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Regulated Nuclear Translocation of the Mig1 Glucose Repressor

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Glucose represses the transcription of many genes in bakers yeast (*Saccharomyces cerevisiae*). Mig1 is a Cys_2-His_2 zinc finger protein that mediates glucose repression of several genes by binding to their promoters and recruiting the general repression complex Ssn6–Tup1. We have found that the subcellular localization of Mig1 is regulated by glucose. Mig1 is imported into the nucleus within minutes after the addition of glucose and is just as rapidly transported back to the cytoplasm when glucose is removed. This regulated nuclear localization requires components of the glucose repression signal transduction pathway. An internal region of the protein separate from the DNA binding and repression domains is necessary and sufficient for glucose-regulated nuclear import and export. Changes in the phosphorylation status of Mig1 are coincident with the changes in its localization, suggesting a possible regulatory role for phosphorylation. Our results suggest that a glucose-regulated nuclear import and/or export mechanism controls the activity of Mig1.

INTRODUCTION

Bakers yeast (*Saccharomyces cerevisiae*) can use several different carbon sources but has evolved mechanisms to ensure that it will preferentially use glucose. Preference for glucose is in part due to repression of transcription of genes not required for growth on glucose, such as genes encoding enzymes for converting other carbon sources to glycolytic intermediates (e.g., *GAL* and *SUC*), gluconeogenesis (e.g., *FBP1* and *PCK1*), and respiration (e.g., *CYC1* and *COX6*) (for reviews, see Johnston and Carlson, 1992; Trumbly, 1992; Ronne, 1995). Mig1 is the repressor responsible for glucose repression of many genes, including *GAL*, *SUC*, and *MAL* (Nehlin and Ronne, 1990; Griggs and Johnston, 1991; Nehlin *et al.*, 1991; Schuller and Entian, 1991; Flick and Johnston, 1992; Johnston *et al.*, 1994; Vallier and Carlson, 1994; Hu *et al.*, 1995; Lutfiyya and Johnston, 1996; Wang and Needleman, 1996). Mig1 is a Cys_2-His_2 zinc finger-containing protein that binds to promoters of glucose-repressed genes and recruits the general repressors Ssn6 and Tup1 (Tzamarias and Struhl, 1994, 1995; Treitel and Carlson, 1995).

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Mig1 function is regulated in response to glucose, and several proteins required for this have been identified. Hxk2 and Reg1 seem to activate the Mig1 repressor, because mutation of either relieves repression (Zimmermann and Scheel, 1977; Entian and Zimmermann, 1980). The role in glucose repression of Hxk2, a hexose kinase, is not understood. Reg1 is thought to be a targeting subunit for the type I protein phosphatase encoded by *GLC7* (Tu and Carlson, 1995). Snf1 is a protein kinase that appears to inhibit Mig1 function in the absence of glucose (Johnston *et al.*, 1994; Vallier and Carlson, 1994). The roles of an opposing kinase and phosphatase in glucose repression suggests that Mig1 function may be regulated by phosphorylation. Indeed, Mig1 has been shown to be more heavily phosphorylated in the absence of glucose than in its presence (Treitel and Carlson, 1995; DeVit, unpublished results), although it has not been shown whether these changes in phosphorylation levels regulate Mig1 activity.

It is not known what activity of Mig1 is regulated by glucose. Its DNA-binding ability does not seem to be regulated: we have found that Mig1 DNA-binding activity is similar in extracts of cells grown in the presence or absence of glucose (DeVit, unpublished results). Furthermore, the ability of a LexA-Mig1 chi-

