Characterization of Three Related Glucose Repressors and Genes They Regulate in *Saccharomyces cerevisiae*

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ABSTRACT

Mig1 and Mig2 are proteins with similar zinc fingers that are required for glucose repression of *SUC2* expression. Mig1, but not Mig2, is required for repression of some other glucose-repressed genes, including the *GAL* genes. A second homolog of Mig1, Yer028, appears to be a glucose-dependent transcriptional repressor that binds to the Mig1-binding sites in the *SUC2* promoter, but is not involved in glucose repression of *SUC2* expression. Despite their functional redundancy, we found several significant differences between Mig1 and Mig2: (1) in the absence of glucose, Mig1, but not Mig2, is inactivated by the Snf1 protein kinase; (2) nuclear localization of Mig1, but not Mig2, is regulated by glucose; (3) expression of *MIG1*, but not *MIG2*, is repressed by glucose; and (4) Mig1 and Mig2 bind to similar sites but with different relative affinities. By two approaches, we have identified many genes regulated by Mig1 and Mig2, and confirmed a role for Mig1 and Mig2 in repression of several of them. We found no genes repressed by Yer028. Also, we identified no genes repressed by only Mig1 or Mig2. Thus, Mig1 and Mig2 are redundant glucose repressors of many genes.

THE yeast *Saccharomyces cerevisiae* has adopted mechanisms to ensure that it efficiently utilizes glucose, its preferred carbon source. One way it achieves this is to repress transcription of genes whose products are dispensable in cells growing on high levels of glucose (for reviews, see Johnston and Carlson 1992; Trumbly 1992; Ronne 1995), such as genes required for utilization of carbon sources other than glucose (*e.g.*, *GAL*, *SUC*, *MAL*), for gluconeogenesis (*e.g.*, *FBP1*, *PCK1*), for enzymes of the Krebs cycle and respiration (*e.g.*, *CYC1*, *COX6*), for high-affinity glucose transporters (*e.g.*, *HXT2*), and for genes involved in sporulation, proteolysis, and peroxisomal function.

Repression of many glucose-repressed genes is executed by Mig1, a zinc-finger DNA-binding protein (Nehlin and Ronne 1990; Klein *et al.* 1998). It represses transcription by recruiting the general repressors Ssn6 and Tup1 (Keleher *et al.* 1992; Treitel and Carlson 1995). Mig1 function is regulated at the level of its nuclear localization: in the absence of glucose, it is located in the cytoplasm; addition of glucose causes it to move rapidly into the nucleus (DeVit *et al.* 1997). Snf1, a protein kinase required for expression of many glucose-repressed genes (Celenza and Carlson 1984, 1986; Schuller and Entian 1987), inhibits Mig1 in the absence of glucose, probably by phosphorylating it, which causes Mig1 to move to the cytoplasm (Treitel and Carlson 1995; DeVit *et al.* 1997).

Mig2 and Yer028 contain two Cys_2His_2 zinc fingers very similar to those of Mig1 (Figure 1), and to the zinc fingers of the mammalian Krox20/Egr and Wilms' tumor proteins (Bohm *et al.* 1997). Like Mig1, Mig2 represses transcription in response to glucose through Ssn6 and Tup1 (Lutfiyya and Johnston 1996). Mig1 and Mig2, like their mammalian homologs, bind to a GC-rich sequence (Nehlin and Ronne 1990; Lutfiyya and Johnston 1996). Mig1 has an additional requirement for an adjacent AT-rich sequence (Lundin *et al.* 1994). It is not clear if Mig2 has a similar sequence requirement for DNA binding. Neither the DNA-binding site, nor the function of Yer028 is known.

Mig1-binding sites reside in the promoters of many glucose-repressed genes, and a role for Mig1 in repression of several of these has been confirmed (for review, see Klein *et al.* 1998): *GAL1* (Nehlin *et al.* 1991; Flick and Johnston 1992), *GAL4* (Griggs and Johnston 1991), *SUC2* (Nehlin and Ronne 1990; Vallier and Carlson 1994), *CAT8* (Hedges *et al.* 1995), and *MAL61*, *MAL62*, and *MAL63* (Hu *et al.* 1995; Klein *et al.* 1996; Wang and Needleman 1996). Mig1 appears to be the sole repressor of the *GAL* genes, because glucose repression of the *GAL* genes is almost completely relieved in a strain lacking *MIG1* (Griggs and Johnston 1991; Nehlin *et al.* 1991; Flick and Johnston 1992). Mig1 and Mig2 collaborate to repress *SUC2* expression (Vallier and Carlson 1994; Lutfiyya and Johnston

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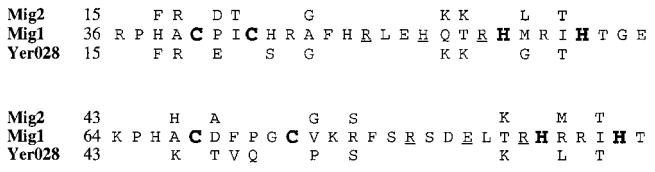


Figure 1.—Zinc fingers in Mig1-related proteins. Alignment of the zinc-finger motifs of Mig2, Mig1, and Yer028. The first zinc finger relative to the amino terminus of each protein is shown on top. All residues except the ones indicated are identical between Mig1 and the other two proteins. The conserved Cys and His residues that comprise the zinc-finger motifs are in bold; the RHR and RER residues believed to make base-specific DNA contacts are underlined (Pavletich and Pabo 1991).

1996). Several other glucose-repressed genes contain Mig1-binding sites in their promoters, but the expression of several of them is not affected by disrupting *MIG1* (Mercado *et al.* 1991; Ronne 1995). Mig2 and Yer028 are good candidates for regulators of these genes because of the similarity of their zinc-finger domains to those of Mig1 (Figure 1; Bohm *et al.* 1997). In an attempt to understand the specific roles played by these three repressors, we analyzed several aspects of their function and identified the genes they regulate.

MATERIALS AND METHODS

Yeast strains, media, and transformations: All strains used in this study are derived from S288C (Table 1). Yeast cells were grown at 30° in standard medium: YEP (rich) medium, or synthetic (minimal) medium lacking the appropriate amino acids (Rose *et al.* 1990). Yeast transformations were done as described by Schiestl and co-workers (Schiestl and Gietz 1989; Schiestl *et al.* 1993).

Gene disruptions were done using PCR products, as previously described (Baudin *et al.* 1993; Niedenthal *et al.* 1996). Briefly, yeast were transformed to His^+ or $G418^R$ with a *HIS3*- or *KanMX*-containing PCR product that included at each end 45 bp upstream and downstream of the region to be disrupted. This resulted in replacement of the target gene (from translation START to STOP codons) with *HIS3*, or *KanMX*. *HSL1*, *YCL024*, and *GIN4* were disrupted in diploid strain YM4919 using the following oligos: OM1150 and OM1151 (*HSL1*), OM1153 and OM1154 (*YCL024*), OM1156 and OM1157 (*GIN4*). The gene disruption was verified by a PCR with primers flanking the disrupted gene (OM1152, *HSL1*; OM1155, *YCL024*; OM1158, *GIN4*) and a primer in *HIS3* (OM483). The *mig1::KanMX* disruption was made as described above using oligos OL937 and OL938; primers OL939 and OM1117 were used in a PCR to confirm the correct disruption. Disruptions of *MIG2* and *YER028* were described previously (Lutfiyya and Johnston 1996). Other gene disruptions included *snf1::URA3*, made using the construct pBM2225 cut with *Bam*HI and *Hin*dIII, and *ura3::LYS2*, made with pBM2265 cut with *Hin*dIII.

Plasmids: Standard procedures for the manipulation of plasmid DNA and transformation into bacteria were followed (Sambrook *et al.* 1989). *Escherichia coli* DH5α was used as the host for all plasmids. All promoter fusions to *lacZ* (except pBM3190, M. DeVit, unpublished data) were made as follows: the promoter region of each gene (approximately 1 kb of sequence upstream from the ATG, including the ATG) was amplified from genomic DNA by a PCR with the oligonucleotides listed in Table 2. Several independent PCR products for each were combined, digested with *Bam*HI and *Eco*RI, and cloned between the *Bam*HI and *Eco*RI sites of YEp357R (pBM2640; Myers *et al.* 1986). The *YDR516* and *HXK1* PCR products were cut with *Bam*HI only and inserted into the *Bam*HI site of YEp357R. All constructs were sequenced to confirm that the ATG was in-frame with *lacZ*.

The Mig1-binding sites were subcloned into the reporter plasmid pBM2832 (Ozcan and Johnston 1996), which has the upstream activation sequence (UAS) fragment of the *LEU2* gene and the TATA box of *HIS3* (with part of the *HIS3* coding region) fused to *lacZ* in YEp356. Two single-stranded oligonucleotides consisting of each Mig1-binding site (OM1041+OM1042, OM1043+OM1044, OM268+OM286, OM270+OM271; see Table 2) were annealed for 15 min at 37°. The double-stranded oligos, which have *Eco*RI "sticky" ends, were then cloned into the *Eco*RI site of pBM2832. The resulting inserts were sequenced to determine the number of inserts. The $LexA_{1\text{-}87}$-Yer028-encoding plasmid, pBM3613, was made as follows. The *YER028* coding region (starting at the ATG) was amplified from genomic DNA by a PCR with oligonucleotides OM1341 and OM1342 as primers. Several independent PCR products were combined, digested with *Eco*RI and *Bam*HI, and cloned between the *Eco*RI and *Bam*HI sites of pSH2-1 (vector containing the *lexA* DNA-binding domain, amino acids 1–87; see Hanes and Brent 1989).

