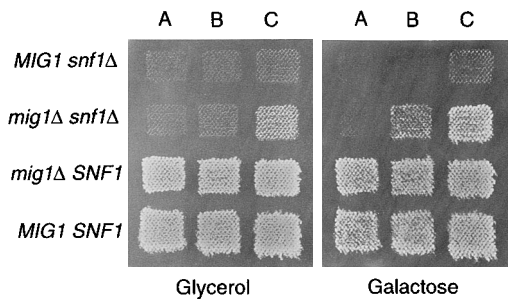

FIG. 4. Partial suppression of the *snf1* growth defect on glycerol and galactose by Mig1-VP16. Centromere plasmids carrying the wild-type *MIG1* gene (A), no insert (B), or the *MIG1-VP16* fusion (C) were transformed into yeast strains W303-1A, H497, H366, and H368 (Table 1). The cells were first grown on uracil-less synthetic 2% glucose medium and then replicated to uracil-less synthetic medium containing either 3% glycerol or 2% galactose. The relevant genotypes of the yeast strains are shown to the left.

Mig1 does repress those genes that are required for gluconeogenic growth but not as the only mechanism (28).

We reasoned that if Mig1 regulates these genes, then the Mig1-VP16 fusion might be able to suppress *snf1* even when a disruption of *MIG1* fails to do so. This is based on the assumption that a strong activator such as Mig1-VP16 would override the Mig1-independent components of glucose repression and cause constitutive expression of those genes that have Mig1-binding sites in their promoters. Suppression of *snf1* by Mig1-VP16 would indicate that constitutive expression of the Mig1-regulated genes is sufficient for growth on a particular carbon source.

To test this hypothesis, we transformed the Mig1-VP16 plasmid into both *snf1 mig1* and *snf1* cells and tested them for growth on different carbon sources. The results are shown in Fig. 4. We found that Mig1-VP16 can partially suppress the *snf1* growth defect on gluconeogenic carbon sources. Thus, Snf1-deficient cells that contain Mig1-VP16 can grow on glycerol but not on lactate, actetate, or ethanol. However, this effect is seen only in the absence of the wild-type Mig1 protein. We conclude that constitutive expression of those genes that are normally repressed by Mig1 is sufficient for growth on glycerol. The fact that the wild-type *MIG1* gene must also be disrupted to permit growth shows that repression by Mig1 is strong enough to interfere with a potent activator such as Mig1-VP16.

A similar effect is seen on galactose. Disruption of the *MIG1* gene in *snf1* cells causes a partial derepression of the *GAL* genes and allows some growth on galactose. However, this growth is strongly enhanced in the presence of Mig1-VP16 (Fig. 4). Also in this case, the effect was only seen in the absence of the wild-type Mig1 protein. We conclude that constitutive expression of the Mig1-regulated genes can suppress the need for the Snf1 kinase in certain cases. However, the failure of these cells to grow on ethanol, acetate, and lactate suggests that Snf1 is also required for the expression of some gene(s) which is not directly controlled by Mig1 and whose expression is necessary for growth on these carbon sources.

DISCUSSION

Effector domain of Mig1. We have shown that a small C-terminal domain in Mig1 comprising its last 24 amino acid residues is necessary for glucose repression and also sufficient for repression when fused to the DNA-binding domain of Mig1. The effector domain thus identified is proline-rich and resembles the consensus motif for binding of SH3 domains (44). However, it lacks the PXXP core motif, which is thought to be crucial for SH3 binding (27). The proline-rich motif is conserved in Mig1 from *K. lactis* (5), which is consistent with the notion that it plays an important role in vivo. Similar motifs are also found in the more distantly related Mig1 homolog CREA (7) and in the Rox1 repressor (2). It is thought that both Mig1 and Rox1 repress transcription by recruiting a general corepressor complex that contains the Ssn6 (or Cyc8) and Tup1 proteins, of which the latter is the active subunit (16, 36–38). One might therefore expect to find similarities between the effector domains of Mig1 and Rox1. In fact, the above similarities are rather limited, being restricted to a few conserved residues. Perhaps this is all that is required to recruit the corepressor complex. The fact that an artificially generated Mig1 C terminus with a similar motif could also repress transcription lends some support to this notion. One should also keep in mind that different domains within Cyc8 have been implicated in mediating different kinds of repression (38). It is therefore difficult to assess how much sequence similarity should be expected between Mig1 and Rox1 based on their proposed interactions with Cyc8 and Tup1.