Table 1. *S. cerevisiae* strains used

Strain	Genotype
YM2175	*MATα ura3-52 his3Δ200 lys2-801 hxk2Δ202 LEU2::GAL1-lacZ*
YM3564	*MATa ura3-52 ade2-101 lys2-801 trp1-901 gal80Δ538 snf1Δ3 ssn6Δ6::URA3*
YM4342	*MATα ura3Δ::LEU2 his3Δ200 trp1-903 lys2-801*
YM4374	*MATa ura3-52 his3Δ200 ade2-101 lys2-801 trp1-901 met⁻ mig1Δ2::LEU2 gal80Δ538 LEU2::GAL1-lacZ*
YM4580	*MATa ura3-52 trp1⁻ leu2⁻ prb1-1122 prc1-407 pep4-3 mig1Δ2::LEU2*
YM4685	*MATα ura3-52 his3Δ200 ade2-101 lys2-801 trp1-901 met⁻ mig1Δ2::LEU2 reg1Δ::hisG gal80Δ538 LEU2::GAL1-lacZ*
YM4901	*MAT? ura3-52 his3Δ200 lys2-801 trp1-901 tyr⁻ ssn6Δ6::ura3Δ::LEU2*
YM4902	*MAT? ura3-52 his3D200 lys2? trp1? gal80? reg1-1*

meric protein to repress transcription via *lexO* sites is regulated by glucose (Treitel and Carlson, 1995; Tzamarias and Struhl, 1995). This leaves transcriptional repression and/or nuclear localization as possible mechanisms for Mig1 regulation. Repression is probably not regulated: replacement of the repression domain of Mig1 with the activation domain of VP16 results in a transcriptional activator whose function is inhibited by Snf1 (Ostling *et al.*, 1996). We have discovered that the nuclear localization of Mig1 is regulated by glucose; we suggest that this is the mechanism by which Mig1 function is regulated.

MATERIALS AND METHODS

Yeast Strains and Growth

Yeast strains are derived from strain S288C (Table 1). Standard methods were used for genetic crosses, sporulation, and tetrad dissection (Rose *et al.*, 1990). Yeast were grown at 30°C in rich (YP) or minimal (YM) medium containing the appropriate carbon source. Yeast transformations were carried out as described by Gietz *et al.* (1992).

Null mutants were made using the following disruption constructions: *ssn6Δ6*, pJS22 cut with *Eco*RI and *Xba*I (Schultz and Carlson, 1987); *mig1Δ2*, pJN22 cut with *Xba*I and *Hin*dIII (Nehlin and Ronne, 1990); *reg1::hisG*, pREG1–4 cut with *Sac*I and *Sal*I (Hoekstra, personal communication).

Plasmids

Standard procedures for the manipulation of plasmid DNA and transformation of bacteria were followed (Sambrook *et al.*, 1989). To assure that no unintentional mutations were introduced into DNA fragments produced by the polymerase chain reaction (PCR) for cloning, the relevant DNA segments were sequenced and/or three independently created clones were examined.

All *MIG1* plasmids (Table 2) are derived from pBM2433 [carries the *MIG1 Xba*I–*Hin*dIII fragment from pMIG1 (Nehlin and Ronne, 1990) inserted between the *Xba*I and *Hin*dIII sites of pRS316, a *URA3-CEN* plasmid (Sikorski and Hieter, 1989)] or pBM2608 (contains the *MIG1 Xba*I–*Kpn*I fragment from pMIG1 inserted between the *Xba*I and *Kpn*I sites of pRS316).

NotI Site at MIG1 ATG (pBM3035). To manipulate the amino terminus of Mig1, a *Not*I site was engineered immediately after the

Table 2. Plasmids used

Plasmid	Description	Parental plasmid
pBM2433	*MIG1 Xba*1–*Hin*dIII fragment in pRS316	pRS316
pBM2608	*MIG1 Xba*I–*Kpn*I fragment in pRS316	pRS316
pBM3034	*Not*I site in polylinker pBM2433 with destroyed	pBM2433
pBM3035	*Not*I site engineered between first and second *MIG1* codons in pBM3034	pBM3034
pBM3076	Three tandem *c-myc* epitopes fused to amino terminus of Mig1	pBM3035
pBM3098	GFP-β-gal fusion in 2μ, *URA3* vector	pVT103-U
pBM3099	SV-40 NLS fused to GFP-β-gal	pBM3098
pBM3182	Mig1 with amino acids 97–391 deleted	pBM2608
pBM3220	Mig1 with amino acids 108–391 deleted	pBM2608
pBM3255	Mig1 with amino acids 174–391 deleted	pBM3182
pBM3315	F64L, S65T variant of GFP fused to amino terminus of Mig1	pBM3035
pBM3350	Mig1 with amino acids 97–173 deleted	pMB2608
pBM3401	Mig1 amino acids 1–167 fused to GFP-β-gal	pBM3098
pBM3403	Mig1 amino acids 261–400 fused to GFP-β-gal	pBM3098
pBM3404	Mig1 amino acids 383–504 fused to GFP-β-galactosidase	pBM3098
pBM3409	GFP-Mig1 with amino acids 40–89 (zinc fingers) deleted	pBM3315
pBM3411	GPF-Mig1 with amino acids 40–89 and 481–504 (repression domain) deleted	pBM3409
pBM3448	GFP-Mig1 with amino acids 108–391 of Mig1 deleted	pBM3315 + pBM3220
pBM3449	GFP-Mig1 with amino acids 174–391 of Mig1 deleted	pBM3315 + pBM3255
pBM3451	GFP-Mig1 with amino acids 97–173 of Mig1 deleted	pBM3315 + pBM3350