The plasmid containing Mig2 (amino acids 81–381) fused to GFP-β-galactosidase (pBM3691) was created by recombination in yeast. *MIG2* was amplified by the PCR using oligonucleotides OM1538 and OM1539 as primers and pBM3091 as template. This product was amplified in a PCR using primers OM1540 and OM1493 to provide homologous sequence for recombination (OM1493 adds 45 nucleotides identical to sequence 5′ of the *Bam*HI site in pBM3098 that is immediately 5′ of the GFP coding sequence in this plasmid; OM1540 adds 42 nucleotides identical to the sequence 3′ to the *Bam*H1 site). This PCR product and *Bam*HI cut pBM3098 (provided by Jim Haseloff, MRC Laboratory of Molecular Biology) were cotransformed into yeast strain YM4342. Plasmids from extracts of Ura^+ transformants were transformed into bacteria for amplification and analysis to confirm the presence of the *MIG2* coding sequence. Plasmid pBM3691 was retransformed into yeast strain YM4342 for visualization of GFP by fluorescent microscopy (DeVit *et al.* 1997).

The *CYC1-lacZ* reporters used in Figure 4 are pLGΔ312s (Guarente and Hoar 1984), which has *lacZ* under control of the wild-type *CYC1* promoter, and JK1621 (Keleher *et al.* 1992), which is identical to pLGΔ312s, except with four LexA-binding sites inserted 5′ of the UAS. In Figure 5, the *GAL1-lacZ* reporters are pLR1Δ1, which contains the *lacZ* gene under the control of the *GAL1* promoter with the UAS deleted (West *et al.* 1984) and pSH18-8, which is derived from pLR1Δ1, but has four LexA-binding sites replacing the UAS (R. Brent, personal communication).

Enzyme assays: β-Galactosidase assays were carried out in permeabilized cells grown to mid-log phase as described previously (Yocum *et al.* 1984), except that cell densities (OD_{600}) and ONPG (OD_{420}) were quantified in microtiter plates on a Molecular Devices (Sunnyvale, CA) plate reader. Yeast were grown in minimal medium lacking uracil (or uracil and histidine) and containing either 4% glucose (repressing conditions) or 5% glycerol and 0.05% glucose (nonrepressing conditions) to mid-log phase (OD_{600} ~1.0). Activities are given in Miller units and are the average of at least four assays of at least two independent transformants. Cells were prepared from exponentially growing cultures for invertase assays. Repressed

TABLE 1

Yeast strains used in this study

Strain	Genotype[a]
YM3696[b]	*MATα lys2-801::BM1499 snf1Δ*
YM3733[c]	*MAT***a** *met⁻ can^R mig1Δ::URA3*
YM4342	*MATα ura3Δ::LEU2 his3Δ200 trp1-903 lys2-801 leu2-3, 2-112*
YM4359[d]	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3, 2-112 trp1-901 gal80Δ can^R*
YM4664[c]	*MAT***a** *met? can? tyr1-501 mig2Δ::HIS3*
YM4725[d]	*MATα met? can? mig1Δ::URA3*
YM4734[c]	*MATα met? can? tyr? yer028Δ::HIS3*
YM4738[d]	*MAT***a** *met? can? mig2Δ::HIS3 mig1Δ::ura3::LYS2*
YM4742[c]	*MATα met? can? tyr1-501 yer028Δ::HIS3 mig2Δ::HIS3*
YM4797[c]	*MAT***a** *met? can? tyr1-501*
YM4804[c]	*MATα met? can? mig1Δ::URA3 mig2Δ::HIS3 yer028Δ::HIS3*
YM4847[c]	*MATα met? can? mig2Δ::HIS3 mig1Δ::ura3::LYS2 yer028Δ::HIS3*
YM4849[c]	*MAT***a** *met? can? tyr1-501 mig2Δ::HIS3 snf1Δ::URA3*
YM4850[c]	*MAT***a** *met- can^R mig1Δ::ura3::LYS2 snf1Δ::URA3*
YM4852[c]	*MAT***a** *met? can? mig2Δ::HIS3 mig1Δ::ura3::LYS2 snf1Δ::URA3*
YM4919[c,d]	*MAT***a**/α *met⁻ / met? can^R/can? mig1Δ::ura3::LYS2/mig1Δ::URA3 snf1Δ::URA3/SNF1*
YM4927[c]	*MAT***a** *met? can? mig1Δ::URA3 hsl1Δ::HIS3*
YM4929[d]	*MAT***a** *met? can? mig1Δ::ura3::LYS2 snf1Δ::URA3 hsl1Δ::HIS3*
YM4931[c]	*MATα met? can? mig1Δ::ura3::LYS2 ycl024Δ::HIS3*
YM4933[c]	*MATα met? can? mig1Δ::URA3 snf1Δ::URA3 ycl024Δ::HIS3*
YM4935[d]	*MATα met? can? mig1Δ::ura3::LYS2 gin4Δ::HIS3*
YM4937[c]	*MAT***a** *met? can? mig1Δ::ura3::LYS2 snf1Δ::URA3 gin4Δ::HIS3*
YM4966[c]	*MAT***a** *met? can? tyr1-501 mig1Δ::KanMX*
YM4968[c]	*MAT***a** *met? can? tyr1-501 mig2Δ::HIS3 mig1Δ::KanMX*
YM4974[c]	*MATα met? can? mig1Δ::URA3 hsl1Δ::HIS3 ycl024Δ::HIS3*
YM4977[d]	*MAT? met? can? migΔ::ura3::LYS2 snf1Δ::URA3 hsl1Δ::HIS3 ycl024Δ::HIS3*
YM4979[c]	*MAT***a** *met? can? mig1Δ::ura3::LYS2 hsl1Δ::HIS3 gin4Δ::HIS3*
YM4981[c]	*MATα met? can? mig1Δ::ura3::LYS2 snf1Δ::URA3 hsl1Δ::HIS3 gin4Δ::HIS3*
YM4983[c]	*MATα met? can? mig1Δ::ura3::LYS2 ycl024Δ::HIS3 gin4ΔHIS3*
YM4984[c]	*MATα met? can? mig1Δ::ura3::LYS2 snf1Δ::URA3 ycl024ΔHIS3 gin4ΔHIS3*
MCY829[e]	*MATα his3Δ200 lys2-801 ura3-52*
MCY1974[e]	*MATα ura3-52 his3Δ200 ade2-101 lys2-801 trp1Δ ssn6Δ9*
MCY2437[e]	*MATα 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 YM4342 and YM4359 contain *ura3-52 his3Δ200 ade2-101 lys2-801 trp1-901 gal80Δ538 LEU2::GAL1-LACZ.*

[b] Does not contain *trp1-901.*

[c] Also contains *gal4::GAL4-CAT-TRP1.*

[d] Also contains *gal4::GAL4-CAT-URA3.*

[e] Strains provided by M. Carlson (Treitel and Carlson 1995).

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 hr (YEP media) or 3 hr (synthetic media). Secreted invertase was assayed in whole cells as described by Goldstein and Lampen (1975) and Celenza and Carlson (1984), except that ABTS [2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)] was substituted for *o*-dianisidine (0.53 mg per reaction), and acid was not added at the end of the reaction. Reactions were incubated at room temperature for 30–60 min to allow the color to develop. Tubes were spun 2–3 min and absorbance was measured at 420 nm in microtiter plates on a Molecular Devices plate reader.

***In vitro* binding site selection:** Oligonucleotide OM1159 (5′ GTAAAACGACGGCCAGTGGATCCataaaaatgcggggaaGAAT TCCCTGTGTGAAATTGTTATCC 3′) was designed so that it would contain a 16-nucleotide degenerate internal region flanked on the 5′ end by a 23-nucleotide sequence of the M13 forward primer followed by a *Bam*HI site, and at the 3′ end by an *Eco*RI site followed by a 26-nucleotide sequence complementary to the M13 reverse primer. The internal region was synthesized by adding at each synthesis step a mixture containing 79% of the wild-type nucleotide of the SUC2-A site and 7% of each of the other 3 nucleotides. A total of 10 pmol of OM1159 was labeled and converted to double strands in a 20-μl reaction containing (final concentration): 1× Taq buffer (Boehringer Mannheim, Indianapolis), 50 μm 3 dNTP mix (minus A), 4 μm dATP, 10 pmol reverse primer (OM558), 20 μCi of [α-^{32}P]dATP, 5 units Taq DNA polymerase (Boehringer Mannheim). This was incubated for 1 min at 94°, 3 min at 51°, and 9 min at 72° for one cycle. The reaction was chased with 50 μm cold dATP for 9 min at 72° and purified on a NucTrap push column (Stratagene, La Jolla, CA). The labeled double-stranded DNA was then purified on a 10% polyacrylamide gel and eluted from the acrylamide by agitation in a buffer containing 0.5 m ammonium acetate, 10 mm MgAc, 1 mm EDTA, and 0.1% SDS, at 37° for 4–15 hr. After elution,

