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Genetics

Repression by SSN6–TUP1 is directed by MIG1, a repressor/activator protein

(transcription/yeast/zinc-finger protein/glucose repression)

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ABSTRACT **The SSN6–TUP1 protein complex represses transcription of diversely regulated genes in the yeast *Saccharomyces cerevisiae*. Here we present evidence that MIG1, a zinc-finger protein in the EGR1/Zif268 family, recruits SSN6–TUP1 to glucose-repressed promoters. DNA-bound LexA–MIG1 represses transcription of a target gene in glucose-grown cells, and repression requires SSN6 and TUP1. We also show that MIG1 and SSN6 fusion proteins interact in the two-hybrid system. Unexpectedly, we found that LexA–MIG1 activates transcription strongly in an *ssn6* mutant and weakly in a *tup1* mutant. Finally, LexA–MIG1 does not repress transcription in glucose-deprived cells, and MIG1 is differentially phosphorylated in response to glucose availability. We suggest a role for phosphorylation in regulating repression.**

Transcriptional repression is an important regulatory mechanism in eukaryotes. Repressors have been shown to inhibit transcription by interfering with various steps in the transcriptional process. Some repressors block the function of specific activators, whereas others interfere with the transcriptional machinery (for review, see ref. 1).

In the yeast *Saccharomyces cerevisiae*, the SSN6 (CYC8)–TUP1 protein complex represses transcription of genes regulated by glucose, cell type, oxygen, DNA damage, and other signals. Mutations in *SSN6* and *TUP1* relieve repression of these genes and also display diverse phenotypes such as clumpiness, temperature-sensitive growth, and defects in sporulation and plasmid maintenance (for reviews, see refs. 2 and 3). These shared phenotypes suggested that SSN6 and TUP1 function together, and biochemical studies showed that the two proteins are associated in a complex (4), herein called SSN6–TUP1. SSN6 and TUP1 contain essential tetratricopeptide (TPR) and β-transducin (WD40) repeats, respectively (5–7).

When bound to DNA, LexA–SSN6 represses transcription of a target gene in a TUP1-dependent manner (8). LexA–TUP1 similarly represses target gene expression but does not require SSN6 (9) and may, therefore, directly mediate repression by the complex. The mechanism of repression is not yet understood. SSN6–TUP1 represses transcription by RNA polymerases I and II, but not RNA polymerase III, suggesting that SSN6–TUP1 interacts with a component common to the RNA polymerase I and II transcription complexes (10). Studies of repression *in vitro* also point to interaction with the general transcriptional machinery (11). Other evidence suggests that repression by SSN6–TUP1 involves positioned nucleosomes that occlude promoter sequences (12, 13).

Repression by SSN6–TUP1 is directed to distinctly regulated genes, yet neither SSN6 nor TUP1 appears to bind DNA (5, 7). It has been proposed that specific DNA-binding proteins recruit the SSN6–TUP1 complex to different promoters (8). Evidence suggests that α2–MCM1 and **a**1–α2 target the SSN6–TUP1 complex to **a**- and haploid-specific genes, respectively (8). Other candidate DNA-binding proteins include MIG1 (14) and ROX1 (15), which may recruit SSN6–TUP1 to glucose-repressible and hypoxic genes, respectively. Here we test this model by examining the role of MIG1 in repression.

MIG1 is a Cys_2His_2 zinc-finger protein related to the mammalian EGR1/Zif268 and WT1 (Wilms tumor) proteins (14). MIG1 binds to the promoters of several glucose-repressible genes, including *SUC2*, *GAL4*, and *GAL1*, and mutation of MIG1 or its binding sites partially relieves glucose repression (14, 16–18). Genetic evidence suggests that MIG1 functions with SSN6–TUP1. *mig1*, *ssn6*, and *tup1* mutations all suppress defects caused by loss of SNF1, a protein kinase required for release from glucose repression, and *mig1* and *ssn6* show no synergistic effects on repression, suggesting that MIG1 acts in the same pathway as SSN6 (7, 19). For *SUC2*, however, *ssn6* and *tup1* relieve repression much more effectively than *mig1*, indicating that repression by SSN6–TUP1 also involves MIG1-independent mechanisms (19). Thus, genetic evidence supports, but does not prove, the model that MIG1 recruits SSN6–TUP1 to glucose-repressible promoters.