Table 3. Oligonucleotides used

Oligonucleotide	Sequence
OM259	GTAAAACGACGGCCAGT (M13-20 primer)
OM377	GCAAGCTTCTAATCGATACTAGAGTTAGACGG
OM558	GGATAACAATTTCACACAGG (in pRS316 polylinker)
OM722	GCGCGCGGCCGCCAAAGCCCATATCCAATG
OM723	GCGCGCGGCCGCCCATGGCTATGGTAGTATG
OM786	GGCGGCGGCCGCAGTAAAGGAGAAGAA
OM787	CGGCGCGGCCGCCTTTGTATAGTTCATCCAT
OM827	GCCCGGCCGTGGTGAACAAAAGTTGATTTCT
OM828	GGCCGGCCGGCGTTCAAGTCTTCTTCTGT
OM978	CCGCCTCGAGGCATGAACCAAAATCACTTG
OM984	GCGCGTCGACCGTCATTCCCACCACTTTC
OM1085	GCCGCTCGAGTCAGTCCATGTGTGGGAA
OM1086	GGCCGTCGACTGCCCACAACCTTCTTCTT
OM1106	GCGGCTCGAGCACCATATATGGTTTCTTCT
OM1108	GCGGCTCGAGCTTCCACTGCTTTGTCT
OM1168	CGCGGATCCATGCAAAGCCCATATCC
OM1177	GCGGGATCCCGCCGATTTCGAATTTGG
OM1178	GCGGGATCCCGTCCATGTGTGGGAAG
OM1180	GCGGGATCCCGGAGAAATGCAAGTGATT
OM1181	GGCAGATCTATGAATTCTCTTTCACCACG
OM1182	GGCAGATCTATGACGAATTTGCACACTTTG
OM1191	CCGGAATTCCAGCATGTTGGTCTTGGC
OM1192	AAACTTGTCAGCGTATCACGACTGAGAAATGGGGG

Relevent restriction sites are underlined.

initiation codon of *MIG1*. First, the *Not*I site in the polylinker of pBM2433 was destroyed by digestion with *Not*I, treated with the Klenow fragment of DNA polymerase I, and religated, creating pBM3034. A new *Not*I site was created by joining the following DNA fragments: 1) an *Xba*I–*Not*I fragment containing the region upstream of *MIG1* and its initiation codon amplified by PCR from pBM2433 with oligonucleotides OM259 (Table 3) and OM723 (adds the *Not*I site after the initiation codon); 2) a *Not*I–*Nar*I fragment of *MIG1* sequence downstream of the initiation codon amplified from pBM2433 with oligonucleotides OM377 and OM722 (adds a *Not*I site before the second codon of *MIG1*); and 3) a 7.4-kb vector fragment produced by digestion of pBM3034 with *Xba*I and *Nar*I. The resulting plasmid pBM3035 contains the *Xba*I–*Hin*dIII *MIG1* fragment as pBM2433 but with the following sequence between the first and second codons of *MIG1*: GGCGGCCGC.

Green Fluorescent Protein (GFP)-Mig1 (pBM3315). The coding sequence of the F64L, S65T variant of the GFP (provided by Lucy Robinson, Louisiana State University, Shreveport, LA) amplified by PCR using primers OM786 and OM787 (which replace the GFP initiation and termination codons with *Not*I sites) was inserted into the *Not*I site of pBM3035.

