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Two Zinc-Finger-Containing Repressors Are Responsible for Glucose Repression of *SUC2* Expression

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Expression of the *SUC2* gene in *Saccharomyces cerevisiae*, which encodes invertase, is repressed about 200-fold by high levels of glucose. Mig1p is a Cys_2His_2 zinc-finger-containing protein required for glucose repression of *SUC2* and several other genes. However, *SUC2* expression is still about 13-fold repressed by glucose in a *mig1* mutant. We have identified a second repressor, Mig2p, containing zinc fingers very similar to those of Mig1p that is responsible for this remaining glucose repression of *SUC2* expression. Overexpression of *MIG2* represses *SUC2* under nonrepressing conditions, and a LexA-Mig2p fusion represses transcription of a *lexO*-containing promoter in a glucose-dependent manner, supporting the idea that Mig2p is a glucose-activated repressor. We have shown that Mig2p binds to the Mig1p-binding sites in the *SUC2* promoter. Even though Mig1p and Mig2p bind to similar sites and share almost identical zinc fingers, they differ in their relative affinities for various Mig1p-binding sites. This could explain our observation that *MIG2* appears to have little role in glucose repression of other promoters with *MIG1*-binding sites.

Transcription of many genes in *Saccharomyces cerevisiae* is repressed during growth on glucose (for reviews, see references 15, 28, and 34). Glucose-repressed genes are of essentially three types: genes for utilization of carbon sources that are alternatives to glucose (e.g., *GAL* and *SUC*), genes encoding proteins of gluconeogenesis (e.g., *FBP1* and *PCK1*), and genes required for oxidation of glucose (e.g., *CYC1* and *COX6*).

Mig1p is a DNA-binding repressor responsible for glucose repression of several of these genes (23). In the absence of glucose, Mig1p function is inhibited, directly or indirectly, by the Snf1p protein kinase, leading to derepression of gene expression (3, 4). Mig1p is thought to mediate repression by recruiting Ssn6p and Tup1p, which are general repressors that act through several diverse DNA-binding proteins in the cell (16, 33). In the absence of Ssn6p and Tup1p, Mig1p is apparently an activator of transcription (33).

Mig1p contains two Cys_2His_2 zinc fingers related to the mammalian Krox20/Egr and Wilms' tumor proteins and, like these proteins, binds to a GC-rich motif. Unique to the Mig1p-binding site is an AT-rich region preceding the GC-rich sequence that is essential for binding (17). Binding sites for Mig1p have been identified in the promoters of several glucose-repressed genes, including *GAL1*, *GAL4*, and *SUC2* (11, 22, 23).

Mig1p binds to the *GAL1* and *GAL4* promoters and is the primary repressor responsible for glucose repression of the *GAL* genes: disruption of *MIG1* relieves nearly all glucose repression of *GAL1* and *GAL4* expression (9, 11). By contrast, while Mig1p binds to the *SUC2* promoter, disruption of *MIG1* only partially relieves glucose repression of *SUC2* expression (35). Furthermore, disruption of *MIG1* has little or no effect on glucose repression of other genes whose promoters contain Mig1p-binding sites (20, 26, 28). These observations suggest that there may be other repressors with roles similar to that of Mig1p. Here we describe such a protein, Mig2p, that contains two zinc fingers highly similar to those of Mig1p. Mig2p acts together with Mig1p to repress *SUC2* expression in response to glucose.

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MATERIALS AND METHODS

Yeast strains, media, and transformations. All of the strains used in this study were derived from S288C (Table 1). Strain constructions followed standard methods for genetic crosses, sporulation, and tetrad dissection (29). Yeast cells were grown at 30°C on standard medium: YEP (rich) medium or synthetic (minimal) medium lacking the appropriate amino acids (29). Yeast transformations were done as described by Schiestl et al. (31, 32).

Gene disruptions were made by a PCR technique described previously (1, 24). Briefly, a *HIS3*-containing PCR product that included at each end 45 bp upstream and downstream of the region to be disrupted was transformed into *S. cerevisiae* by replacing the target region with *HIS3*. The initial *MIG2* disruption, *mig2*Δ*-1*, was made in diploid strain YM4551 with oligonucleotides OM532 (5′AGCGACTCGTGCTCCTCCTCATTACTACCACTGCTGCTGTTATTG GGCCTCCTCTAGTACACTC 3′) and OM533 (5′CATCTTCCAAATGGTAA GCAAACATCAAGTAGACTTAAGCGGGTCGCGCGCCTCGTTCAG AATG 3′). This disruption was made before the DNA sequence was completely determined and was later found to disrupt all of the *MIG2* coding region except the 3′ 100 bp and a small portion of an upstream open reading frame. A precise disruption of only the *MIG2* coding region, *mig2*Δ*-2*, constructed in YM3733 by using primers OM951 (5′ ATGCCTAAAAAGCAAACGAATTTCCCAGTAG ATAACGAAAACAGAGGCCTCCTCTAGTACACTC 3′) and OM952 (5′ TTAAACTCTTTTGGGACCGTTGAAAACATCAATTTGTTTCAGTAGCG CGCCTCGTTCAGAATG 3′), behaved identically to *mig2*Δ*-1*. A PCR with a primer flanking *MIG2*, OM636 (5′ GATAAAAAGGGGCCGTAAAGG 3′), and a primer in *HIS3*, OM483 (5′ GCCTCATCCAAAGGCGC 3′), was used to confirm correct gene disruption. Disruption of *YER028* was constructed in YM4551 as described above, with oligonucleotides OM890 (5′ TTTGCTCTTA GTAGGTGCACATCGGCGATCCTTTTCTTAAATTCCGGCCTCCTCTAG TACACTC 3′) and OM891 (5′ CCCATTGTTATTTTCCTTCTTATATTCTA TACGTTTTATTTACTGGCGCGCCTCGTTCAGAATG 3′). The *mig1*::*URA3* disruption in these strains has already been described (23).