To test its function as a transcriptional repressor, we fused MIG1 to the LexA DNA-binding domain so that binding could be directed to target genes containing *lexA* operators. We show that LexA–MIG1 represses target gene transcription and that repression requires SSN6 and TUP1. These studies also revealed an unexpected transcriptional activation capability for MIG1 when SSN6 and TUP1 are absent. Finally, we provide evidence for differential phosphorylation of MIG1 in response to glucose availability.

MATERIALS AND METHODS

Strains and Genetic Methods. *S. cerevisiae* MCY829 (*MATα his3 lys2 ura3*), MCY1974 (*MATα ssn6-Δ9 ade2 his3 lys2 trp1 ura3*), MCY2437 (*MATα tup1-Δ1::TRP1 his3 lys2 ura3 trp1 LEU2::GAL1-lacZ? gal80?*), and MCY3507 (*MATα pep4::URA3 his3*) are derivatives of S288C. MCY3565 is MCY1974 with *tup1-Δ1::TRP1*. *ssn6-Δ9* and *tup1-Δ1::TRP1* are null alleles (5, 7). CTY10.5D (*MAT***a** *ura3::lexAop-lacZ ade2 trp1 leu2 his3 gal4 gal80*) was constructed by R. Sternglanz (State University of New York, Stony Brook). MCY3567 (*MAT***a** *tup1-Δ1::TRP1 ura3::lexAop-lacZ his3 leu2 ade2 gal4? gal80?*) is a segregant from the cross MCY2437 × CTY10.5D. Standard genetic methods were followed (20). *Escherichia coli* XL-1 Blue was used as host for plasmids.

β-Galactosidase Assays. Freshly transformed colonies were grown to logarithmic phase in SC medium (20) containing the indicated carbon source and lacking appropriate supplements to maintain selection for plasmids. β-Galactosidase activity was assayed (20) in permeabilized cells or in protein extracts (21).

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Abbreviations: UAS, upstream activation sequence; GAD, GAL4 activation domain.

Plasmids. pLexA-MIG1 was constructed by using two primers to direct synthesis of *MIG1* by the polymerase chain reaction (PCR) with pMIG1 (14) as template. The primer 5′-CGGGATCCCCATGCAAAGCCCATATCC incorporated a *Bam*HI site 5′ to the initiating ATG codon, and the primer 5′-GGGTCGACGTCCATGTGTGGGAAGGG was complementary to nt 1495–1512 with an incorporated *Sal* I site. The amplified DNA was digested with *Bam*HI and *Sal* I and cloned into pSH2-1 (22). LexA–MIG1 contains LexA-(1–87) fused via six additional residues to the entire MIG1 coding sequence, followed by six residues. The construct uses the *ADH1* promoter and transcriptional termination sequence. LexA–MIG1 provides MIG1 function, as judged by its ability to confer 2-deoxyglucose sensitivity for growth on sucrose to *mig1* mutants.

pGAD-SSN6 was constructed by using primers to direct synthesis of *SSN6* sequence by PCR with cloned DNA (23) as template. The primer 5′-CGGGATCCAAATGAATCCGGGCGGT incorporated a *Bam*HI site 5′ to the initiating ATG codon, and the primer 5′-GGGTCGACTTCTCTTATGTGAAC was complementary to nt 1195–1209 with an incorporated *Sal* I site. Amplified DNA was digested with *Bam*HI and *Sal* I and cloned into pGADNOT (24). GAD–SSN6 contains the GAL4 activation domain residues 768–881 and the N-terminal 403 residues of SSN6.

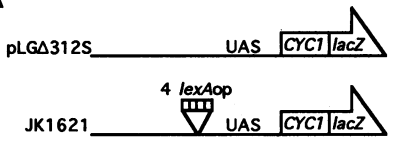
Preparation of Cell Lysates and Phosphatase Treatment. Whole cell lysates were prepared as described (21), except that for samples used in Fig. 3*B*, the breaking buffer was 20 mM Tris·HCl, pH 7.5/1 mM EDTA/400 mM NaCl/5% (vol/vol) glycerol/0.5 mM dithiothreitol/chymostatin (2 μg/ml)/2 μM pepstatin A/0.6 μM leupeptin/2 mM benzamidine/1 mM phenylmethylsulfonyl fluoride. Phosphatase treatment was performed as described (25) except extracts were treated with 10 or 20 units of calf intestinal phosphatase (Boehringer Mannheim, 20 units/μl). Some samples were treated with a phosphatase inhibitor mixture (5 mM sodium fluoride/5 mM sodium phosphate/10 mM sodium pyrophosphate/5 mM EDTA/5 mM EGTA).