(c-myc)$_3$-Mig1 (pBM3076). Three *c-myc* epitopes amplified by PCR of pBM2955 (three tandem *c-myc* epitopes in pUC119, provided by David Pellman, Whitehead Institute) with oligonucleotides OM827 and OM828 (which add *Eag*I sites just before and after the *c-myc* coding sequences) were inserted into the *Not*I site of pBM3035. The resulting *c-myc* epitope-tagged Mig1 protein is fully functional for repression of *GAL1* (our unpublished result).

GFP-Mig1 Deleted for Amino Acids 108–391 (pBM3448). First, the sequence between the *Xho*I and *Hin*dIII sites of pBM2608 was replaced with *MIG1* DNA (*Xho*I–*Hin*dIII) amplified by PCR from pBM2433 using oligonucleotides OM978 (introduces a *Xho*I site before codon 392) and OM558. The resulting plasmid pBM3182 was further modified by replacing the sequence between the *Xho*I and *Nar*I sites with a PCR-amplified *Sal*I–*Nar*I fragment of pBM2433 using oligonucleotides OM1086 (introduces a *Sal*I site after codon 107) and OM375, producing pBM3220. Finally, the GFP coding sequence was introduced by recombination in yeast: A 1.5-kb *Nae*I–*Xho*I fragment of pBM3315 was cotransformed with the 7- to 8-kb vector fragment produced by digestion of pBM3220 with *Xba*I and *Nar*I. Plasmids from extracts of Ura$^+$ transformants were transformed into bacteria for amplification and analysis to confirm the presence of the deletion and the GFP coding sequence.

GFP-Mig1 Deleted for Amino Acids 173–391 (pBM3449). The sequence between the *Nar*I and *Xho*I sites of pBM3182 was replaced with a *Sal*I–*Nar*I *MIG1* fragment amplified by PCR from pBM2433 with oligonucleotides OM375 and OM984 (introduces a *Sal*I site after codon 173) to produce pBM3255. The GFP coding sequence was introduced by recombination in yeast as described for pBM3448.

GFP-Mig1 Deleted for Amino Acids 96–173 (pBM3451). Sequence between the *Xho*I and *Cla*I sites of pBM2608 was replaced with a *Xho*I–*Cla*I DNA fragment generated by PCR amplification of pBM2433 with oligonucleotides OM1085 and OM1106 (introduces an *Xho*I site before codon 174), creating pBM3350. The GFP coding sequence was introduced by recombination in yeast as described for pBM3448.

GFP-Mig1 Deleted for Amino Acids 40–89 and 481–504 (pBM3411). First, the sequence between the *Xba*I and *Eco*RI sites of pBM2433 was replaced with a PCR-derived fragment (*Xba*I–*Eco*RI) amplified from pBM3315 with oligonucleotides OM259 and OM1191 (introduces an *Eco*RI site after codon 39 of *MIG1* in the *GFP-MIG1* fusion), producing pBM3409. The second deletion was made by replacing the sequence between the two *Spe*I sites in pBM3409 with a *Spe*I DNA fragment deleted for *MIG1* codons 481–504. This fragment was produced by two PCR amplifications: First, a fragment of pBM2433 was amplified with oligonucleotides OM1108 and OM1192 (anneals to codons 475–480, and to the termination codon plus the immediate 3′ untranslated sequence, thereby deleting codons 481–504). This DNA fragment was extended in a second PCR with oligonucleotides OM558 and OM1108, and pBM3255

(oligonucleotide OM1108 cannot anneal in pBM3255, preventing synthesis of wild-type product from this template).

GFP-β-Galactosidase (GFP-β-gal) Chimera. pBM3098 (2μ, *URA3* selectable marker, provided by Jim Haseloff, Medical Research Council Laboratory of Molecular Biology) contains a fusion of the coding sequence of wild-type GFP upstream of the *Escherichia coli lacZ* gene in vector pVT103-U (Vernet *et al.*, 1987). A *Bam*HI site located between the *ADH1* promoter and the GFP coding sequence is used to make fusions. It was necessary to introduce an initiation codon upstream of sequences inserted at this site.