Regulation of Mig1 activity. Our results show that the 20-fold repressing activity of Mig1 can be separated into two components. First, there is a 10-fold repression which is dependent on the effector domain and which is seen only in the presence of glucose. Second, there is a twofold repression which requires only the DNA-binding domain and which is seen both in the presence and in the absence of glucose. It seems likely that the former component reflects specific repression by Mig1, while the twofold constitutive effect may be due to some other mechanism, such as competition between Mig1 and activator proteins for overlapping sites. Our results further show that negative control of Mig1 activity in the absence of glucose is mediated by two internal elements in the Mig1 protein, R1 and R2 (Fig. 1).

Role of the Snf1 protein kinase. The mechanism(s) by which these elements inhibit Mig1 activity remains to be determined, but it is likely that this regulation is somehow mediated by the Snf1 protein kinase. The evidence for this is genetic: loss of Mig1 is partially epistatic over loss of Snf1. However, it should be noted that a Mig1-independent mechanism is also involved in glucose repression downstream of Snf1 (28). The nature of this mechanism remains to be elucidated, but there is evidence that it involves the recently discovered mediator complex of RNA polymerase II (3, 13, 19).

The presence of this Mig1-independent mechanism makes it difficult to study directly whether the activity of Mig1 is regulated by Snf1. We therefore chose an alternative approach, in which we constructed a Mig1-VP16 fusion that functions as an activator in *S. cerevisiae*. We found that Mig1-VP16 can activate transcription from promoters that contain Mig1-binding sites. Most significantly, we found that the activity of Mig1-VP16 is under negative control by the Snf1 kinase. Thus, a deletion of *SNF1* caused a fivefold increase in Mig1-VP16 activity under derepressing conditions. Deletion mapping within the Mig1-VP16 protein revealed that this control is mediated by the internal part of the Mig1 sequence. Moreover, a deletion of one of the two regulatory elements discussed above, R1, caused a complete release from negative control by Snf1, and a deletion of R2 caused a partial release. These results are all consistent with the notion that negative control of Mig1 activity in the absence of glucose is mediated by the Snf1 kinase. However, it still remains to be shown that the wild-type Mig1 repressor is regulated by Snf1 in the same way as Mig1-VP16. It also remains to be determined if this control

is achieved by direct phosphorylation of Mig1 or by some other indirect mechanism. The R1 domain contains at least two possible target sites for serine-specific protein kinases (Fig. 2A), but neither of them closely resembles the target site of the AMP-activated protein kinase, which is known to be recognized by Snf1 (43).

Mechanism of regulation. The above results show that negative control by the Snf1 kinase acts on a part of Mig1 that is distinct from the effector domain. This would argue against models in which phosphorylation of the latter directly blocks its interaction with the corepressor complex. Instead, it seems likely that negative control by Snf1 is achieved in some other way. One possible mechanism is if phosphorylation by Snf1 blocks nuclear entry of Mig1. This is how the Cdc28 kinase inhibits the activity of another yeast zinc finger protein, Swi5 (23). However, it is also possible that some other aspect of Mig1 function is inhibited by Snf1. In this context, it should be noted that the twofold repressing effect which is dependent on the DNA-binding domain alone is seen under both repressed and derepressed conditions (Fig. 1). This suggests that Mig1 may also be present in the nucleus under derepressed conditions, which would argue against nuclear entry or DNA binding being regulated by glucose. This notion is also supported by our finding that some Mig1-VP16 plasmids are able to activate transcription from Mig1 sites in the absence of glucose. However, further experiments are necessary to ascertain whether nuclear entry of Mig1 is regulated by Snf1 or by glucose.

Suppression of *snf1* by Mig1-VP16. The fact that Mig1-VP16 can suppress the inability of Snf1-deficient cells to grow on glycerol and galactose shows that constitutive expression of some genes that are normally repressed by Mig1 is sufficient to overcome certain aspects of the Snf1 defect. It does not rule out that other mechanisms downstream of Snf1 also act on these genes, but if so, then Mig1-VP16 is a strong enough activator to overcome these mechanisms. However, Snf1-deficient cells that contain Mig1-VP16 are still unable to grow on ethanol, lactate, or acetate. This shows that expression of some genes that are critical for gluconeogenic growth requires a Snf1-dependent mechanism which cannot be bypassed by Mig1-VP16. One possible candidate for such a mechanism is provided by the zinc cluster protein Cat8, which functions as an activator of the gluconeogenic genes (12). Transcription of *CAT8* is repressed by Mig1, but in order to activate transcription, Cat8 is also thought to require a posttranscriptional step which is dependent on the Snf1 (Cat1) kinase (12). Some targets of the Cat8 activator, such as *FBP1*, contain strong Mig1-binding sites (20) and are therefore likely to be activated by Mig1-VP16. However, any genes that require Cat8 for their expression and which are not directly regulated by Mig1 themselves would fail to be expressed in Snf1-deficient cells that carry the Mig1-VP16 plasmid. A failure to express such genes may account for the inability of these cells to grow on acetate, ethanol, and lactate.

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