Isolation of *MIG2*. *MIG2* was isolated in a "one-hybrid" screen for genes that bind to the *GAL4* upstream activating sequence (UAS). Briefly, we fused the *GAL4* promoter to *HIS3* and replaced the *GAL4* basal promoter element (UES) with a TATA box. The resulting promoter is inactive because the *GAL4* UAS does not activate transcription through a TATA box (11). We then transformed a strain containing this construct (YM4212) to His^+ with a plasmid library of yeast DNA fragments fused to the Gal4p transcriptional activation domain (a "two-hybrid" library obtained from Stan Fields [8]). Since the Gal4p transcriptional activation domain activates transcription through a TATA box, this selects for DNA-binding domains that recognize the *GAL4* UAS. However, two observations suggest that Mig2p does not act at *GAL4*: *MIG2* was not fused to the

TABLE 1. Yeast strains used in this study

Strain	Genotype[a]
YM2061	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 met⁻ LEU2*::*GAL1-lacZ*
YM2169	*MAT***a** *met⁻*
YM3733[b]	*MAT***a** *trp1-901 met⁻ can1*^{R} *mig1Δ*::*URA3*
YM4212[c]	*MAT***a** *leu2-3,2-112 trp1-901 can1*^{R} *gal4*::*GAL4*(UES→TATA)-*HIS3-URA3*(pBM2406)
YM4359[c,d]	*MAT***a** *leu2-3,2-112 trp1-901 can1*^{R}
YM4551[b,d]	*MAT***a**/α *trp1-901*/*trp1-901 met⁻*/*MET tyr1-501*/*TYR1 can1*^{R}/*CAN*^{S}
YM4662[d]	*MAT*α *trp1-901 met*? *can1*? *tyr1-501 mig2Δ-1*::*HIS3*
YM4663[d]	*MAT***a** *trp1-901 met*? *can1*? *tyr1-501 mig2Δ-1*::*HIS3*
YM4721[b]	*MAT***a** *trp1-901 met*? *can1*? *mig1Δ*::*URA3 mig2Δ-1*::*HIS3*
YM4727	*MAT*α *trp1-901 met*? *can1*? *mig2Δ-1*::*HIS3 GAL4*
YM4734[b]	*MAT*α *trp1-901 met*? *can1*? *tyr*? *yer028Δ*::*HIS3*
YM4738[b]	*MAT***a** *trp1-901 met*? *can1*? *mig1Δ*::*ura3*::*LYS2 mig2Δ-1*::*HIS3*
YM4740[d]	*MAT*α *trp1-901 met*? *can1*? *mig1Δ*::*URA3 yer028Δ*::*HIS3*
YM4743[b]	*MAT***a** *trp1-901 met*? *can1*? *tyr1-501 mig2Δ1*::*HIS3 yer028Δ*::*HIS3*
YM4744[d]	*MAT*α *trp1-901 met*? *can1*? *tyr*? *yer028Δ*::*HIS3*
YM4797[b]	*MAT***a** *trp1-901 tyr1-501 met*? *can1*?
YM4801[b]	*MAT***a** *trp1-901 met⁻ can1*^{R} *mig1Δ*::*URA3 mig2Δ-2*::*HIS3*
YM4804[b]	*MAT*α *trp1-901 met*? *can1*? *mig1Δ*::*URA3 mig2Δ-1*::*HIS3 yer028Δ*::*HIS3*
YM4807[b]	*MAT***a** *trp1-901 met⁻ can1*^{R} *mig1Δ*::*ura3*::*LYS2*
YM4808[d]	*MAT***a** *trp1-901 met⁻ can1*^{R} *mig1Δ*::*ura3*::*LYS2 GAL4*::*URA3*
YM4809	*MAT***a** *trp1-901 met*? *can1*? *mig1Δ*::*ura3*::*LYS2 mig2Δ-1*::*HIS3 GAL4*::*URA3*
YM4853[c,d]	*MAT*? *leu2-3,2-112 trp1-901 met*? *can1*? *mig1Δ*::*URA3*
MCY829[e]	*MAT*α *his3Δ200 lys2-801 ura3-52*
MCY1974[e]	*MAT*α *ura3-52 his3Δ200 ade2-101 lys2-801 trp1Δ ssn6Δ9*
MCY2437[e]	*MAT*α *his3Δ200 lys2-801 ura3-52 trp1Δ tup1Δ*::*TRP1*

[a] The *met* and *can* markers are segregating in these strains, but in many cases their phenotypes were not scored (indicated by ?). All strains except YM2061 contain *ura3-52 his3Δ200 ade2-101 lys2-801 gal80Δ538 LEU2*::*GAL1-lacZ*.
[b] Also contains *gal4*::*GAL4-CAT-TRP1*.
[c] Does not contain *LEU2*::*GAL1-lacZ*.
[d] Also contains *gal4*::*GAL4-CAT-URA3*.
[e] Strain provided by M. Carlson (33).

Gal4p activation domain in the clone we obtained, and *mig2* mutations do not affect *GAL4* expression or regulation.

Enzyme assays. β-Galactosidase assays were carried out in permeabilized cells grown to mid-log phase as described by Yocum et al. (37). For invertase assays, glucose-repressed and derepressed cells were prepared from exponentially growing cultures. Repressed cultures were grown overnight in media containing 4% glucose. For derepression, cells were shifted to media containing 5% glycerol and 0.05% glucose for 2.5 h (YEP media) or 3 h (synthetic media). Secreted invertase was assayed in whole cells as described by Goldstein and Lampen (10) and Celenza and Carlson (3), with the following variation: for the color reaction, ABTS [2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] was substituted for *o*-dianisidine (0.53 mg per reaction mixture). Reaction mixtures were incubated at room temperature for 30 to 60 min to allow the color to develop. Acid was not added at this step. Tubes were spun for 2 to 3 min, and the A_{420} was measured.

Sequencing. *MIG2* was cloned in pBM2613 from a two-hybrid library of genomic fragments (8). A 2-kb insert was used for hybridization to λ filters (27a), mapping it to lambda 1207 on the left arm of chromosome VII. The gene was further mapped to a 5-kb *Hin*dIII fragment on this lambda, which was subcloned into pBluescript to yield pBM2663. The sequence was then determined by shotgun sequencing, as previously described (13). Briefly, DNA from pBM2663 was sheared and approximately 1-kb fragments were subcloned into M13mp18. Approximately 200 random clones were sequenced on an ABI 373A automated sequencer with a dye-labeled M13 universal primer. The sequence was assembled and managed with Staden's XBAP program (5). Both strands of about 4.6 kb (including *MIG2*) were determined, with an average depth of sequence coverage of 4.