Immunoblot Analysis. Proteins were separated by SDS/PAGE and electroblotted to nitrocellulose. Blots were incubated with polyclonal LexA antibody (gift of J. Kamens and R. Brent, Massachusetts General Hospital, Boston) at a dilution of 1:2000. The primary antibody was detected with goat anti-rabbit IgG coupled to alkaline phosphatase and the Protoblot reagents (Promega) or donkey anti-rabbit IgG conjugated to horseradish peroxidase and the ECL reagents (Amersham).

RESULTS

DNA-Bound LexA–MIG1 Represses Transcription. To test MIG1 for function as a transcriptional repressor, we expressed a LexA–MIG1 fusion protein containing the LexA DNA-binding domain (residues 1–87) from plasmid pLexA-MIG1. Wild-type cells were cotransformed with pLexA-MIG1 and *CYC1–lacZ* target genes containing zero or four *lexA* operators located 5′ to the *CYC1* upstream activation sequence (UAS) (8, 26) (Fig. 1*A*). Transformants were assayed for β-galactosidase activity after growth in glucose. Expression of the reporter gene containing LexA binding sites was 20-fold lower than that of the reporter with no binding sites (Fig. 1*B*), indicating that DNA-bound LexA–MIG1 represses transcription. Repression requires binding to the *lexA* operators, thereby excluding indirect mechanisms of action. LexA–SSN6 represses transcription 34-fold in this assay (8).

Repression by LexA–MIG1 Requires SSN6 and TUP1. To examine whether repression by LexA–MIG1 depends on SSN6 and TUP1, we assayed repression in *ssn6* and *tup1* mutants after growth in glucose. DNA-bound LexA–MIG1 did not significantly repress transcription of the target gene, relative to



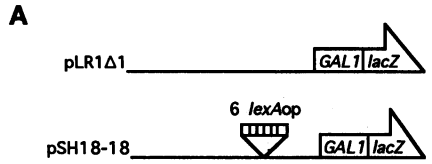
B

Relevant genotype	Expressed protein	Growth condition	No. *lexAop* 0	No. *lexAop* 4	Fold repression
WT	LexA-MIG1	High Glu	450	22	20
	LexA1-87		330	180	1.8
ssn6Δ	LexA-MIG1	High Glu	59	220	0.27
	LexA1-87		88	44	2.0
	none		60	30	2.0
tup1Δ	LexA-MIG1	High Glu	85	35	2.4
	LexA1-87		88	25	3.5
	none		56	26	2.2
WT	LexA-MIG1	Low Glu	900	290	3.1
	LexA1-87		1100	480	2.3
WT	LexA-MIG1	Gal	1880	1020	1.8
	LexA1-87		2150	810	2.6

FIG. 1. Transcriptional repression by LexA–MIG1. (*A*) Target plasmids. pLGΔ312S contains the *lacZ* gene under control of the *CYC1* promoter and UAS (27). JK1621 is derived from pLGΔ312S and contains four *lexA* operators (op) 5′ to the UAS (8). (*B*) Repression of target gene expression by the indicated LexA protein in wild-type (WT) and mutant strains. Strains were MCY829, MCY1974, and MCY2437. Expression plasmids were pLexA–MIG1 or pSH2-1. Transformants were grown selectively to midlogarithmic phase in 2% glucose (High Glu) and shifted to 0.05% glucose (Low Glu) for 3 h or grown in 2% galactose (Gal). β-Galactosidase activity was assayed in permeabilized cells and expressed in Miller units. Values represent averages for 3–12 transformants. Standard errors were <12%.

controls, in either *ssn6* or *tup1* mutant hosts (Fig. 1*B*). Surprisingly, in an *ssn6* mutant LexA–MIG1 appeared instead to activate transcription: β-galactosidase activity was nearly 4-fold higher when the target gene contained *lexA* operators (0.27-fold repression). These data indicate that repression by LexA–MIG1 requires SSN6 and TUP1 (see Fig. 4*A*) and further suggest that LexA–MIG1 functions as a transcriptional activator in the absence of SSN6.