TABLE 2

Plasmids used

Plasmid	Description[a]	Recipient plasmid
pBM2636	*HXT1* promoter fused to *lacZ*[b]	
pBM3190	*MIG1* promoter fused to *lacZ*[c]	pBM2640
pBM3295	*MIG2* promoter fused to *lacZ* (OM1132, OM1133)	pBM2640
pBM3437	1 copy of Mig1-binding site SUC2-A in a heterologous promoter (OM1041, OM1042)	pBM2832
pBM3439	1 copy of Mig1-binding site SUC2-B in a heterologous promoter (OM1043, OM1044)	pBM2832
pBM3441	1 copy of Mig1-binding site URS-A in a heterologous promoter (OM268, OM286)	pBM2832
pBM3444	1 copy of Mig1-binding site URS-C in a heterologous promoter (OM270, OM271)	pBM2832
pBM3459	*REG2* promoter fused to *lacZ* (OM1201, OM1202)	pBM2640
pBM3461	*YDR516* promoter fused to *lacZ* (OM1203, OM1204)	pBM2640
pBM3465	*HXT13* promoter fused to *lacZ* (OM1207, OM1208)	pBM2640
pBM3469	*YKR075* promoter fused to *lacZ* (OM1211, OM1212)	pBM2640
pBM3471	*HXT15* promoter fused to *lacZ* (OM1213, OM1214)	pBM2640
pBM3473	*TPS1* promoter fused to *lacZ* (OM1215, OM1216)	pBM2640
pBM3475	*HXT17* promoter fused to *lacZ* (OM1219, OM1220)	pBM2640
pBM3497	*SSA4* promoter fused to *lacZ* (OM1278, OM1279)	pBM2640
pBM3498	*YHR054* promoter fused to *lacZ* (OM1290, OM1291)	pBM2640
pBM3499	*YBR101* promoter fused to *lacZ* (OM1282, OM1283)	pBM2640
pBM3501	*DOG2* promoter fused to *lacZ* (OM1266, OM1267)	pBM2640
pBM3502	*YFL054* promoter fused to *lacZ* (OM1288, OM1289)	pBM2640
pBM3503	*HSP26* promoter fused to *lacZ* (OM1268, OM1269)	pBM2640
pBM3504	*RIM9* promoter fused to *lacZ* (OM1298, OM1299)	pBM2640
pBM3505	*SSE2* promoter fused to *lacZ* (OM1280, OM1281)	pBM2640
pBM3506	*YEL070* promoter fused to *lacZ* (OM1286, OM1287)	pBM2640
pBM3507	*HSP30* promoter fused to *lacZ* (OM1270, OM1271)	pBM2640
pBM3508	*YLR042* promoter fused to *lacZ* (OM1294, OM1295)	pBM2640
pBM3509	*HSP82* promoter fused to *lacZ* (OM1274, OM1275)	pBM2640
pBM3510	*YEL050* promoter fused to *lacZ* (OM1284, OM1285)	pBM2640
pBM3511	*HSP60* promoter fused to *lacZ* (OM1272, OM1273)	pBM2640
pBM3512	*YLR264* promoter fused to *lacZ* (OM1296, OM1297)	pBM2640
pBM3513	*HXK1* promoter fused to *lacZ* (OM1307, OM1308)	pBM2640
pBM3514	*AHT1* promoter fused to *lacZ* (OM1264, OM1265)	pBM2640
pBM3613	*YER028* fused to the LexA DNA-binding domain (OM1341, OM1342)	pSH2-1
pBM3643	*YER028* fused to the MalE protein (OM1341, OM1342)	p*MAL*
pBM3691	Mig2-GFP-β-Gal fusion plasmid	pBM3098

[a] Oligonucleotides used in constructing these plasmids are in parentheses.
[b] Described previously (Ozcan and Johnston 1995).
[c] M. DeVit, unpublished results.

the DNA was ethanol precipitated, dried, and resuspended in 100 μl of ddH_2O. The specific activity was 3×10^5 cpm/pmol.

For the gel shift assays, approximately 1×10^5 cpm of DNA (0.3 pmol) was incubated with 1–5 μl of Mig2 or Mig1 for 10 min at 4° in a 25-μl reaction in the following buffer: 50 mm Tris-HCl (pH 7.5), 10% glycerol, 35 mm $MgCl_2$, 200 mm KCl, 10 μm $ZnSO_4$, 2.5 mm DTT, and 0.5 μg of poly(dI:dC). Mig1 and Mig2 proteins were produced in *E. coli* as previously described (Lutfiyya and Johnston 1996). Protein-DNA complexes were separated on a nondenaturing 6% (30:0.8) polyacrylamide gel (containing 3% glycerol) run at 4° at 13 V/cm in 0.5× Tris-borate-EDTA buffer (Sambrook *et al.* 1989), after which the gel was exposed to X-OMAT (Eastman Kodak, Rochester, NY) film for 10 hr at −70°. The shifted band was excised and the DNA eluted as described above. After elution, the DNA was extracted once with phenol/chloroform, once with chloroform, ethanol precipitated, washed once with 70% ethanol, dried, and resuspended in 20 μl of ddH_2O.

To amplify the DNA recovered from the gel shift experiment, 1 μl was used in a PCR using primers OM259 (universal forward primer) and OM558 (universal reverse primer). Reactions were incubated for 1 min at 94°, 1 min at 51°, and 1 min at 72° for 25 cycles. The PCR products were digested with *Bam*HI and *Eco*RI, cloned between the *Bam*HI and *Eco*RI sites of pBluescript SK^+ (Stratagene), and the resulting plasmids were sequenced on an ABI 373A automated sequencer using dye-labeled terminators.

Protein preparation: The entire Yer028 protein was fused to the bacterial MalE protein by amplifying *YER028* in a PCR with oligonucleotides OM1341 and OM1342 as primers, combining several independent reactions, digesting them with *Eco*RI and *Bam*HI, and inserting the fragment between the *Eco*RI and *Bam*HI sites of p*MAL* (New England Biolabs, Beverly, MA), generating pBM3643. Cells were grown, and protein was purified on a maltose affinity column according to the manufacturer's protocol.

DNA-binding assays: A labeled probe of the *SUC2* promoter region was made by combining PCR products from several independent PCR reactions (using as primers OM526 and OM534), digesting with *Eco*RI, purifying the digested product on a nondenaturing 10% polyacrylamide gel, and labeling with [^{32}P]dATP by filling in with the Klenow fragment of DNA

polymerase I (Sambrook *et al.* 1989). Oligonucleotide probes were annealed, labeled with [^{32}P]dATP by filling in the sticky ends with the Klenow fragment of DNA polymerase I, and purified on a NucTrap push column (Stratagene). The oligonucleotides used were SUC2-A (OM1041 and OM1042) and SUC2-B (OM1043 and OM1044); Mig1 binds to both of these sites (Nehlin and Ronne 1990; Lutfiyya and Johnston 1996). The gel shift assay was carried out as described above.

Computer methods: searching the yeast genome for Mig1-binding sites: A pattern search program, RNABOB (http://genome.wustl.edu/eddy/#rnabob/), was used to search for Mig1 binding sites in a database consisting of only the regions between the predicted open reading frames (ORFs) in the yeast genome (S. Eddy, personal communication). The search was limited to sites similar to the SUC2-A site: AAAAA T GCGGGG. The criteria used were: (1) any sites that matched *exactly* in the GC-box *and* allowed up to three changes in the AT-box, or (2) any sites that had a GC-box sequence of GTGGGG, or CCGGGG, *and* any one change in the AT-box. This allowed for flexibility in the AT-box, while maintaining a strict requirement in the GC-box for sites that Mig1 is known to bind to (Lundin *et al.* 1994).

Screening an array of yeast genes for Mig1, Mig2, and Yer028 regulated genes: Total yeast RNA was isolated from YM4797 (wild type) and YM4804 (*mig1*Δ *mig2*Δ *yer028*Δ) cultures grown in rich medium containing 4% glucose, as described previously (Elder *et al.* 1983). Poly(A)$^+$ RNA was prepared using the poly(A) spin mRNA isolation kit from New England Biolabs. Fluorescently labeled cDNA was prepared from the mRNA and hybridized to glass slides to which a DNA array of approximately 3325 yeast ORFs was attached (Derisi *et al.* 1997).

RESULTS

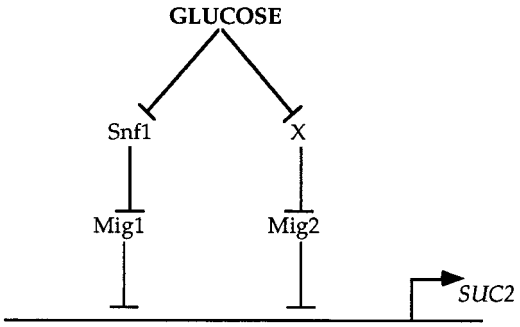
Mig1 and Mig2 function is regulated differently: The Snf1 protein kinase inhibits Mig1 under nonrepressing conditions (Vallier and Carlson 1994), probably by phosphorylating it, leading to its exit from the nucleus (Treitel and Carlson 1995; DeVit *et al.* 1997). Because of this, *SUC2* expression is abolished in a *snf1*Δ mutant (Figure 2, line 5; also see Vallier and Carlson 1994). Mig1 is clearly responsible for this, because deletion of *MIG1* (Figure 2, line 7), but not *MIG2* (Figure 2, line 6) restores expression. Snf1 appears not to inhibit Mig2 function. This is clearly seen in a *snf1 mig1* mutant (line 7), in which Mig2 is primarily responsible for the 10-fold glucose repression of *SUC2* expression observed in this mutant (compare lines 2 and 4 or lines 7 and 8): Mig2-mediated repression and derepression of *SUC2* expression are not affected by loss of *SNF1* (compare lines 2 and 7). Thus, it appears that Snf1 does not inactivate Mig2. Nevertheless, Mig2 function is regulated in response to glucose (lines 2 and 7), suggesting that the regulator of Mig2 (X in Figure 2A) responds to glucose. Similar results were reported by Vallier *et al.* (1994).

We thought that the protein responsible for inactivating Mig2 under nonrepressing conditions could be one of the Snf1 homologs encoded in the yeast genome. Loss of the protein that inactivates Mig2 would cause Mig2 to repress *SUC2* expression even under derepressing conditions (in the absence of glucose, as loss of *SNF1* causes Mig1 to repress under these conditions). However, disruption of the genes encoding three of the closest homologs of Snf1 (*HSL1*, *YCL024*, and *GIN4*) in a *mig1*Δ *snf1*Δ strain had no effect on *SUC2* expression (Table 3). Thus, none of these three Snf1 homologs appears to regulate Mig2.

Regulation of Mig1 and Mig2 function occurs by different mechanisms. The nuclear localization of Mig1 is regulated by glucose (DeVit *et al.* 1997), but Mig2 is located in the nucleus both in the presence and absence of glucose (Figure 3). This is consistent with the observation that Snf1, whose action causes Mig1 to move to the cytoplasm, does not regulate Mig2 activity.