Plasmids. Standard procedures for the manipulation of plasmid DNA and transformation into bacteria were followed (18). *Escherichia coli* DH5α was used as the host for all plasmids. $LexA_{1-87}$-Mig2p-encoding plasmids pBM3090 and pBM3091 were made as follows. The *MIG2* coding region (starting at the ATG) was amplified from genomic DNA by a PCR with oligonucleotides OM862 (5′ CGCGGATCCTTATGCCTAAAAAGCAAACGAA 3′) and OM863 (5′ CGC GGATCCTTAAACTCTTTTGGGACCGT 3′) as primers. Several independent PCR products were combined, digested with *Bam*HI, and cloned into the *Bam*HI site of pSH2-1 (vector containing the *lexA* DNA-binding domain, amino acids 1 to 87; see reference 14). $LexA_{1-87}$-Mig1p (pBM3216; reference 5a) included Mig1p amino acids 96 to 504 (amplified by the PCR with oligonucleotides OM375 [5′ GCGCCATGGAAAGCCCATATCCAATGACAC 3′] and OM1085 [5′ GCCGCTCGAGTCAGTCCATGTGTGGGAA 3′] with pBM2220 as the template [23]). Several independent PCR products were combined, digested with *Xho*I, and cloned into the *Sal*I site of pSH2-1.

The *HXT2-lacZ* reporter (see Table 2) was obtained from S. Özcan (26); the *FBP1-lacZ* and *PCK1-lacZ* reporters were provided by J. M. Gancedo (20). The *CYC1-lacZ* reporters (see Fig. 3) were pLGΔ312s (12), which has *lacZ* under the control of the wild-type *CYC1* promoter, and JK1621 (16), which is identical to pLGΔ312s, except with four *lex* operators inserted 5′ to the UAS. The GAL1-lacZ reporters (see Fig. 4) were pLR1Δ1, which contains the *lacZ* gene under the control of the *GAL1* promoter with the UAS deleted (36), and pSH18-8, which is derived from pLR1Δ1 but has four *lex* operators replacing the UAS (2a).

Overexpression of *MIG2*. *MIG2* was overexpressed by using high-copy FAT plasmid pBM2666, which contains the defective *leu2-d* gene (7) that allows selection for a very high copy number. *MIG2* was amplified by a PCR with oligonucleotides OM1087 (5′ GAAGATCTCCTGCGGAGCTTGCGTCA 3′) and OM1088 (5′ GAAGATCTGGGCTGGATGACTTGTCG 3′) as primers. Several PCR products were combined, digested with *Bgl*II, and subcloned into the *Bam*HI site of pBM2666 to generate pBM3214 and pBM3215, which behave identically. Cells were first grown overnight in synthetic (complete) media lack-

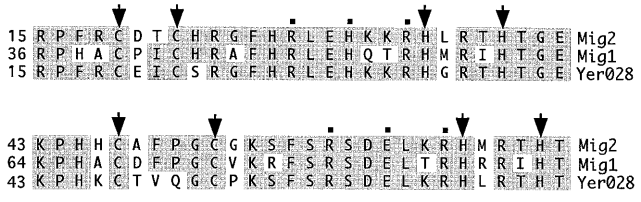

FIG. 1. Zinc fingers in Mig1p-related proteins. Alignment of the zinc finger motifs of Mig2p, Mig1p, and Yer028p. Shaded residues are identical between Mig2p and the other two proteins. The first zinc finger relative to the amino terminus of each protein is shown on top. The arrows point to the conserved Cys and His residues that make up the zinc finger motifs. The RHR and RER residues that are believed to make base-specific DNA contacts are marked by black squares (27).

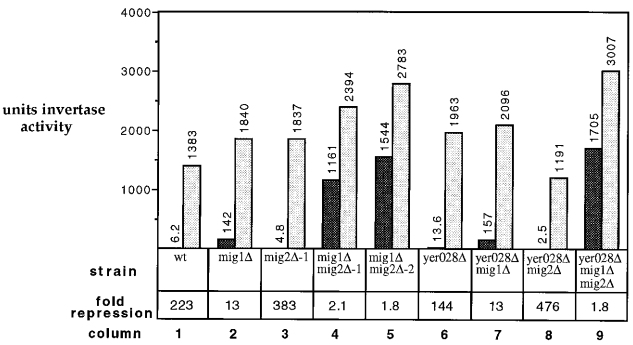

FIG. 2. Effects of *mig1*Δ, *mig2*Δ, and *yer028*Δ on *SUC2* expression. Assays were done in YEP medium under conditions of repression (4% glucose; dark bars) and derepression (5% glycerol and 0.05% glucose; light bars). The data are from 2 to 16 assays of at least two different strains. Standard errors were usually less than 26%. Strains: wild type (wt), YM4359; *mig2*Δ*-1*, YM4662; *mig1*Δ, YM3733; *mig1*Δ *mig2*Δ*-1*, YM4721; *mig1*Δ *mig2*Δ*-2*, YM4801; *yer028*Δ, YM4734; *mig1*Δ *yer028*Δ, YM4740; *mig2*Δ*-1 yer028*Δ, YM4743; *mig1*Δ *mig2*Δ*-1 yer028*Δ, YM4804.

ing tryptophan and then overnight in synthetic media lacking leucine to select for a high copy number.

Protein preparation. The entire Mig2 protein was fused to the bacterial MalE protein by amplifying *MIG2* in a PCR with oligonucleotides OM930 (5′ CGCG GATCCATGCCTAAAAAGCAAACG 3′) and OM863 (5′ CGCGGATCCTT AAACTCTTTTGGGACCGT 3′) as primers, combining several independent reactions, digesting them with *Bam*HI, and inserting the fragment into the *Bam*HI site of p*MAL* (New England Biolabs), generating pBM3178 and pBM3179. Cells were grown, and protein was purified on a maltose affinity column in accordance with the manufacturer's protocol. The Mig2 protein that was obtained had a molecular mass of approximately 55 kDa, suggesting that it is partially proteolyzed. Since the MalE protein was fused to the N terminus of Mig2p and since the purified protein binds to DNA (see Results), it is likely that the truncated protein includes the zinc finger DNA-binding domain. We estimate that it also includes about 50 amino acids adjacent to the DNA-binding domain.