LexA–MIG1 Strongly Activates Transcription in an *ssn6* Mutant. We next tested the ability of LexA–MIG1 to stimulate transcription of a *GAL1–lacZ* target gene, in which UAS_G was replaced by *lexA* operators (Fig. 2*A*). In glucose-grown *ssn6* mutant cells, LexA–MIG1 strongly activated target gene expression, increasing β-galactosidase activity >1000-fold relative to controls (Fig. 2*B*). Activation depended on the binding of LexA–MIG1 to the promoter as no expression was detected from a target gene lacking *lexA* operators. LexA-(1–87) did not activate transcription in an *ssn6* mutant nor did other control LexA fusions (data not shown). LexA–MIG1 also did not activate transcription in the wild type, consistent with its role as a repressor in the presence of SSN6. These data indicate that LexA–MIG1 is a potent activator when the SSN6 protein is absent, suggesting that SSN6 masks an activation domain of MIG1 or blocks the interaction of MIG1 with an activator protein (see Fig. 4*B*).



B

Relevant genotype	Expressed protein	No. lexAop 0	6
ssn6Δ	LexA-MIG1	1	1330
	$LexA_{1-87}$	1	<1
tup1Δ	LexA-MIG1	2	61
	$LexA_{1-87}$	<1	2
ssn6Δ tup1Δ	LexA-MIG1	2	1190
	$LexA_{1-87}$	1	<1
WT	LexA-MIG1	1	<1
	$LexA_{1-87}$	<1	1

FIG. 2. LexA–MIG1 activates transcription in *ssn6* and *tup1* mutants. (*A*) Target plasmids. pLR1Δ1 contains the *lacZ* gene under control of the *GAL1* promoter with UAS_G deleted (28). pSH18-18 is derived from pLR1Δ1 and contains six *lexA* operators 5′ to the promoter, replacing UAS_G (S. D. Hanes and R. Brent, personal communication). (*B*) Activation of target gene expression by the indicated LexA protein in wild type (WT) and mutants. Strains and expression plasmids were as in Fig. 1. Cultures were grown in 2% glucose. β-Galactosidase activity was assayed in permeabilized cells and expressed in Miller units. Values are averages for 4–14 transformants. Standard errors were <15%.

LexA–MIG1 Weakly Activates Transcription in a *tup1* Mutant. Mutation of *TUP1* converted LexA–MIG1 to a weak activator. In a *tup1* mutant, LexA–MIG1 significantly activated transcription but did so 20-fold less effectively than in an *ssn6* mutant (Fig. 2*B*). It is possible that SSN6 is slightly less stable in the absence of TUP1 (4), so that some LexA–MIG1 molecules are no longer associated with SSN6. These findings indicate that SSN6 and TUP1 have distinct relationships to MIG1 and that TUP1 is not required for the association of SSN6 with MIG1.

MIG1 and SSN6 Interact in the Two-Hybrid System. To confirm the interaction of MIG1 and SSN6, we used the two-hybrid system (29). GAD–SSN6 contains the GAL4 activation domain (GAD) fused to the N-terminal tetratricopeptide repeat domain of SSN6 (residues 1–403), which is sufficient for SSN6 function (5). LexA–MIG1 recruited GAD–SSN6 to the promoter of a *GAL1–lacZ* reporter with *lexA* operators replacing UAS_G (Table 1). LexA–MIG1 and GAD–SSN6 together stimulated β-galactosidase expression >10-fold relative to controls. Interaction of these hybrid proteins was also detected in a *tup1* mutant, although the background was higher due to activation by LexA–MIG1.

LexA–MIG1 Does Not Repress Transcription in Glucose-Deprived Cells. SSN6 and TUP1 repress diversely regulated genes, whereas MIG1 functions specifically in glucose repression. Thus, MIG1 seemed a likely target for regulatory signals regarding glucose availability. Consistent with this idea, we detected no repression by LexA–MIG1 in cells shifted to low glucose (0.05%) or grown in galactose (Fig. 1*B*). It is possible that LexA–MIG1 did not bind to the operators. In galactose-grown cells, LexA–MIG1 did not interfere with transcription of a target gene with an operator located 3′ to the UAS (CK30; ref. 8), whereas LexA-(1–87) did (data not shown), suggesting that LexA–MIG1 either did not bind or contributed to activation.

Table 1. Interaction of MIG1 and SSN6 in the two-hybrid system

DNA-binding hybrid	Activation hybrid	β-Galactosidase activity WT	*tup1*
LexA–MIG1	GAD	0.7	13
LexA–MIG1	GAD–SSN6	11	57
LexA-(1–87)	GAD–SSN6	1	4
None	None	0.6	ND

Strains were CTY10.5D (WT) or MCY3567 (*tup1Δ*). Expression plasmids were pLexA-MIG1, pSH2-1, pGAD2F (30), and pGAD-SSN6. Cultures were grown in 2% glucose. β-Galactosidase activity was assayed in protein extracts (21) and expressed as units per mg of protein (31). Values are averages for 4–14 transformants. Standard errors were <7%. ND, not determined.