***MIG1* and *MIG2* expression are regulated differently:**

A



B

relevant genotype	Avg invertase activity (U) ± SD: Repressed	Derepressed
1 *WT*	6 ± 4	586 ± 81
2 *mig1Δ*	88 ± 20	1182 ± 159
3 *mig2Δ*	2 ± 1	765 ± 196
4 *mig1Δ mig2Δ*	1178 ± 230	2213 ± 236
5 *snf1Δ*	<1	<1
6 *mig2Δ snf1Δ*	<1	<1
7 *mig1Δ snf1Δ*	150 ± 41	1313 ± 363
8 *mig1Δ mig2Δ snf1Δ*	1029 ± 188	2062 ± 202

Figure 2.—Snf1 does not inactivate Mig2. (A) Regulation of Mig1 and Mig2 function inferred from this, and other data (Lutfiyya and Johnston 1996). X is a hypothetical protein (possibly a protein kinase) that inhibits Mig2 function in non-glucose-grown cells. (B) Invertase assays were done in YEP medium under conditions of repression (4% glucose) and derepression (5% glycerol and 0.05% glucose). The data are from three to seven assays of at least two different strains. Strains: wild type (WT), YM4359; *mig1*Δ, YM4725; *mig2*Δ, YM4664; *mig1*Δ *mig2*Δ, YM4738; *snf1*Δ, YM3696; *mig2*Δ *snf1*Δ, YM4849; *mig1*Δ *snf1*Δ, YM4850; *mig1*Δ *mig2*Δ *snf1*Δ, YM4852; *mig1*Δ *snf1*Δ *hsl1*Δ, YM4929; *mig1*Δ *snf1*Δ *ycl024*Δ, YM4933; *mig1*Δ *snf1*Δ *gin4*Δ, YM4937; *mig1*Δ *snf1*Δ *hsl1*Δ *ycl024*Δ, YM4977; *mig1*Δ *snf1*Δ *hsl1*Δ *gin4*Δ, YM4981; *mig1*Δ *snf1*Δ *ycl024*Δ *gin4*Δ, YM4984.

TABLE 3

Disruption of *SNF1* homologs has no effect on *SUC2* expression

Relevant genotype[a]	Average invertase activity[a] (units ± SD) Repressed	Derepressed
1 WT	6 ± 4	586 ± 81
2 *mig1*Δ	88 ± 20	1182 ± 159
3 *mig1*Δ *snf1*Δ	150 ± 41	1313 ± 363
4 *mig1*Δ *snf1*Δ *hsl1*Δ	178 ± 43	1116 ± 147
5 *mig1*Δ *snf1*Δ *ycl024*Δ	175 ± 59	861 ± 59
6 *mig1*Δ *snf1*Δ *gin4*Δ	177 ± 41	1261 ± 247
7 *mig1*Δ *snf1*Δ *hsl1*Δ *ycl024*Δ	136 ± 9	820 ± 72
8 *mig1*Δ *snf1*Δ *hsl1*Δ *gin4*Δ	237 ± 7	1468 ± 117
9 *mig1*Δ *snf1*Δ *ycl024*Δ *gin4*Δ	242 ± 3	1608 ± 144

[a] Assays were done as in Figure 2. See Figure 2 legend for strains used.

Expression of *MIG1* and *MIG2* is regulated differently. *MIG1* expression is repressed about 12-fold in the presence of glucose; *MIG2* is expressed constitutively (Table 4). Mig1 and Mig2 are primarily responsible for repression of *MIG1* expression: repression is slightly relieved in *mig1*Δ, *mig2*Δ, and *yer028*Δ single mutants, and almost completely relieved in a *mig1*Δ *mig2*Δ strain. Yer028 plays no significant role in repression of *MIG1*, because deleting *YER028* in the *mig1*Δ *mig2*Δ mutant has little effect on expression. Thus, Mig1, together with Mig2, represses its own expression in the presence of glucose.

YER028 may encode a glucose-dependent transcriptional repressor: Because Yer028 possesses a DNA-binding domain very similar to those of Mig1 and Mig2, and is also similar to Mig2 outside of the zinc-finger region, it seemed likely that Yer028 has a function similar to Mig1 and Mig2. Indeed, a LexA-Yer028 chimeric protein is a glucose-dependent repressor of gene expression (Figure 4, lines 1–4). Like Mig1 and Mig2, Yer028 also requires Ssn6 and Tup1 to repress gene expression (Figure 4, lines 6 and 8). In the absence of *TUP1* and *SSN6*, LexA-Yer028 activates transcription (Figure 5, lines 1 and 3). This is like LexA-Mig1 (Treitel and Carlson 1995), but unlike LexA-Mig2 (Lutfiyya and Johnston 1996).

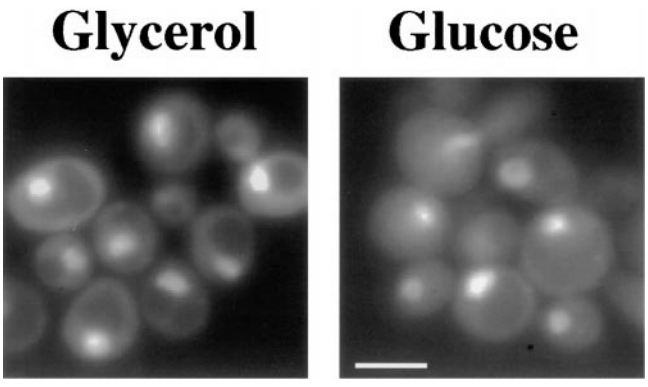


Figure 3.—Subcellular localization of Mig2(81-381)-GFP-β-Gal. The portion of Mig2 included in the chimeric protein is sufficient to provide glucose-regulated repression of gene expression. Yeast strain YM4342 expressing Mig2-GFP-β-Gal from a plasmid (pBM3691) was grown on YM-Uracil + 2% glucose (repressing conditions) or YM-Uracil + 5% glycerol (derepressing conditions). Bar, 5 μm.

Yer028 binds to the Mig1-binding sites in the *SUC2* promoter: To determine if Yer028 binds to the same DNA sequence as Mig1 and Mig2, it was produced in *E. coli* (see materials and methods) and assayed for binding to the Mig1-binding sites in the *SUC2* promoter (Figure 6). Yer028 binds well to a fragment of the *SUC2* promoter containing both Mig1-binding sites and also to oligonucleotides of the individual Mig1-binding sites in the *SUC2* promoter (SUC2-A and SUC2-B).

Function of individual Mig1 DNA-binding sites *in vivo*: Mig1 and Mig2 have different relative affinities for different binding sites in the *GAL1* and *SUC2* promoters, which may explain the different sensitivities of these genes to the two repressors (Lutfiyya and Johnston 1996). To determine if the affinities of Mig1 and Mig2 for various binding sites *in vitro* are correlated with the amount of transcriptional repression caused by those sites *in vivo*, their ability to repress expression through the Mig1-binding sites in the *GAL1* and *SUC2* promoters was tested (Figure 7). The SUC2-A and GAL1-A sites cause the most repression, consistent with the observation that Mig1 has the highest affinity for these sites and that Mig2 has the highest affinity for SUC2-A. The SUC2-B and GAL1-C sites, to which Mig1 and Mig2 bind less strongly, directed much less repression [though two copies of these sites caused 20- to 100-fold repression (data not shown)]. Mig1 plays the major role in repression caused by all four sites (line 2); Mig2 is responsible for most, or all, of the remaining repression (line 4). Thus, the relative affinities of Mig1 and Mig2 for their binding sites, measured *in vitro*, correlates well with their ability to repress transcription through these sites *in vivo*.

Two other observations are notable in the experiment presented in Figure 7. First, GAL1-A causes a small but probably significant amount of glucose repression in a *mig1*Δ *mig2*Δ *yer028*Δ strain (data not shown), suggesting that another protein binds to this site to mediate repression. This is similar to the situation for *GAL1*, whose expression is ~3-fold repressed by glucose in the triple mutant strain (data not shown). Second, in the absence of *MIG1* and *MIG2*, SUC2-A causes 5 to 10-fold activation on glycerol, suggesting that an activator binds to this site (data not shown). This is consistent with the observations of others (Sarokin and Carlson 1984; Bu and Schmidt 1998).

Defining binding site specificities for Mig1 and Mig2: In an attempt to define the binding sites preferred by Mig1 and Mig2, sequences bound by each protein were

TABLE 4

***MIG1* and *MIG2* expression**

	Mean β-galactosidase activity (Miller units ± SD)[a]					
	MIG1-LacZ			*MIG2-LacZ*		
Relevant genotype	R	D	D/R	R	D	D/R
WT	27 ± 8	325 ± 51	12	2478 ± 441	3969 ± 357	1.6
*mig1*Δ	144 ± 38	631 ± 129	4	ND	ND	—
*mig2*Δ	64 ± 11	342 ± 96	5	ND	ND	—
*yer028*Δ	71 ± 14	365 ± 49	5	ND	ND	—
*mig1*Δ *mig2*Δ	400 ± 113	535 ± 76	1.3	ND	ND	—
*mig2*Δ *yer028*Δ	85 ± 5	296 ± 45	3.5	ND	ND	—
*mig1*Δ *mig2*Δ *yer028*Δ	547 ± 87	824 ± 134	1.5	2688 ± 294	2268 ± 210	0.8

Strains: WT, YM4797; *mig1*Δ, YM4966; *mig2*Δ, YM4664; *yer028*Δ, YM4734; *mig1*Δ*mig2*Δ, YM4738; *mig2*Δ *yer028*Δ, YM4742; *mig1*Δ *mig2*Δ *yer028*Δ, YM4847. Plasmids: pBM3190, pBM3295.

[a] Cells were grown in synthetic media lacking only uracil and containing either 4% glucose (R), or 5% glycerol and 0.05% glucose (D). The values are Miller units of β-galactosidase activity averaged from two to three independent tranformants assayed in duplicate. D/R, fold depression; ND, not done.

selected *in vitro* (materials and methods; see Horwitz and Loeb 1986; Oliphant and Struhl 1987; Wright and Funk 1993). An oligonucleotide with a degenerate sequence of the SUC2-A strong Mig1-binding site (Figure 7A) was synthesized, with each position of the binding site seeded with 79% of the wild-type nucleotide and 7% of each of the other three nucleotides. Oligonucleotides bound by Mig1 or Mig2 were selected in a gel mobility shift experiment (Figure 8A), eluted from the gel, cloned in a plasmid, and their sequence determined.