The Mig1 protein was fused to bacterial Gst by amplifying the *MIG1* coding region with a PCR using oligonucleotides OM376 (5′ GCGAAGCTTGGATC CAGCGTATCAGTCCATGTGTGG 3′) and OM380 (5′ CGGGGATCCAAA GCCCATATCCAATGAC 3′). Several independent reaction products were combined, digested with *Bam*HI, and inserted into the *Bam*HI site of pGEX3X (Pharmacia) to generate pBM2421. pBM2421 was then digested with *Sty*I, and the vector was religated to generate pBM2445. This resulted in a 654-bp deletion of *MIG1* that removed residues 243 to 462, which are well outside of the DNA-binding domain, which ends at amino acid 90. This deletion was necessary to produce a stable fusion protein. The resultant protein, which was purified with a glutathione affinity column, had approximately the expected molecular weight. Cells were grown, and protein was prepared in accordance with the manufacturer's protocol (Pharmacia).

Gel shifts. Labeled PCR probes were made by combining several PCR products for each probe, digesting them with the appropriate enzyme, and labeling them with [^{32}P]dATP by filling in with the Klenow fragment of DNA polymerase (18). Labeled DNA was purified on a nondenaturing 10% polyacrylamide gel. Oligonucleotide probes were annealed, labeled with [^{32}P]dATP by filling in with the Klenow fragment of DNA polymerase, and purified on a NucTrap push column (Stratagene). The oligonucleotides used were SUC2-A (OM1041 and OM1042), SUC2-B (OM1043 and OM1044) (see Fig. 7A for sequences), URS-A (OM268 and OM286), URS-B (OM396 and OM397), and URS-C (OM270 and OM271) (see reference 9 and Fig. 7A for sequences). All of these double-stranded oligonucleotides have the overhang 5′ AATT 3′.

For the gel shift assay, 1 to 3 ng of labeled DNA (30,000 to 100,000 cpm) was incubated with 1 to 5 μl of Mig2p or Mig1p for 10 min at 4°C in a 25-μl reaction mixture in a buffer containing 50 mM Tris-HCl (pH 7.5), 10% glycerol, 35 mM $MgCl_2$, 200 mM KCl, 10 μM $ZnSO_4$, 2.5 mM dithiothreitol, and 0.5 μg of poly(dI-dC). Protein-DNA complexes were separated on a nondenaturing 6% (acrylamide-bisacrylamide, 30:0.8) polyacrylamide gel (containing 3% glycerol) run at 4°C and 13 V/cm in 0.5× Tris-borate-EDTA buffer (18).

Nucleotide sequence accession numbers. The GenBank accession numbers of the sequences reported here are as follows: *MIG2*, U54564; *YER028*, U18778.

RESULTS

***MIG2* encodes a Cys_2His_2 zinc-finger-containing protein.** *MIG2* was included on a plasmid we isolated in a screen for genes that affect *GAL4* expression. However, its isolation appears to have been fortuitous, since it appears to play no role in *GAL4* expression (see Materials and Methods). *MIG2* maps to lambda clone 1207 (27a) on the left arm of chromosome VII. We determined the DNA sequence of a 5-kb *Hin*dIII fragment that contains *MIG2* (see Materials and Methods). *MIG2* is predicted to encode a protein of 383 amino acids with two zinc fingers of the Cys_2His_2 type that are highly similar to those of Mig1p (71% identical) and Yer028p (82% identical), a protein with an unknown function (identified by the genome sequencing project). Residues that are thought to make base-specific contacts to DNA (RHR in finger 1 and RER in finger 2) (Fig. 1) are conserved in all three proteins. Like Mig1p and Yer028p, Mig2p is serine rich (14% serine residues). The remainder of the protein is somewhat similar to Yer028p but not to Mig1p. This region of the protein has no significant homologies to proteins in the databases and contains no other recognizable sequence motifs.

***MIG2* is involved in glucose repression of *SUC2* expression.** Disruption of *MIG1* only partially relieved glucose repression of *SUC2* expression (Fig. 2, column 2, and reference 35). *MIG2* is responsible for most of the remaining glucose repression observed in a *mig1* mutant, since further disruption of *MIG2* almost completely relieved glucose repression of *SUC2* expression (columns 4 and 5). Because single disruptions of *MIG1* and *MIG2* had a partial effect or no effect on repression of *SUC2* (columns 2 and 3), these proteins seem to be redundant repressors of *SUC2* expression. By contrast, *YER028* plays no role in the regulation of *SUC2* expression (columns 6 to 9).

TABLE 2. Effect of *mig2*Δ on other glucose-repressed promoters[a]

Reporter	Mean β-galactosidase (Miller units) or fold repression[b]											
	Wild type			*mig1*Δ			*mig2*Δ			*mig1*Δ *mig2*Δ		
	R	D	D/R	R	D	D/R	R	D	D/R	R	D	D/R
PCK1-lacZ	<1	19	>19	1*	34	34	ND	ND		<1	23	>23
FBP1-lacZ	<1	43	>43	4	35	9	ND	ND		2	30	15
HXT2-lacZ	20	280	14	99	367	3.7	ND	ND		132	233	1.8
GAL1-lacZ	9*	414	46	50	173	3.5	6*	322	54	138	399	2.9

[a] For *HXT2*, *FBP1*, and *PCK1*, the strains were as follows: wild type, YM4797; *mig1*Δ, YM4807; *mig1*Δ *mig2*Δ, YM4738. For *GAL1-lacZ*, the strains were as follows: wild type, YM2169; *mig1*Δ, YM4808; *mig2*Δ, YM4727; *mig1*Δ *mig2*Δ, YM4809. Wild-type and *mig2*Δ mutant strain values are the averages for the strains listed here and also strains obtained from a cross between YM4662 and YM3733 (Table 1).

[b] For *HXT2*, *FBP1*, and *PCK1*, cells were grown in synthetic media lacking only uracil and containing either 4% glucose (repressed [R]), 5% glycerol (derepressed [D] for *FBP1* and *PCK1*), or 5% glycerol and 0.1% glucose (derepressed for *HXT2*); for *GAL1*, cells were grown in rich media containing either 4% glucose (repressed) or 5% glycerol and 0.1% glucose (derepressed). The values are Miller units (21) and are averages of at least two assays of at least two (usually three) independent strains. Standard errors were less than 25%, except for those values marked with an asterisk, which were less than 50%. D/R, fold repression; ND, not done.