Simian Virus 40 (SV-40) NLS-GFP-β-gal. pBM3099 contains the coding sequence of the SV-40 nuclear localization signal inserted at the *Bam*HI site (provided by Jim Haseloff, Medical Research Council Laboratory of Molecular Biology).

Mig1 Amino Acids 1–167-GFP-β-gal (pBM3401). *MIG1* sequence amplified from pBM2433 with oligonucleotides OM1168 (introduces a *Bam*HI site before the first codon of *MIG1*) and OM1177 (introduces a *Bam*HI site after *MIG1* codon 167) was inserted into the *Bam*HI site of pBM3098.

Mig1 Amino Acids 261–400-GFP-β-gal (pBM3403). *MIG1* sequence amplified from pBM2433 with oligonucleotides OM1181 (introduces a *Bgl*II site and an initiation codon before *MIG1* codon 261) and OM1180 (introduces a *Bam*HI site after *MIG1* codon 400) was inserted into the *Bam*HI site of pBM3098.

Mig1 Amino Acids 383–504-GFP-β-gal (pBM3404). *MIG1* sequence amplified from pBM2433 with oligonucleotides OM1182 (introduces a *Bgl*II site and an initiation codon before *MIG1* codon 383) and OM1178 (introduces a *Bam*HI site after *MIG1* codon 504) was inserted into the *Bam*HI site of pBM3098).

Measurement of Glucose Repression

Repression by Mig1 deletions was determined by measuring β-gal expressed from a *GAL1-lacZ* fusion (pRY181) integrated at the *LEU2* locus as described previously (Yocum *et al.*, 1984), except cell densities (OD_{600}) and product formation (OD_{420}) was quantified in microtiter plates on a Molecular Devices plate reader. Yeast were grown in minimal medium lacking uracil and containing 2% glucose (repressing conditions) or 5% glycerol (nonrepressing conditions) to midlogarithmic phase (OD_{600} of about 1.0). Duplicate yeast cultures from at least three independent clones were assayed for each plasmid construction.

Imaging of GFP-Mig1

Yeast strains containing GFP-Mig1 fusions were grown to early logarithmic phase (OD_{600} of less than 0.5) in medium described in the figures for each experiment. The strains used for imaging are *ADE2*$^+$ or are grown in the presence of 20 mg/l adenine to reduce the background fluorescence observed in *ade2* mutants. Cells from 1-ml cultures were harvested by centrifugation, washed with water containing the appropriate carbon source to remove fluorescent compounds from the medium, and then resuspended in a small volume (about 50 μl) of water plus carbon source. One microliter of the cell suspension was placed on an agarose pad on a microscope slide as previously described (Waddle *et al.*, 1996). The agarose pad contains the same carbon source present during growth except where indicated. Because *snf1*Δ, *ssn6*Δ strains grow poorly, especially in the absence of glucose, these strains were grown in rich medium containing glucose. Plasmid was maintained in this strain in the absence of selection in a sufficient fraction of cells for imaging. Derepressing conditions were established for the *snf1*Δ, *ssn6*Δ strain by transferring cells to rich medium containing glycerol for 2 h before imaging. To visualize nuclear DNA cells were grown overnight in the presence of 0.5 μg/ml 4,6-diamidino-2-phenylindole (DAPI).

To determine the effect of glucose concentration on the subcellular localization of Mig1 (see Figure 3), 1-ml cultures were first grown overnight on medium containing 5% glycerol. The cells were collected by centrifugation then incubated for 2 min in 1 ml of fresh medium containing glucose concentrations indicated in Figure 3. The cells were concentrated and placed on agarose pads containing the same concentration of glucose and imaged within 5 min.