A total of 147 sites were identified for Mig2 and 106 for Mig1. The results, summarized in Figure 8B, show that the two proteins prefer almost identical binding sites. The most conserved sequence element is a GC-rich sequence, and the requirements in the GC-box appear to be almost identical for the two proteins. It is known that Mig1 requires for binding an AT-rich sequence directly upstream of the GC-box (Lundin *et al.* 1994), which is evident from this experiment, because only 12% of the sites bound by Mig1 contain two or more G's or C's within the AT-box (Figure 8C). In contrast, 22% of the sites bound by Mig2 contain two or more G's and C's within the AT-box: more G's and C's are found in positions 2, 3, 4, and 7 as compared to Mig1. Thus, Mig2 may not have as strict a requirement as Mig1 for the AT-rich sequence for binding. We conclude that Mig1 and Mig2 probably recognize the same sequences. Thus, the different effects of these proteins on the *GAL1* and *SUC2* genes are probably not due to

Line	Strain	Expressed Protein	Carbon Source	*CYC1-lacZ* Reporter		Fold Repression
				UAS	$LexA_{op}$-UAS$_4$	
1	WT	$LexA_{1-87}$	glu	1669	888	**1.9**
2		LexA-Yer028p		1242	105	**12**
3	WT	$LexA_{1-87}$	gly	4598	2823	**1.6**
4		LexA-Yer028p		4102	2117	**1.9**
5	*ssn6Δ*	$LexA_{1-87}$	glu	247	89	**2.8**
6		LexA-Yer028p		288	498	**0.6**
7	*tup1Δ*	$LexA_{1-87}$	glu	237	102	**2.3**
8		LexA-Yer028p		235	112	**2.1**

Figure 4.—Yer028 is a transcriptional repressor. The *CYC1-lacZ* reporters used in this experiment were pLGΔ312s (UAS; Guarente and Hoar 1984) and JK1621 ($LexA_{op}$-UAS; Keleher *et al.* 1992). Cells expressed either the $LexA_{1-87}$ DNA-binding domain (pSH2-1; Hanes and Brent 1989), or the $LexA_{1-87}$-Yer028 fusion (pBM3613). 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 0.05% glucose. The values are Miller units of β-galactosidase activity and are averages of at least four assays of two or three independent transformants. Standard errors were less than 25%. WT, wild type.

Line	Strain	Expressed Protein	*GAL1-lacZ* Reporter	
			ΔUAS	$LexA_{op}$$_4$
1	*ssn6Δ*	LexA-Yer028	5	906
2		$LexA_{1-87}$	2	10
3	*tup1Δ*	LexA-Yer028	5	66
4		$LexA_{1-87}$	3	5
5	WT	LexA-Yer028	<1	<1
6		$LexA_{1-87}$	<1	<1

Figure 5.—Yer028 activates transcription in the absence of Ssn6 or Tup1. The *GAL1-lacZ* reporters used were pLR1Δ1 (ΔUAS; West *et al.* 1984) and pSH18-8 ($LexA_{op}$; derived from pLR1Δ1, but with four lex operators replacing the UAS). The strains are the same as those in Figure 3. The cells expressed either $LexA_{1-87}$ (pSH2-1), or $LexA_{1-87}$-Yer028 (pBM3613). 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 two or three independent transformants assayed in duplicate. Standard errors were less than 25%. WT, wild type.

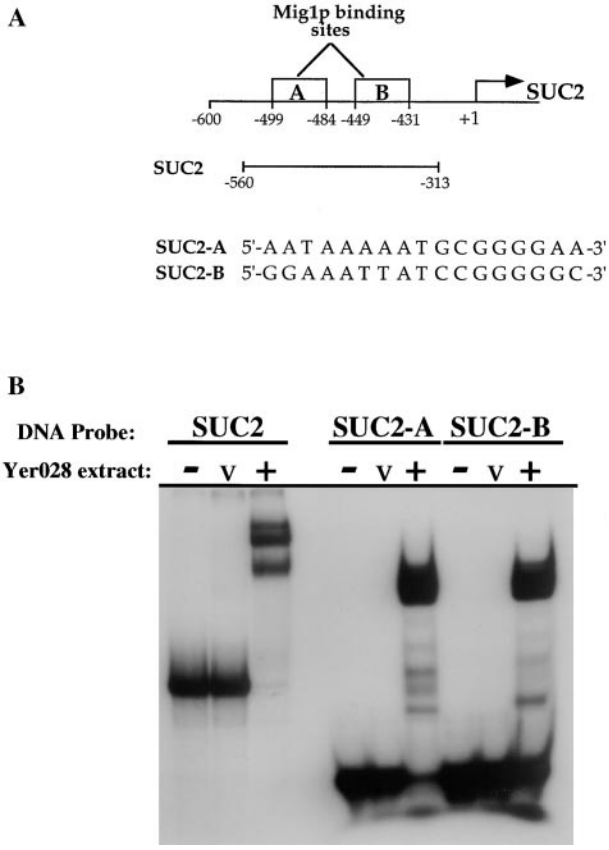
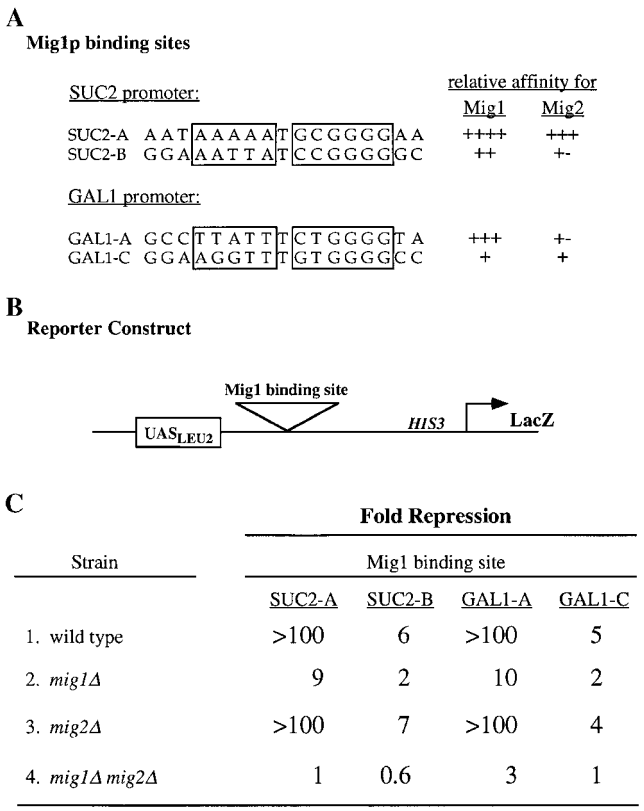


Figure 6.—Yer028 binds to the Mig1-binding sites in the *SUC2* promoter. (A) Probes used in a gel mobility shift assay of Yer028. A fragment within the *SUC2* promoter (SUC2) was generated in a PCR and end labeled with [^{32}P]dATP by filling in the *Eco*RI-digested product with the Klenow fragment of DNA polymerase I. The positions of the two Mig1-binding sites within this fragment, SUC2-A and SUC2-B, are indicated. Labeled ([^{32}P]dATP), double-stranded oligonucleotides constituting SUC2-A and SUC2-B were also generated by filling in with the Klenow fragment of DNA polymerase I. (B) Gel mobility shift assay. Yer028 was produced in *E. coli* as a fusion to the MalE protein (see materials and methods). A minus or plus sign indicates the absence or presence, respectively, of Yer028 in the reaction mixture. V, protein prepared from *E. coli* bearing the *MAL* vector alone (*i.e.*, not fused to Yer028).



C

Strain	Fold Repression: Mig1 binding site			
	SUC2-A	SUC2-B	GAL1-A	GAL1-C
1. wild type	>100	6	>100	5
2. *mig1Δ*	9	2	10	2
3. *mig2Δ*	>100	7	>100	4
4. *mig1Δ mig2Δ*	1	0.6	3	1

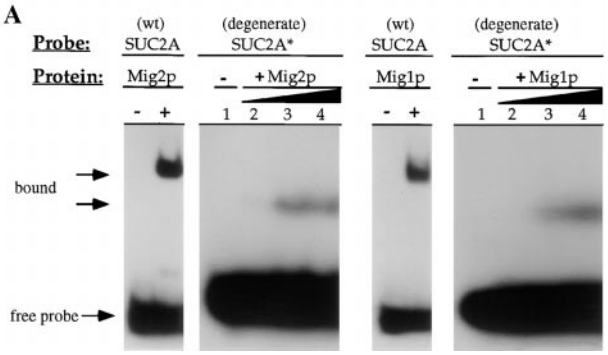
Figure 7.—*In vivo* characterization of Mig1-binding sites. (A) Mig1-binding sites in the *SUC2* and *GAL1* promoters. The relative affinities of Mig1 and Mig2 for each site is indicated (see Lutfiyya and Johnston 1996). (B) Mig1-binding sites were inserted into a nonglucose-regulated reporter vector, pBM2832 (Ozcan and Johnston 1996), which contains the UAS fragment of the *LEU2* gene and the TATA box with part of the *HIS3* coding region fused to *lacZ*. (C) Fold repression is the repression *relative to the vector alone* (*i.e.*, vector alone/vector + insert) based on β-galactosidase assays measuring expression levels in glucose-grown cells. β-Galactosidase activity was averaged from at least two independent transformants assayed in duplicate. Cells were grown in synthetic media lacking only uracil and containing either 4% glucose, or 5% glycerol and 0.05% glucose. There was little or no repression in the glycerol-grown cells: the level of β-galactosidase expressed from the reporter gene without any Mig1/Mig2-binding sites from glucose-grown cells averaged 177 units in all assays (range 115–248 units) and from glycerol-grown cells averaged 406 units in all assays (range 260–536). Standard errors were usually less than 25%. The plasmids assayed were: pBM3437, pBM3439, pBM3441, and pBM3444 (Table 2). The strains used were: wild type, YM4797; *mig1*Δ, YM4966; *mig2*Δ, YM4664; *mig1*Δ *mig2*Δ, YM4968; *mig1*Δ *mig2*Δ *yer028*Δ, YM4847.

differences in binding site *specificity* but rather to their affinities for different sites.