TABLE 3. Overexpression of *MIG2* represses *SUC2* expression under derepressing conditions[a]

Plasmid	Avg invertase activity (U) ± SD[b]			
	Wild type		*mig1*Δ	
	R	D	R	D
Vector only (FAT vector)	<2	790 ± 42	124 ± 60	1,091 ± 186
Very-high-copy *MIG2* (FAT)	26 ± 11	171 ± 56	36 ± 16	135 ± 33
High-copy *MIG2* (2μm)	6 ± 5	588 ± 100	30 ± 7	688 ± 40

[a] Wild type, YM4359; *mig1*Δ, YM4853. Very-high-copy *MIG2* (FAT), BM3214 and BM3215; high-copy *MIG2* (2μm), BM3090 and BM3091. Plasmid constructions are described in Materials and Methods.

[b] Invertase assays were done in synthetic media as described in Materials and Methods. The data are from 2 to 13 assays of at least two different strains. R, repressed; D, derepressed.

Thus, *MIG1* and *MIG2*, but not YER028, encode glucose repressors of *SUC2* expression.

***MIG2* does not act on other glucose-repressed genes.** Expression of several other genes in *S. cerevisiae* that are glucose repressed and have Mig1p-binding sites in their promoters is either unaffected or only partially affected by disruption of *MIG1* (19, 26, 35). The effect of a *mig2* disruption on glucose repression of some of these genes was tested. The results shown in Table 2 reveal that *MIG2* plays little, if any, role in glucose repression of *PCK1*, *FBP1*, *HXT2*, or *GAL1*. Neither Mig1p nor Mig2p is responsible for repression of *PCK1*, since glucose repression of expression of this gene is intact in *mig1*Δ and *mig1*Δ *mig2*Δ mutants (Table 2). Mig1p may play a minor role in repression of *FBP1*, since its disruption had a modest effect on *FBP1* repression, but further disruption of *MIG2* had no effect on *FBP1* expression (Table 2). While Mig1p plays a major role in glucose repression of *HXT2* and *GAL1* (9, 11, 22, 26), we consistently observed a small amount of repression of these genes in a *mig1*Δ mutant (Table 2). This residual repression is probably not due to Mig2p, since most of it was still apparent in a *mig1*Δ *mig2*Δ mutant (Table 2). We also tested these promoters for relief of repression in a *mig1*Δ *mig2*Δ *yer028*Δ triple mutant. There was no effect on *FBP1* or *PCK1* (data not shown), but glucose repression of *HXT2* was almost completely relieved (14-fold repression in the wild type and 1.2-fold repression in the triple mutant). Thus, Mig2p, and possibly also Yer028p, may have a minor role in glucose repression of *HXT2* expression.

***MIG2* encodes a glucose-dependent repressor.** Multiple copies of *MIG2* reduce *SUC2* expression under derepressing conditions, consistent with a role for Mig2p as a repressor (Table 3). Very high levels of *MIG2* reduced derepressed levels of *SUC2* expression about fivefold in wild-type cells and about eightfold in a *mig1*Δ mutant. Unexpectedly, very high levels of *MIG2* caused an increase in repressed levels of *SUC2* expression in wild-type cells. We imagine that this was due to titration of the general repressors Ssn6p and/or Tup1p. This effect was not observed in a *mig1*Δ mutant, which was expected, since Mig1p is the main glucose repressor of *SUC2.*

To test more directly the function of Mig2p as a transcriptional repressor, it was fused to the LexA DNA-binding domain and assayed for repression of a *CYC1-lacZ* reporter gene containing four LexA-binding sites upstream of the UAS. Results are shown in Fig. 3. LexA-Mig2p repressed gene expression 13-fold (line 2), but only in glucose-grown cells (line 4). Thus, Mig2p appears to be a glucose-dependent repressor of gene expression. Nearly identical results have been interpreted to mean that Mig1p is a glucose-activated repressor (33). Like Mig1p, Mig2p requires Ssn6p and Tup1p to repress (lines 6 and 8).

LexA-Mig2p weakly activates transcription in the absence of *SSN6*. A LexA-Mig1p fusion protein activates transcription in the absence of *SSN6* (33). Since Mig1p and Mig2p have similar functions as glucose repressors, LexA-Mig2p may also activate transcription in *ssn6* and/or *tup1* mutants. This was tested by using a *GAL1-lacZ* reporter with four *lex* operators replacing the UAS (Fig. 4). In the absence of *SSN6*, LexA-Mig2p activated transcription, but much more weakly than LexA-Mig1p (lines 1 and 2). In the absence of *TUP1*, LexA-Mig2p did not activate transcription of the reporter and LexA-Mig1p activated it weakly (lines 4 and 5). Thus, the function of Mig2p diverges from that of Mig1p with respect to transcriptional activation.

Mig2p binds to the *SUC2* promoter. To determine if Mig2p binds directly to the *SUC2* promoter, Mig2p produced in *E. coli* (see Materials and Methods) was assayed by the gel mobility shift assay for binding to several DNA fragments spanning the *SUC2* promoter (Fig. 5). The only fragments to which there was significant binding of Mig2p are those that contain

LINE	STRAIN	EXPRESSED PROTEIN	CARBON SOURCE	CYC1/LACZ REPORTER: UAS	CYC1/LACZ REPORTER: LexAop$_4$-UAS	FOLD REPRESSION
1	WT	$LexA_{1-87}$	glu	155	106	**1.5**
2		LexA-Mig2p		168	13	**13.0**
3	WT	$LexA_{1-87}$	gly	380	250	**1.5**
4		LexA-Mig2p		350	100	**3.5**
5	*ssn6Δ*	$LexA_{1-87}$	glu	30	11	**2.7**
6		LexA-Mig2p		19	13	**1.5**
7	*tup1Δ*	$LexA_{1-87}$	glu	15	7	**2.1**
8		LexA-Mig2p		13	7	**1.9**

FIG. 3. LexA-Mig2p is a transcriptional repressor. The *CYC1-lacZ* reporters in this experiment were pLGΔ312s (12) in column 1 and JK1621 (16) in column 2. Cells expressed either the $LexA_{1-87}$ DNA-binding domain (pSH2-1; reference 14) or the $LexA_{1-87}$-Mig2p fusion (pBM3091). The strains used were MCY829, MCY1974, and MCY2437 (Table 1). Cells were grown in synthetic media lacking only uracil and histidine and containing either 4% glucose or 5% glycerol and 0.1% glucose. The values are Miller units of β-galactosidase activity and are averages of 5 to 28 assays of two or three independent transformants. Standard errors were less than 28%. WT, wild type.