LexA–MIG1 Is Differentially Phosphorylated in Response to Glucose Availability. We next examined the LexA–MIG1 protein for differential modification in glucose-repressed and derepressed cells. Immunoblot analysis showed that LexA–MIG1 from glucose-repressed cells migrates as several distinct species, whereas the protein from derepressed cells migrates heterogeneously and with slower mobility (Fig. 3*A*). The same pattern was observed in glucose- and galactose-grown *ssn6* mutants (data not shown). The modification appears specific to the MIG1 moiety because another LexA fusion migrated the same in all samples (data not shown).

To determine whether these migration patterns reflect differential phosphorylation of LexA–MIG1, we treated extracts with calf intestinal phosphatase (Fig. 3*B*). This treatment converted the slow-migrating forms to a major form migrating close to 66 kDa, the predicted size of the fusion protein. This conversion did not occur in mock-treated extracts and was blocked by phosphatase inhibitors. Thus, LexA–MIG1 is phosphorylated to different extents in glucose-repressed and derepressed cells. We cannot exclude, however, that the state of the protein also differs with respect to other modifications.

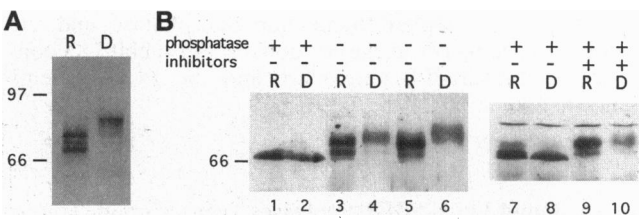


FIG. 3. Immunoblot analysis of LexA–MIG1 from glucose-repressed and derepressed cells. Glucose-repressed (R) cultures of wild-type strains carrying pLexA-MIG1 were grown to midlogarithmic phase in selective SC medium containing 2% glucose. Derepressed (D) cells were prepared by shifting to medium containing 0.05% glucose for 3 h (*A*) or 1 h (*B*). Altered mobility of LexA–MIG1 was apparent 0.5 h after shift to low glucose. (*A*) Proteins (25 μg) were separated by SDS/PAGE on a 7.5% gel and subjected to immunoblot analysis using LexA antibody and colorimetric detection. The strain was MCY829. Molecular size markers are indicated. (*B*) Protein extracts (5 μg) were treated with 10 units of calf intestinal phosphatase (lanes 1 and 2), mock-treated (lanes 3 and 4), or mock-treated without incubation at 37°C (lanes 5 and 6). In control experiments to rule out contamination of the phosphatase with proteases, samples were treated with phosphatase (20 units) in the absence (lanes 7 and 8) or presence (lanes 9 and 10) of phosphatase inhibitors. Proteins were separated by SDS/PAGE on 6% (lanes 1–6) or 7.5% (lanes 7–10) gels. Immunoblot analysis was carried out by using anti-LexA and the chemiluminescence detection method. The strain was MCY3507. LexA–MIG1 migrated at the position predicted for the unmodified protein (66 kDa) in the lanes containing phosphatase-treated samples.

These results strongly suggest that MIG1 is differentially phosphorylated in response to glucose availability.

DISCUSSION

Here we present evidence that the MIG1 zinc-finger protein recruits the SSN6–TUP1 repressor complex to specific promoters. We show that LexA–MIG1 represses transcription of a target gene when bound 5′ to the UAS and that repression requires both SSN6 and TUP1 (Fig. 4*A*). LexA–SSN6 also represses in this assay and requires TUP1 (8). In addition, we detected interaction between MIG1 and SSN6 in the two-hybrid system. Thus, our findings support the model that MIG1 recruits SSN6–TUP1 to promoters containing MIG1 binding sites. This model does not exclude the possibility that other proteins assist MIG1 in tethering SSN6–TUP1.