Identifying genes regulated by Mig1, Mig2, and Yer028: Despite the fact that Mig1 and Mig2 are glucose-dependent transcriptional repressors that appear to bind to similar DNA sequences, they do not always regulate the same genes. With the hope of shedding light on this paradox, and in an attempt to identify genes regulated by Yer028, we sought to identify genes regulated by each protein. In addition to revealing the genes that are regulated by these proteins, differences in the binding sites of regulated genes might provide clues to why these repressors act on different genes.

Two approaches were taken to identify genes that are regulated by Mig1, Mig2, and Yer028. The first approach involved searching the yeast genome for known Mig1-binding sites (Nehlin and Ronne 1990; Nehlin *et al.* 1991; Lundin *et al.* 1994; Hu *et al.* 1995). A pattern search program was used to find Mig1-binding sites in the regions between ORFs in the yeast genome (see materials and methods). More than 100 genes with candidate Mig1-binding sites in their promoters were identified. Several of these, listed in Table 5 (lines 1–7), were tested for regulation by Mig1 and Mig2. The pro-

A



B

Mig2p

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
G%	16	14	19	12	10	7	17	7	**95**	3	**95**	**98**	**94**	**93**	28	30
A%	**73**	9	**54**	**63**	**83**	**85**	**70**	3	1	1	1	1	3	1	**51**	**50**
T%	10	**67**	18	16	3	5	9	**81**	1	5	3	1	2	3	13	12
C%	1	10	8	10	3	1	4	8	3	**91**	1	1	1	4	8	7
consensus	A	T	A	A	A	A	A	T	G	C	G	G	G	G	A	A

Mig1p

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
G%	13	12	10	8	9	10	10	6	**93**	3	**100**	**94**	**88**	**91**	20	24
A%	**77**	6	**72**	**77**	**83**	**77**	**80**	4	2	1	0	1	4	1	**61**	**63**
T%	8	**76**	9	9	6	8	5	**86**	1	8	0	3	8	7	12	8
C%	2	6	8	6	2	4	5	5	4	**88**	0	2	0	2	7	4
consensus	A	T	A	A	A	A	A	T	G	C	G	G	G	G	A	A

C

% "other" bases observed

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	A	T	A	A	A	A	A	T	G	C	G	G	G	G	A	A
Mig2p	27	33	46	37	17	14	30	18	5	9	5	2	6	7	49	49
Mig1p	23	24	28	23	19	23	20	14	7	12	0	6	12	9	39	36

% of sequences with ≥2 Gs and Cs in the A-T box: Mig2p: 22%, Mig1p: 12%

% of sequences with >1 change in the G-C box: Mig2p: 5%, Mig1p: 10%

Figure 8.—*In vitro* binding site selection for Mig1- and Mig2-binding sites. (A) Gel mobility shift assay. The probe was a double-stranded, labeled oligonucleotide consisting of either (1) a wild-type SUC2-A site (OM1041 and OM1042) or (2) a degenerate SUC2-A site, OM1159 (see materials and methods). The wild-type probe is 43 nucleotides shorter than the degenerate probe. Mig1 and Mig2 were produced in *E. coli* as fusions to bacterial Gst and MalE, respectively, as previously described (Lutfiyya and Johnston 1996). A minus or plus sign indicates the absence or presence of protein. Lanes 2–4 of each gel (Mig1 or Mig2) contain increasing amounts of the protein indicated. In each gel, the amount of protein used in the corresponding lanes shifted roughly the same amount of probe. Also, in each experiment, the same amount of protein was used with the wild-type probe (+ lane) as with the degenerate oligonucleotide in lane 3. (B) A compilation of the selection data yields the consensus binding site for each protein. The highest frequency at each position is shown in bold. (C) The frequency of "other" bases found at each position; *i.e.*, the frequency at which the protein tolerates a change in that position. The number of sequences selected that contain at least two G's or C's within the AT-box, or more than one change in the GC-box is indicated.

moter regions (~1 kb of sequence upstream of the ATG codon) of these genes were fused to *lacZ* and expression assayed in wild-type and various mutant strains. The results are illustrated in Figure 9, A–E and summarized in Table 5. Four of the seven genes tested, *YKR075*, *REG2*, *YDR516*, and *HXT13* are regulated by Mig1 and Mig2. Glucose repression of *YKR075* and *YDR516* expression (encoding a protein homologous to *REG1* and a protein similar to Glucokinase 1, respectively) is slightly relieved by deletion of *MIG1* or *MIG2*, and almost completely relieved when both genes are deleted (A and C). *REG2* (B) and *HXT13* (D) are partially derepressed in a *mig1*Δ *mig2*Δ strain, but still exhibit significant (three- to fourfold) repression in the double mutant. Yer028 plays little role in glucose repression of any of these genes, and thus cannot account for the repression remaining in the double mutant. No expression was detected for *HXT15* and *HXT17*, which encode proteins similar to hexose transporters. Glucose repression of *TPS1* (E) was not clearly affected by any of the three mutations.

To identify more comprehensively genes regulated by Mig1, Mig2, and Yer028, expression of more than half of the predicted ORFs in the yeast genome was measured in wild-type (YM4797) and *mig1*Δ *mig2*Δ *yer028*Δ (YM4804) strains using DNA microarrays (Derisi *et al.* 1997; see also materials and methods). Of the ~3325 genes analyzed in this way, 235 exhibit expression at least twofold higher in the triple mutant compared to wild type (Table 6; see materials and methods). Thirty-eight genes have the opposite expression pattern, being at least twofold more highly expressed in the wild-type strain than in the triple mutant. Many of these genes possess at least one (usually several) potential Mig1/Mig2-binding sites. The promoter regions of 17 of the genes in the first class (Table 5, lines 9–25), and 1 of the genes in the second class (Table 5, line 8) were fused to *lacZ* (see materials and methods) and their expression analyzed to determine which of the three repressors affects their expression (data for 7 of these are shown in Figure 10, A–G).

Ten of the 18 genes analyzed in more detail were removed from the analysis for various reasons. Expression of 4 genes (*RIM9*, *AHT1*, *YEL050*, and *YHR054*) could not be detected by the β-galactosidase assay. Six genes yielded results that either did not agree with results from the array [expression levels were no higher in the triple mutant than in wild type (*HSP82*, *HSP60*, and *YLR264*)], or that varied significantly from one experiment to the next (*SSA4*, *HSP26*, and *HSP30*). Five of these 6 genes encode heat-shock proteins that are induced by many conditions, so it is easy to imagine that they could give variable results from experiment to experiment.

Five genes (*HXK1*, *DOG2*, *YEL070*, *YLR042*, *YFL054*; Figure 10, A–E) are clearly regulated by both Mig1 and Mig2 and are repressed to varying degrees by glucose;

TABLE 5

Promoters assayed for regulation by Mig1, Mig2, and Yer028

Promoter	Gene function / homology	Regulation by Mig1, Mig2, or Yer028[a]
1. YKR075[b]	Homologous to *REG1*	Mig1 + Mig2
2. REG2[b]	Homologous to *REG1*	Mig1 + Mig2
3. YDR516[b]	Homologous to *GLK1*	Mig1 + Mig2
4. HXT13[b]	Hexose transporter	Mig1 + Mig2
5. TPS1	Trehalose-6-phosphate synthase	None
6. HXT15	Hexose transporter	Not expressed
7. HXT17	Hexose transporter	Not expressed
8. HXT1[b]	Hexose transporter	Mig1 + Mig2
9. HXK1[b]	Hexokinase I	Mig1 + Mig2
10. DOG2[b]	2-Deoxyglucose-6-phosphate phosphatase (confers resistance to 2-deoxyglucose)	Mig1 + Mig2
11. YEL070[b]	Homologous to d-mannitol 2-dehydrogenase from *Rhodobacter sphaeroides*	Mig1 + Mig2
12. YFL054[b,c]	Similarity to (glycerol, water) channel proteins	Mig1 + Mig2
13. YLR042[b]	No homology	Mig1 + Mig2
14. YBR101[b]	No homology	Mig1 + Mig2
15. SSE2	Heat-shock gene	?
16. SSA4	Heat-shock gene	?
17. HSP26	Heat-shock gene	?
18. HSP30	Heat-shock gene	?
19. HSP82	Heat-shock gene	None
20. HSP60	Heat-shock gene	None
21. YLR264	Ribosomal protein	None
22. RIM9	Regulator of IME2 (meiosis)	Not expressed
23. AHT1	No homology	Not expressed
24. YEL050	Similarity to ribosomal protein	Not expressed
25. YHR054	No homology	Not expressed

[a] See Figures 9 and 10.
[b] Genes regulated by Mig1 and Mig2.
[c] YFL054 and YFL053 (similar to Citrobacter 2-dihydroxyacetone kinase) are divergently expressed from a promoter of ~600 bp. YFL053 is also a candidate gene on the microarray.

this repression is partially relieved by a *mig1*Δ mutation and almost completely relieved by further deletion of *MIG2*. For *HXK1*, *DOG2*, *YEL070*, and *YLR042* (Figure 10, A–D), the effect of deleting *MIG2* is only apparent if *MIG1* is also deleted (as for *SUC2*). For *YFL054* (Figure 10E), deletion of *MIG2* by itself has a modest effect on glucose repression that is much more apparent if *MIG1* is also deleted. Deletion of *YER028* had little or no effect on expression of any of these genes and thus appears not to be involved in their regulation. Thus, all of these genes, like *SUC2*, appear to be regulated by both Mig1 and Mig2, with Mig1 being the primary repressor. No genes solely sensitive to Mig2 or to Yer028 were identified.