To determine the rate of Mig1 nuclear import, cells were grown overnight on medium containing 5% glycerol, washed, and concentrated as described above. A 1-μl aliquot was placed on an agarose pad containing 2% glucose and the slide was rapidly placed on the microscope stage and imaging started within 30 s after the cells were placed on the pad. To determine the rate of nuclear export, cells were grown overnight in medium containing 5% glycerol, and then 2% glucose was added to induce nuclear import (*MIG1* is subject to glucose repression, so Mig1 levels are higher, and therefore more easily detectable, after growth on glycerol). The cells were washed and concentrated in a volume of about 30 μl of water containing 2% glucose. A 1-μl aliquot was then placed on an agarose pad containing no glucose. The resulting dilution of the glucose into the agarose pad is sufficient to induce nuclear export. The slide was rapidly placed onto the microscope stage and images were recorded at the time points indicated in Figure 2B after placing the cells on the agarose.

Cells were viewed at room temperature on a BMax-60F microscope (Olympus, Lake Success, NY) equipped with Nomarski differential interference contrast and fluorescence optics. GFP was visualized with filter set 41014 (Chroma Technology, Brattleboro, VT). High dynamic range still images (12 bits per pixel) were taken with a 512 × 1024 back-illuminated thinned electronic frame transfer charge-coupled device camera with 15-μm-square pixels (Princeton Instruments, Trenton, NJ). The camera was controlled by an Optiplex GXPro200 computer (Dell Computer, Austin, TX) running WinView software (v. 1.6.2.1, Princeton Instruments). After acquisition, the raw 12-bit WinView images were transferred to a Power Macintosh computer, linearly scaled to 8 bits ([max pixel value − min pixel value]/256), inverted to conform to the Macintosh greyscale range, and saved as tagged image file format (TIFF) files by using a batch file conversion program (Spe2Tiff, written by and freely available on request from jwaddle@genetics.wustl.edu). The brightness and contrast of each image was optimized by using NIH Image 1.60 (NIH Image was written by Wayne Rasband at the National Institutes of Health and is available by anonymous ftp at zipp.nimh.nih.gov). Image sets were assembled and annotated using Canvas 3.5.4 (Deneba Systems, Miami FL) and printed on a Tectronix Phaser 440 printer (Tectronix, Wilsonville OR). All images were taken with 100× magnification. Scale bars represent 5 μm.

For time-lapse imaging, the analog video signal (RS-170) from the Pentamax camera was used as input to an AG-5 frame grabber (Scion, Fredrick, MD) in a Power Macintosh 8100/80 computer. One frame per second movies were made by using the NIH Image 1.6 "Make movie" command. Camera exposure times were held constant at 1 s as cells were monitored over a 100-s interval. Six frames from 20-s intervals are shown in Figure 2A.

Phosphorylation of Mig1

To determine the rate of phosphorylation of Mig1, a 50-ml culture of a protease-deficient strain (YM4580) expressing a triple *c-myc-*

Figure 1 (facing page). Subcellular localization of GFP-Mig1 under repressing (glucose) or derepressing (glycerol) conditions. Yeast strain YM4342 expressing GFP-Mig1 from a plasmid (pBM 3315) was grown on YM-uracil + 2% glucose (A, C, and E) or YM-uracil + 5% glycerol (B, D, E and F). The cells were stained with DAPI and then imaged for GFP fluorescence (A and B), for DAPI fluorescence (C and D), and by Nomarski optics (E and F). Arrows indicate examples of nuclear localization (compare overlapping location of GFP and DAPI fluorescence at arrowheads in A and C) or the apparent nuclear exclusion of Mig1 on glycerol (compare the absence of fluorescence at arrowhead in B with location of DAPI