Unexpectedly, we found that LexA–MIG1 is a strong activator, rather than a repressor, in an *ssn6* mutant. This result suggests that SSN6 not only functions with MIG1 in repression but also masks an activation domain of MIG1 or blocks interaction with an activator protein (Fig. 4*B*). Mutation of *TUP1* alone converted LexA–MIG1 to a weak activator (Fig. 4*C*). Perhaps TUP1 also masks an activation domain of MIG1; alternatively, in the absence of TUP1, SSN6 may be less stable so that some LexA–MIG1 molecules are no longer associated with SSN6. This evidence that SSN6 and TUP1 have distinct relationships to MIG1 strengthens the argument that interaction with MIG1 is both physical and functional. Furthermore, TUP1 cannot be required for the association of SSN6 with MIG1, as was also indicated by two-hybrid experiments. Our results are compatible with the proposal (9) that SSN6 mediates the association of TUP1 with MIG1 but do not exclude direct interaction between TUP1 and MIG1.

In the wild type, it is possible that MIG1 contributes to transcriptional activation of genes controlled by glucose repression. Although *mig1* mutants show no defects in activation of the *SUC2* or *GAL* genes (14, 17, 19), such a role for MIG1 is not excluded because other proteins may function redundantly. Indeed two activators with similar zinc fingers are known to affect *SUC2* expression (32). One possible model is that the interaction of SSN6–TUP1 with MIG1 is disrupted or altered in the absence of glucose, thereby converting MIG1 from a repressor to an activator. In preliminary studies, we found that LexA–MIG1 did not activate transcription in galactose-grown wild type (unpublished results); however, the reporter lacked a UAS, whereas the MIG1 binding sites at natural promoters may be adjacent to sites for activators that modulate the association of MIG1 with SSN6 in response to glucose.

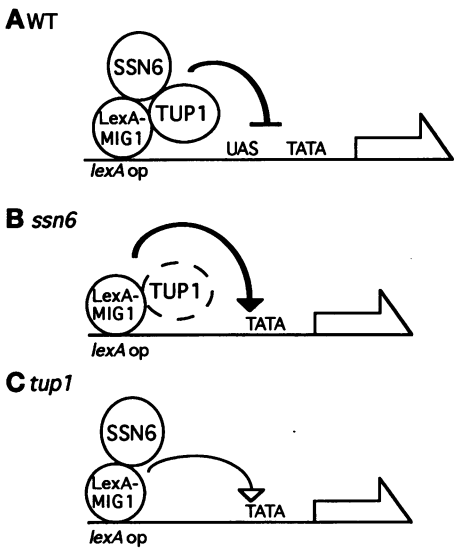


FIG. 4. Models for repression and activation by LexA–MIG1. (*A*) In wild type (WT), LexA–MIG1 recruits SSN6–TUP1 to repress transcription of a promoter containing a UAS. Other proteins may assist in the association of SSN6–TUP1 with MIG1, and it is not clear whether MIG1 directly contacts both SSN6 and TUP1. (*B*) In an *ssn6* mutant, LexA–MIG1 strongly activates transcription of a promoter with no UAS. The association of TUP1 with MIG1 is uncertain, as indicated by the dashed line; however, TUP1 is not required because activation was observed in an *ssn6 tup1* strain. (*C*) In a *tup1* mutant, LexA–MIG1 is associated with SSN6 and only weakly activates transcription. It is not excluded that activation results from partial instability of SSN6.

Interestingly, the related zinc-finger protein WT1, which recognizes a similar G+C-rich sequence, also can function as a transcriptional repressor or activator (33, 34). WT1 and p53 are physically associated and appear to work together in repression; in the absence of p53, WT1 activates transcription (34). Another protein with similar zinc fingers, EGR1/Zif268, also contains both repression and activation domains (35). Perhaps this class of zinc-finger proteins functions in either repression or activation, depending on the chromosomal and cellular context.

What is the role of MIG1 in regulating repression in response to glucose availability? It seems likely that MIG1 and other DNA-binding proteins serve to regulate repression of specific genes by SSN6–TUP1. For example, the regulated synthesis of **a**1, α2, and ROX1 could control repression of cell-type-specific and hypoxic genes, respectively (8, 15). For MIG1, its differential modification in response to glucose availability suggests a possible mechanism for regulation of its repressor function. Phosphorylation of the native MIG1 could potentially affect its binding to DNA, its association with SSN6–TUP1, or the ability of the complex to repress transcription. Our observation that LexA–MIG1 does not repress in galactose-grown cells is compatible with any of these mechanisms. Genetic evidence that *mig1* is a suppressor of *snf1* (19) suggests a role for the SNF1 protein kinase in phosphorylation of MIG1.

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