LINE	STRAIN	EXPRESSED PROTEIN	GAL1/LACZ REPORTER ΔUAS	GAL1/LACZ REPORTER $LexA_{op}$ 4
1	*ssn6Δ*	LexA-Mig2p	<1	6
2		LexA-Mig1p	ND	81
3		$LexA_{1-87}$	<1	<1
4	*tup1Δ*	LexA-Mig2p	<1	<1
5		LexA-Mig1p	ND	4
6		$LexA_{1-87}$	<1	<1
7	WT	LexA-Mig2p	< 1	<1
8		LexA-Mig1p	ND	<1
9		$LexA_{1-87}$	<1	<1

FIG. 4. LexA-Mig2p weakly activates transcription in an *ssn6*Δ mutant strain. The *GAL1-lacZ* reporters used were pLR1Δ1 (36) in column 1 and pSH18-8 (derived from pLR1Δ1 but with four *lex* operators replacing the UAS [2a]) in column 2. The strains are the same as those in Fig. 3. The cells expressed $LexA_{1-87}$ (pSH2-1), $LexA_{1-87}$-Mig2p (pBM3091), or $LexA_{1-87}$-Mig1p (pBM3216). Cells were grown in synthetic media lacking only uracil and histidine and containing 4% glucose. The values are Miller units of β-galactosidase activity for three independent transformants assayed in duplicate. Standard errors were less than 25%. ND, not done; WT, wild type.

the Mig1p-binding sites (probes a and b). It is possible that Mig2p also binds weakly to several other sites in the *SUC2* promoter, on the basis of the fastest-migrating shifted bands for probes c, d, e, and g. However, these DNA fragments did not compete for the strong binding of Mig2p to fragments a and b (data not shown), suggesting that the major Mig2p-binding sites are contained within fragment b. The most slowly migrating bands for these probes (and for probe f) are likely to be due to nonspecific binding, since they did not appear in all of the protein preparations and appeared to have the same mobility regardless of the size of the probe. The Mig2p that was assayed was fused to the *E. coli* maltose-binding protein, and we verified that the maltose-binding protein does not bind to a fragment containing the Mig1p-binding sites (probe a, middle lane).

To delimit further the sites to which Mig2p binds, gel shift experiments were carried out by using as probes oligonucleotides of the two Mig1p-binding sites in the *SUC2* promoter. Mig2p bound well to Mig1p-binding site A of the *SUC2* promoter and very weakly to Mig1p-binding site B (Fig. 6A). Thus, Mig2p and Mig1p bind to the same sites in the *SUC2* promoter. By contrast, Mig2p bound very poorly or not at all (Fig. 6B) to the two Mig1p-binding sites in the *GAL1* promoter (URS-A and URS-C, as well as URS-B, which does not appear to contain a Mig1p-binding site). This is not surprising, since disruption of *MIG2* did not affect *GAL1* repression (Table 2).

Since Mig2p does not act upon all promoters with Mig1p-binding sites, it seems likely that the two proteins differ in relative affinity for and recognition of different sites. To address this possibility, competitive gel mobility shift experiments were carried out by using as the probe ^{32}P-labeled oligonucleotides of the SUC2-A site, with oligonucleotides of each of the four Mig1p-binding sites from the *GAL1* and *SUC2* promoters as unlabeled competitors (Fig. 7A). The results presented in Fig. 7B show that Mig1p has the highest affinity for the SUC2-A site, less but clearly significant affinity for URS-A, and relatively little, if any, affinity for SUC2-B and URS-C. Mig2p also has the highest affinity for the SUC2-A site but much less affinity for the other three sites (Fig. 7C). The different relative affinities of the two proteins for these binding sites may explain their different effects on the various glucose-repressed promoters.

DISCUSSION

SUC2 gene expression is repressed about 200-fold by glucose. We have shown that this is due to two repressors, Mig1p and Mig2p, that contain very similar Cys_2His_2 zinc fingers (Fig. 1). These two proteins have overlapping functions: deletion of either one alone had a modest to no effect on *SUC2* glucose repression, and deletion of both almost completely relieved repression (Fig. 2). Thus, like that of *GAL1* expression, stringent glucose repression of *SUC2* expression is achieved by two mechanisms. In the case of *GAL1*, a single repressor, Mig1p, acts at several levels to repress expression (9, 11); for *SUC2*, two different repressors are responsible for stringent glucose repression.

Mig1p is more important than Mig2p for *SUC2* repression, since it is sufficient to achieve complete repression. Mig2p appears to play no role in *SUC2* repression when Mig1p is present, since *mig2*Δ alone has no effect on *SUC2* expression in a *MIG1* strain (Fig. 2). One possible explanation is that *MIG2* gene expression is repressed by Mig1p. Alternatively, Mig2p may bind to the *SUC2* promoter only in the absence of Mig1p.

It appears that the role of Mig2p in the cell is as a transcriptional repressor. *MIG1* was cloned as a multicopy inhibitor of *GAL1*, suggesting a role for Mig1p as a repressor (23). Similarly, when *MIG2* was introduced in high copy into wild-type