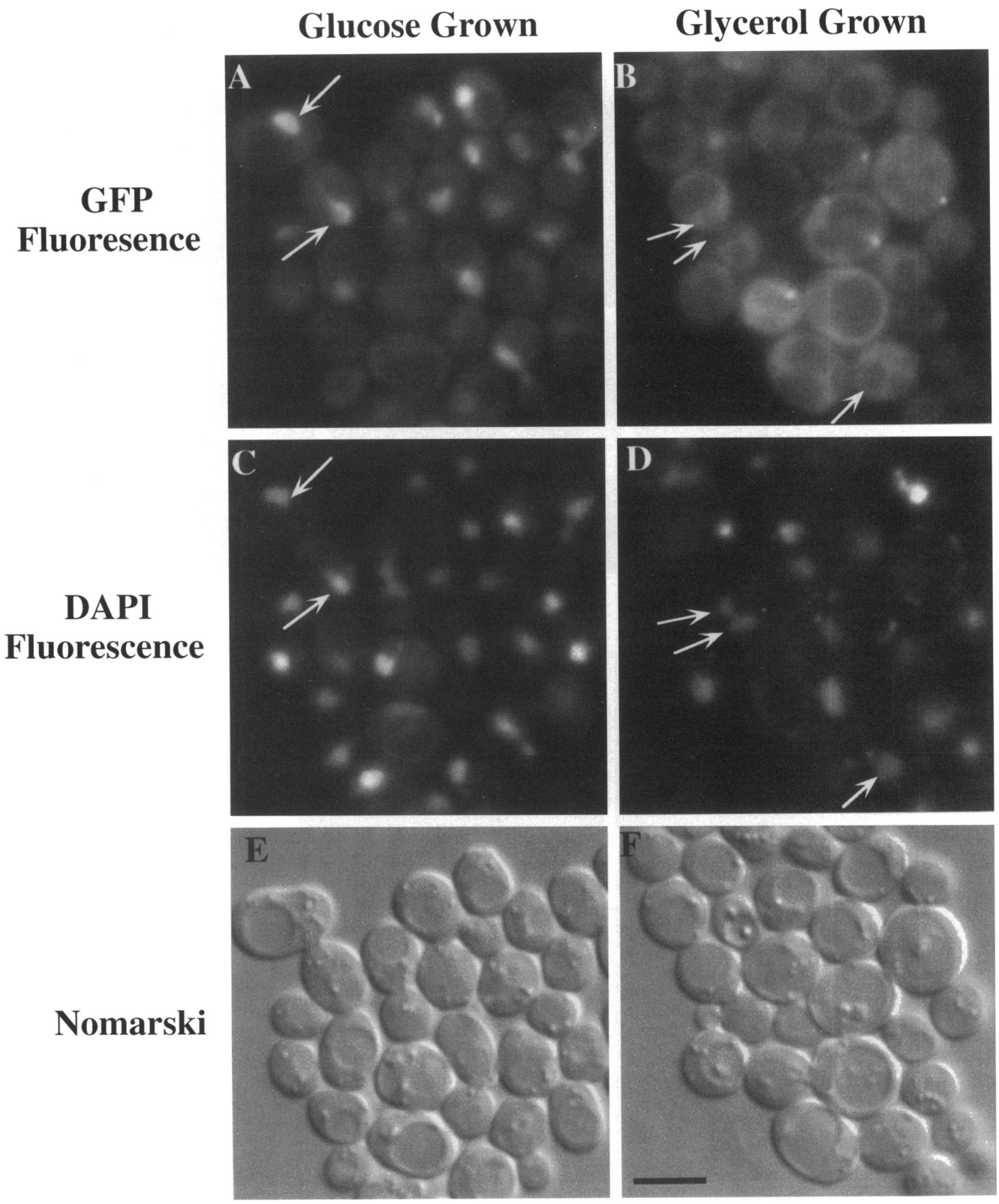


Figure 1 (cont). fluorescence at arrowhead in D). Bar, 5 μm. The cells that do not show strong nuclear fluorescence have their nuclei in a different focal plane: nuclear fluorescence is apparent by changing the focus.