The effect on *YBR101* (Figure 10F) expression of deleting *MIG1* and *MIG2* was unusual: glucose repression of this gene is not apparent, and expression is higher in the *mig1*Δ *mig2*Δ mutant, both in the presence and absence of glucose [*SSE2* exhibits a similar pattern of expression (data not shown)]. It is unclear why Mig1 and Mig2 action on this gene is not sensitive to the levels of glucose. A higher level of expression of the other genes in glycerol-grown *mig1*Δ *mig2*Δ cells is also apparent. This is likely due to residual repression activity of Mig1 and Mig2 under nonrepressing conditions. Expression of *HXT1* was reduced almost threefold in the *mig1*Δ *mig2*Δ mutant, under both inducing and noninducing conditions (Figure 10G). Thus Mig1 and Mig2 appear to be modest activators of *HXT1* expression. In fact, *HXT1* expression is induced by high levels of glucose (Ozcan and Johnston 1995), conditions that cause Mig1 and Mig2 to act as transcriptional repressors. While it remains to be seen if Mig1 and Mig2 bind directly to *HXT1*, we note that it contains Mig1-binding sites in its promoter.

DISCUSSION

Mig1 and Mig2 are redundant transcriptional repressors: Mig2 was initially identified as a repressor that collaborates with Mig1 to cause glucose repression of *SUC2* expression (Lutfiyya and Johnston 1996). Because it appeared to have no effect on glucose repression of *GAL1* expression, we wondered if there are other

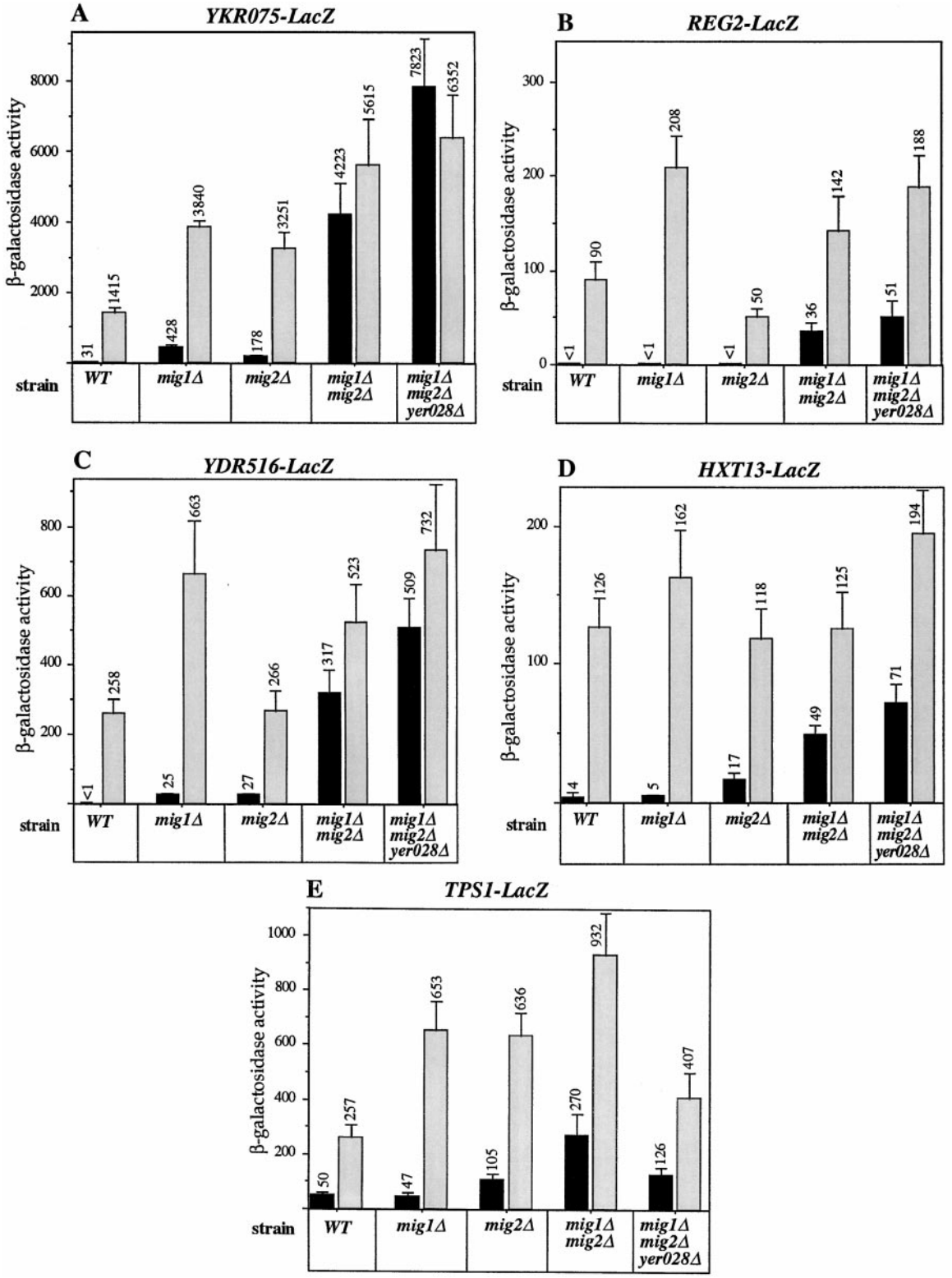


Figure 9.—Regulation of promoters containing Mig1-binding sites. (A–E) β-Galactosidase activity indicating expression levels of the indicated gene promoter fused to *lacZ*. The plasmids assayed were: (A) pBM3469, (B) pBM3459, (C) pBM3461, (D) pBM3465, (E) pBM3473 (Table 2). The strains used were: wild type (WT), YM4797; *mig1*Δ, YM4966; *mig2*Δ, YM4664; *mig1*Δ *mig2*Δ, YM4968; *mig1*Δ *mig2*Δ *yer028*Δ, YM4847. Cells were grown in synthetic media lacking only uracil and containing either 4% glucose (dark bars), or 5% glycerol and 0.05% glucose (light bars). The values are Miller units of β-galactosidase activity averaged from two to three independent transformants assayed in duplicate. Standard errors were usually <25% and except for two were always <30%.

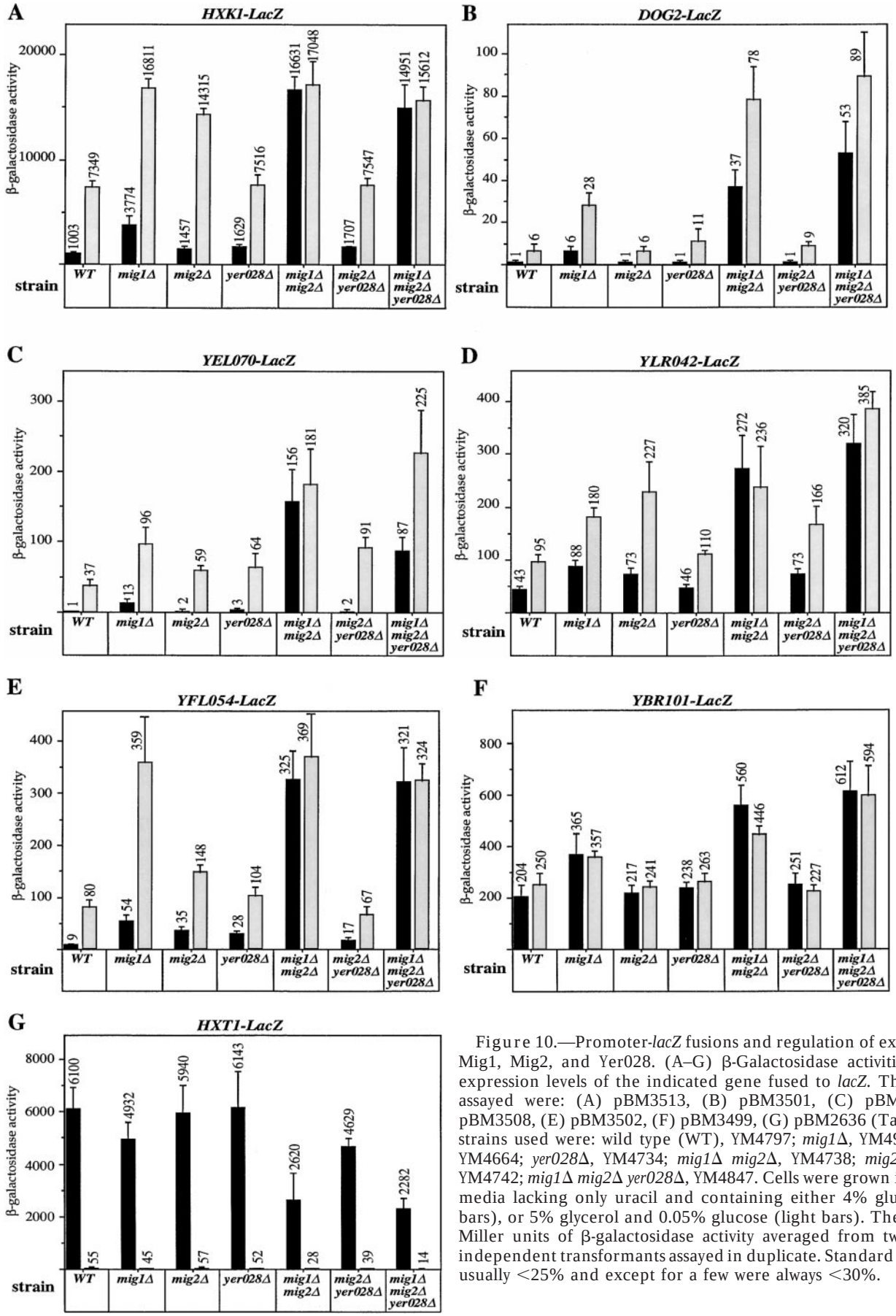


Figure 10.—Promoter-*lacZ* fusions and regulation of expression by Mig1, Mig2, and Yer028. (A–G) β-Galactosidase activities indicate expression levels of the indicated gene fused to *lacZ*. The plasmids assayed were: (A) pBM3513, (B) pBM3501, (C) pBM3506, (D) pBM3508, (E) pBM3502, (F) pBM3499, (G) pBM2636 (Table 2). The strains used were: wild type (WT), YM4797; *mig1*Δ, YM4966; *mig2*Δ, YM4664; *yer028*Δ, YM4734; *mig1*Δ *mig2*Δ, YM4738; *mig2*Δ *yer028*Δ, YM4742; *mig1*Δ *mig2*Δ *yer028*Δ, YM4847. Cells were grown in synthetic media lacking only uracil and containing either 4% glucose (dark bars), or 5% glycerol and 0.05% glucose (light bars). The values are Miller units of β-galactosidase activity averaged from two to three independent transformants assayed in duplicate. Standard errors were usually <25% and except for a few were always <30%.