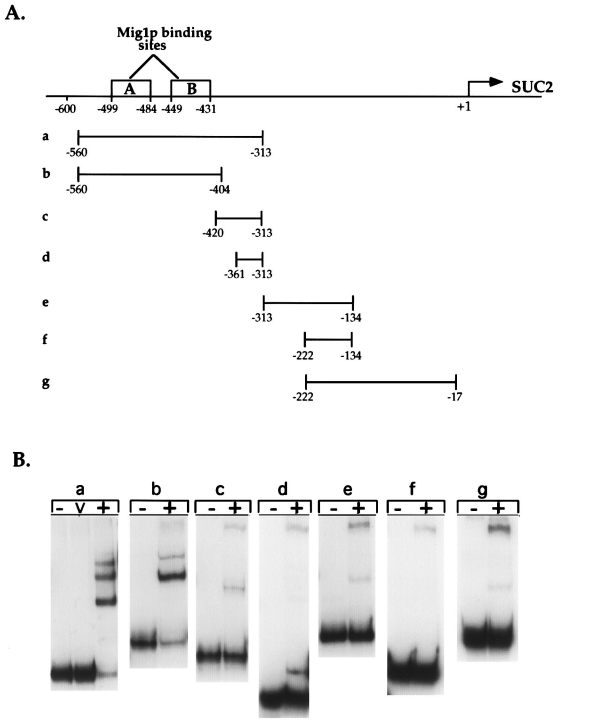

FIG. 5. Mig2p binds to the *SUC2* promoter. (A) Fragments within the *SUC2* promoter (a to g) generated by a PCR and end labeled with [^{32}P]dATP by filling in digested products with the Klenow fragment of DNA polymerase. The positions of the two Mig1p-binding sites in the promoter, SUC2-A and SUC2-B, are indicated. (B) Gel mobility shift assays. Mig2p was produced in *E. coli* as a fusion to the MalE protein (see Materials and Methods). The letters refer to the fragments shown in panel A. A minus or plus sign indicates the absence or presence, respectively, of Mig2p in the reaction mixture. V, protein prepared from *E. coli* bearing the *MAL* vector alone (i.e., not fused to *MIG2*). The gel shift assay with probe a, which contains the Mig1p-binding sites, resulted in three shifted bands, but that with probe b, which contains the same sites, resulted in only two shifted bands. It is possible that another, weak Mig2p-binding site is present within probe a but not probe b.

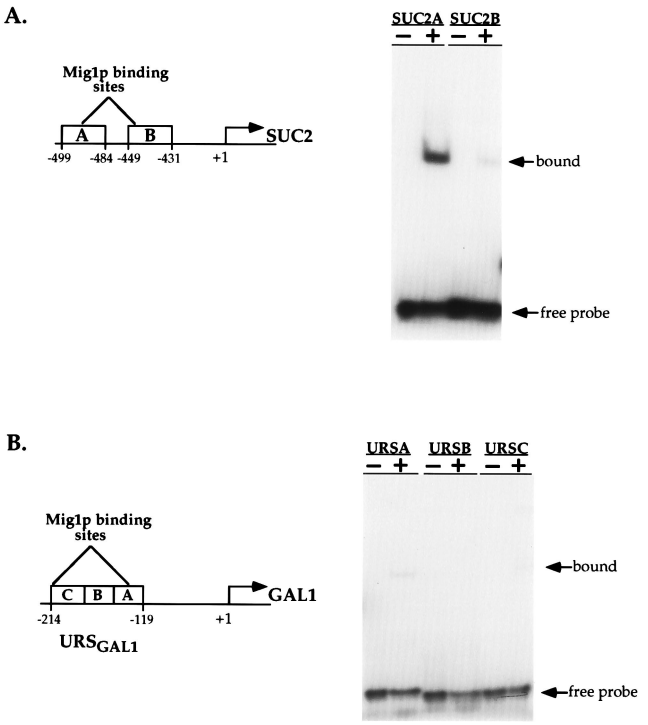

FIG. 6. Mig2p binds strongly to Mig1p-binding sites in the *SUC2* promoter and weakly to those in the *GAL1* promoter. (A) Mobility shift assay of Mig2p with oligonucleotides constituting Mig1p-binding sites in the *SUC2* promoter. (B) Mobility shift assay of Mig2p with oligonucleotides constituting sequences in the upstream repression sequence (URS) of the *GAL1* promoter. The sequences of the labeled double-stranded oligonucleotides used are listed in Materials and Methods. (A and B) Double-stranded oligonucleotides were labeled with [^{32}P]dATP by filling in with the Klenow fragment of DNA polymerase. The same Mal-Mig2p protein was used as for Fig. 5. A minus or plus sign indicates the absence or presence of Mig2p.

cells, *SUC2* expression was reduced under nonrepressing conditions (Table 3). The role of Mig2p as a repressor was further established by the ability of a LexA-Mig2 protein fusion to repress transcription of a *CYC1-lacZ* reporter (13-fold; Fig. 3). Repression occurs only in the presence of glucose, confirming a role for Mig2p as a glucose-activated repressor. Mig1p functions as both a repressor and an activator of transcription (reference 33 and Fig. 4). While LexA-Mig2p activates transcription in the absence of Ssn6p (Fig. 4), this activation is much weaker than with Mig1p. It is not clear if transcriptional activation by either Mig2p or Mig1p is physiologically relevant.

Since Mig1p and Mig2p have such similar zinc fingers, it was expected that they would bind very similar sites. Mig2p, indeed, bound directly to the two Mig1p-binding sites in the *SUC2* promoter (Fig. 6). We observed much stronger binding to site A than to site B but very little binding to the Mig1p-binding sites in the *GAL1* promoter. Mig1p, on the other hand, bound relatively well to site A in the *SUC2* promoter and to URS-A in the *GAL1* promoter and less strongly to its other sites in either promoter (Fig. 7). The relative affinities of Mig1p and Mig2p for these four binding sites are thus different (Fig. 7), and this probably explains the different effects of these proteins on different promoters. Mig2p may bind weakly to other regions of the *SUC2* promoter (fragments c, e, and g, Fig. 5) that possibly contribute to repression, but the effects of these promoter sequences on glucose repression are minor (30). Thus, it appears that Mig1p and Mig2p act primarily through the same sites in the *SUC2* promoter.

Several genes in *S. cerevisiae* are glucose repressed by Mig1p. However, several promoters that have obvious Mig1p-binding sites (as defined by Lundin et al. [17]) are not affected by a *mig1* disruption (e.g., *FBP1*, *PCK1*, and *HAP4* [19, 28]). There are also several promoters, such as *GAL1* and *HXT2*, for which Mig1p appears not to be the sole repressor, since some repression remains in a *mig1* mutant (reference 26 and Table 2). Since Mig2p appears to bind to some of the same sites as Mig1p, it is a good candidate for an additional repressor of these genes. However, a *mig2* disruption had little or no effect on the other glucose-repressed genes whose expression we examined (Table 2). Nevertheless, it seems likely that there are other promoters that Mig2p represses. The third member of this protein family, Yer028p, also does not appear to play a major role in repression of these genes, although it may play a

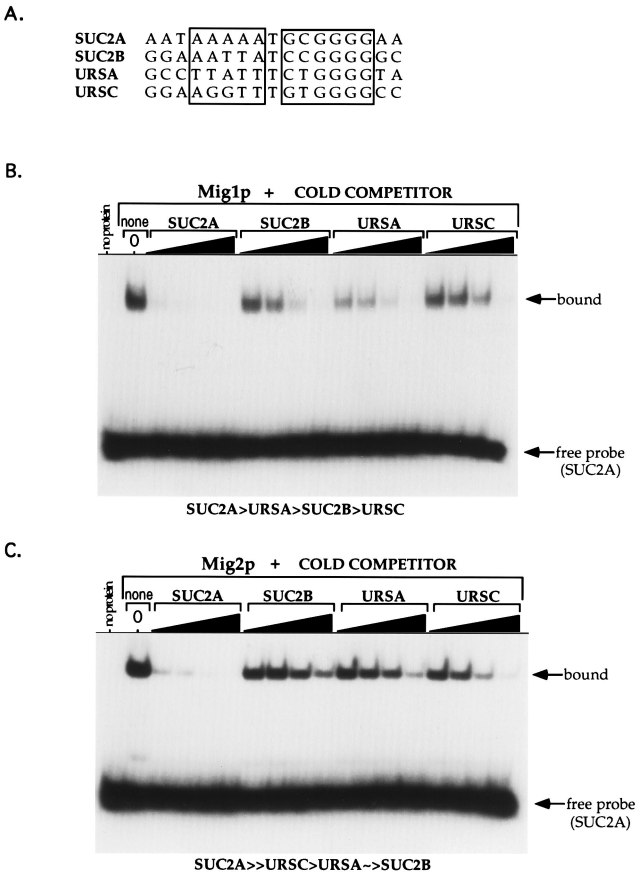

FIG. 7. Mig1p and Mig2p have different relative affinities for the various Mig1p-binding sites in the *SUC2* and *GAL1* promoters. (A) Sequences of the four oligonucleotides constituting the Mig1p-binding sites in the *SUC2* and *GAL1* promoters. The boxed sequences indicate the AT- and GC-rich regions determined to be part of the Mig1p-binding site. (B) Competition for Mig1p binding to SUC2-A. (C) Competition for Mig2p binding to SUC2-A. (B and C) The lanes are organized exactly the same in both gels. All lanes had the same amount of the [^{32}P]dATP-labeled SUC2-A probe and 0.5 μg of a nonspecific dA-dT competitor. Lane 1 contained no protein. The reaction mixtures loaded on the rest of the lanes in each gel contained the same amount of either Mig1p (B) or Mig2p (C). (In both cases, the two proteins shifted roughly the same amount of free probe [~20%], as determined by quantitation of the radioactivity in the gel.) Lane 2 contains no unlabeled competitor DNA. Each set of four lanes after lane 2 had increasing amounts of unlabeled competitor DNA (site SUC2-A, SUC2-B, URS-A, or URS-C) at molar ratios of 1:5, 1:10, 1:25, and 1:100 (probe to unlabeled competitor). The Mal-Mig2p fusion is the same as that used for the experiments whose results are described in Fig. 5 and 6; Mig1p was made in *E. coli* as a fusion to Gst (see Materials and Methods).

minor role in repression of *HXT2* expression, which is fully derepressed in a *mig1*Δ *mig2*Δ *yer028*Δ triple mutant. One explanation for the fact that these proteins have little or no effect on different promoters with Mig1p-binding sites is suggested by recent work of Dohrmann et al. (6), who showed that Ace2p and Swi5p activate the transcription of different genes even though they bind to the same site. Negative regulators acting at the different promoters appear to determine the promoter specificity of these transcriptional activators.

Since Mig1p and Mig2p are similar only in their zinc fingers, sequences outside the DNA-binding domain could contribute to their different DNA-binding specificities. One candidate sequence is a highly conserved basic region of 10 amino acids just downstream of the zinc fingers of Mig1p and *Kluyveromyces lactis* Mig1p (25) that is not conserved in Mig2p and Yer028p (although both proteins are somewhat basic in the same region). Alternatively, the few amino acid differences between the Mig2p and Mig1p zinc fingers could account for their different relative DNA-binding specificities. Amino acids in the Mig1p zinc fingers believed to be important for DNA recognition can be inferred on the basis of the structure of a Mig1p homolog, Zif268, bound to its recognition site (27). Amino acids within each finger of Mig1p (RHR at positions 15, 18, and 21 in finger 1 and RER at positions 15, 18, and 21 in finger 2) that recognize specific bases in a GC box are conserved in both Mig2p and Yer028p. How these two proteins distinguish between their sites may provide insight into zinc-finger-binding specificity and may prove useful in the development of a more comprehensive set of rules for predicting binding sites on the basis of the amino acid sequence of the binding protein (2).

SSN6 and *TUP1* encode general repressors in the cell that are recruited by several DNA-binding proteins (including Mig1p) to establish repression at various promoters (16). While a *mig1* mutant only partially relieves repression of *SUC2* expression, an *ssn6* mutant almost completely alleviates this repression (35). It seemed likely, therefore, that Mig2p-mediated repression would occur through these proteins. Indeed, a LexA-Mig2p fusion represses transcription of the *CYC1* promoter only on glucose and only in the presence of *SSN6* and *TUP1* (Fig. 3). Therefore, it appears that Mig2p, like Mig1p, recruits a complex that includes Ssn6p and Tup1p to the *SUC2* promoter to establish repression. However, Mig1p and Mig2p are not similar outside of the zinc finger region. This is not surprising considering that other proteins that mediate repression presumably by recruiting Ssn6p and Tup1p do not share any obvious sequence motifs.

Repression caused by Mig1p is relieved in the absence of glucose by a mechanism that requires the Snf1p protein kinase (3, 4), which is required for derepression of *SUC2* expression. *SUC2* expression in a *snf1* mutant is partially restored by a *mig1* disruption, but the considerable amount of repression of *SUC2* expression due to *MIG2* that remains in a *snf1 mig1* mutant is still regulated by glucose (35). This suggests the existence of a second protein (possibly also a protein kinase) that regulates Mig2p activity and may play a role in the derepression of other glucose-repressed genes that are unaffected by a *snf1* mutant.

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