genes repressed only by Mig1, or some genes repressed only by Mig2. Among several genes in the yeast genome whose promoters contain potential Mig1-binding sites, we found none that are regulated only by Mig1 (Figure 9; Table 5). A more comprehensive survey of the yeast genome revealed many genes apparently regulated by Mig1, Mig2, and/or Yer028 (Table 6), but all of the ones we analyzed in more detail are regulated by both Mig1 and Mig2 (Figure 10). Thus, as far as we can tell, Mig2 always works in conjunction with Mig1.

However, Mig1 appears to be more important than Mig2, since it is sufficient to cause nearly full repression of most of the genes it regulates. An effect of deleting *MIG2* alone is observed only for a few genes. Perhaps Mig2 binds to DNA with lower affinity than Mig1, causing it to repress gene expression only in the absence of Mig1. Alternatively, Mig2 could be regulated post-transcriptionally, so that it is present only at low levels in the presence of Mig1. In any event, it is difficult to believe that Mig2 evolved to repress gene expression only when Mig1 is absent.

DNA-binding specificities of Mig1, Mig2, and Yer028: Why does Mig1 seem to act alone at some promoters (*e.g.*, *GAL1*, *GAL4*, *HXT4*, and *CAT8*; Griggs and Johnston 1991; Flick and Johnston 1992; Hedges *et al.* 1995; Ozcan and Johnston 1995), but with Mig2 at others? One possibility is that these proteins differ in the DNA-binding sites they recognize, despite possessing very similar DNA-binding domains. This is the case for some other related DNA-binding proteins. For example, a single amino acid in the recognition helix of the Bcd homeodomain determines its DNA-binding specificity, thereby distinguishing its binding site from that of similar homeodomain proteins (Treisman *et al.* 1989; Gehring *et al.* 1994). The Elk-1 and SAP-1a ETS-domain transcription factors also recognize slightly different sites due to subtle differences in their DNA-binding domains (Shore *et al.* 1996). The results of the *in vitro* selection for Mig1- and Mig2-binding sites (Figure 8) suggest that the two proteins bind to the same sequence, though there may be subtle differences between them that were not detected in our experiment (we recognize, however, that our experiment would not uncover Mig2-binding sites that differ greatly from the consensus Mig1-binding site). For example, it is not clear if Mig2, like Mig1, requires for binding an AT-rich sequence preceding the GC-box. Similar difficulties in determining subtle differences in binding site specificities between very similar proteins using *in vitro* binding site selections have been noted (Shore *et al.* 1996). Mig1 and Mig2 appear to differ in their relative affinities for their sites (Lutfiyya and Johnston 1996), like two other very similar zinc-finger proteins, NGFI-A and NGFI-C (Swirnoff and Milbrandt 1995), and these affinities correlate well with the ability of these sites to repress gene expression *in vivo* (Figure 7).

Amino acids in the Mig1 zinc fingers important for DNA recognition can be inferred on the basis of the structure of the Mig1 homolog Zif268 (NGFI-A), bound to its recognition site (Pavletich and Pabo 1991). These amino acids (see Figure 1) are identical in Mig1, Mig2, and Yer028. Amino acids other than those contacting the DNA may also have a significant effect on binding site recognition. For example, although Krox-20 and Mig1 share similar zinc-finger domains, Krox-20 has a stricter requirement for its site than does Mig1 (Nardelli *et al.* 1992; Lundin *et al.* 1994). Differences in DNA-binding affinities of NGFI-A and NGFI-C have been shown to be determined by the protein context of the DNA-binding domain, and not by the zinc fingers themselves (Swirnoff and Milbrandt 1995). In addition, some residues important for defining the binding site specificity of Elk-1, an ETS-domain transcription factor, do not contact DNA (Shore *et al.* 1996). Instead, these residues probably affect the way other residues interact with DNA. A true understanding of the different DNA-binding abilities of Mig1 and Mig2 awaits knowledge of their structures.

Yer028 appears to be a glucose-dependent transcriptional repressor: Due to the sequence similarity between Yer028 and Mig1 and Mig2, it seemed likely that Yer028 would function similarly to these proteins. Indeed, LexA-Yer028 is a glucose-regulated transcriptional repressor (Figure 4), and Yer028 binds to the same sites as Mig1 and Mig2 (Figure 6). It is surprising, then, that it does not appear to be functionally redundant with either Mig1 or Mig2. Yer028 appears to have evolved a role in regulating expression of a set of genes separate from those regulated by Mig1 and Mig2.

Because LexA-Yer028 is a glucose-activated repressor that binds to Mig1/Mig2-binding sites, we are mystified by the fact that Yer028 does not cause glucose repression of a reporter gene containing those sites (Figure 7). This is unlikely to be due to lack of expression of *YER028*, because it appears as a strong signal on the expression arrays (data not shown). Since the repression ability of LexA-Yer028 is regulated by glucose, we can only surmise that the DNA-binding ability of native Yer028 is inhibited in glucose-grown cells.

It is surprising that Mig1 and Mig2 appear to be more similar in function than Mig2 and Yer028, because Mig2 is more similar to Yer028 than to Mig1, both within and outside of the zinc fingers. In addition, Mig2 and Yer028 are clearly more closely related, because they arose by duplication of a chromosomal region (Wolfe and Shields 1997). Mig2 and Yer028 are also similar in size, ~380 amino acids, compared to the 504-amino-acid Mig1 protein. It is possible that Mig2 and Yer028 function redundantly in expression of some genes not detected in our DNA microarrays.

Differential regulation of Mig1 and Mig2: Although Mig1 and Mig2 are functionally redundant repressors, there are significant differences in their regulation: *MIG1* expression is glucose repressed about 10-fold by

TABLE 6

Summary: Classification of genes more highly expressed in a *mig1*Δ *mig2*Δ *yer028*Δ strain

Category	No. of genes
(1) Hypothetical (no informative homologies)	100
(2) Metabolism	
(a) Carbohydrate	18
(b) Transporter	5
(c) Other	10
(3) Mitochondrial, respiratory	30
(4) Heat shock, stress response	13
(5) Mating type	4
(6) Other	
(a) Protein synthesis, translation, ribosomal proteins	12
(b) Cell cycle, mitosis, DNA synthesis	10
(c) Protein transport and translocation, vacuolar sorting	8
(d) Transcription	8
(e) Kinase	7
(f) Cytoskeletal	6
(g) Proteasome, protease, ubiquitin	4
Total	235

Mig1 and Mig2, while *MIG2* is constitutively expressed at a higher level than *MIG1* (Table 4). This is surprising considering that Mig1 and Mig2 seem to have equivalent roles in repression of many genes (Figures 9 and 10), with Mig1 apparently playing a more important role in repression.

Mig1 and Mig2 proteins also appear to be regulated differently. The Snf1 protein kinase clearly regulates Mig1 function (Vallier and Carlson 1994; DeVit *et al.* 1997), but does not appear to affect Mig2 (Figure 2; see also Vallier and Carlson 1994). This is consistent with the fact that Mig1 and Mig2 are also regulated at different levels; Mig1 function is regulated by its nuclear localization (DeVit *et al.* 1997), while Mig2 is always present in the nucleus, regardless of carbon source (Figure 3). We thought that proteins similar to Snf1 might act on Mig2, but three of the closest homologs to Snf1 (Hsl1, Gin4, and Ycl024) appear not to affect Mig2 function (Table 3). The role in regulation of Mig2 function of three other kinases more distantly related to Snf1 (Kin1, Kin2, and Kin4) remains to be tested.

Genes regulated by Mig1 and Mig2: We identified 235 genes that exhibit at least twofold higher expression in the *mig1 mig2 yer028* triple mutant strain compared to the wild-type strain. These fall into several functional categories (Table 6). Almost 30% of the genes play a role either in metabolic pathways, or in mitochondrial functions. This is reasonable, because these are two major classes of genes that are known to be glucose repressed (Johnston and Carlson 1992; Trumbly 1992; Ronne 1995). All four of the genes we analyzed in more detail that have known or probable roles in metabolic pathways (*HXK1*, *DOG2*, *YEL070*, and *YFL054*) were glucose repressed by Mig1 and Mig2. Another well-represented class of genes are heat-shock or stress-response genes. About half of the Mig1/Mig2-regulated genes are of unknown and unpredictable function.

Expression of ~11% of yeast genes (~710) is increased by a factor of at least two as glucose is progressively depleted from the medium (*i.e.*, are glucose-repressed genes; Derisi *et al.* 1997). On the basis of analysis of about half of the genes in yeast, we estimate that 10–15% of these genes are regulated by Mig1 and Mig2. Thus, while it is clear that Mig1 and Mig2 are important for glucose repression, other repressors that play a major role in glucose repression of gene expression remain to be identified.

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