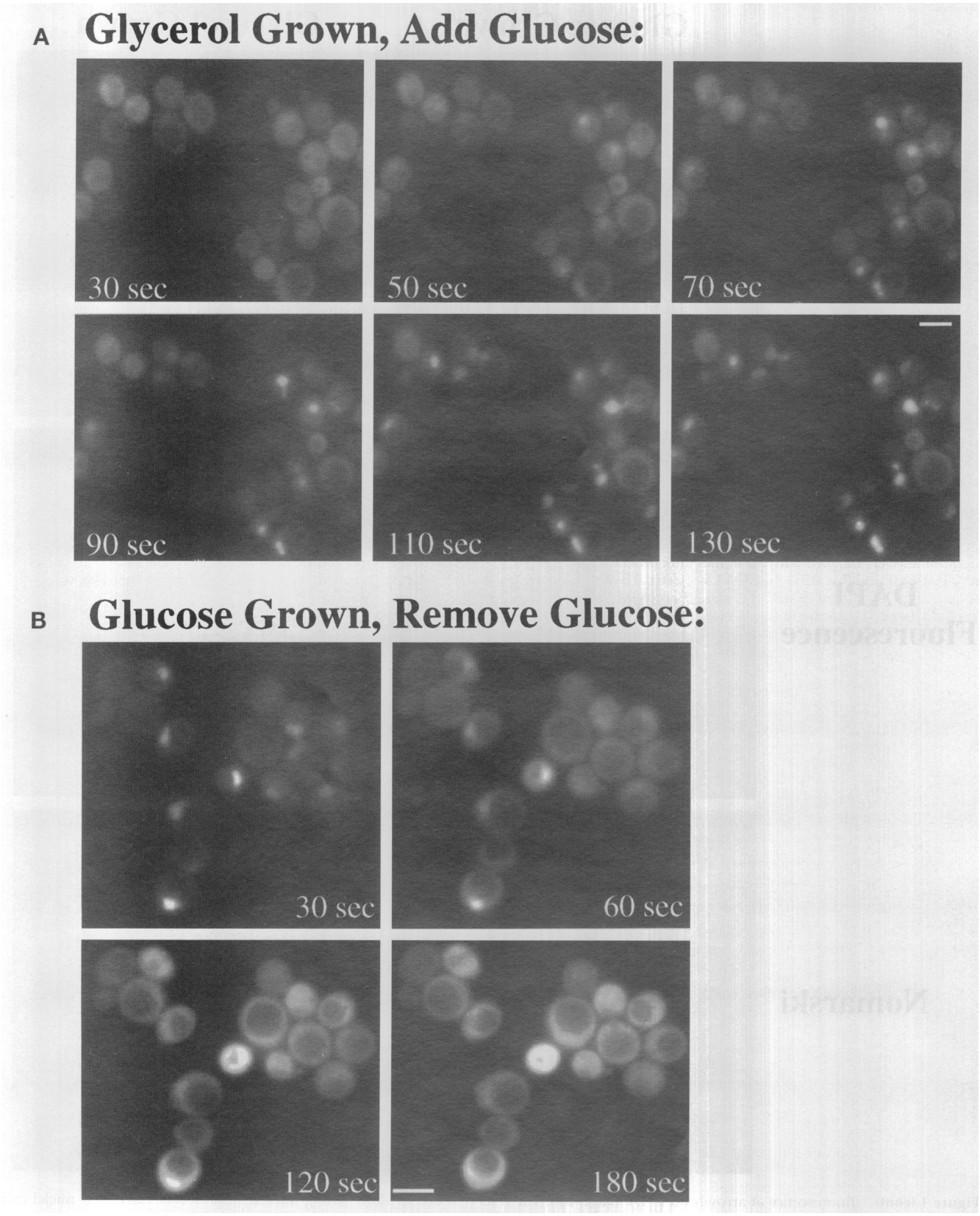

A
Glycerol Grown, Add Glucose:
30 sec
50 sec
70 sec
90 sec
110 sec
130 sec
B
Glucose Grown, Remove Glucose:
30 sec
60 sec
120 sec
180 sec

Figure 2.

epitope tagged Mig1 was grown in YM-uracil containing 2% glucose to midlogarithmic phase (OD_{600} about 1.0). The cells were concentrated by centrifugation to a volume of 3 ml in the same medium and a 200-μl sample was removed and processed for immunoblotting, as described below. The remaining cells were centrifuged and the medium was replaced with YM-uracil containing 5% glycerol. Samples were taken at the time points indicated in Figure 4B.

The rate of dephosphorylation of Mig1 was determined in a similar manner, except that the cells were grown to an OD_{600} of 0.8 and then transferred to medium containing 5% glycerol for 6 h. The cells were concentrated into a volume of 3 ml and a 200-μl sample was removed for immunoblotting, as described below. To the remaining cells, 300 μl of 40% glucose was added and samples were taken at time points indicated in Figure 4A.

Samples were immediately lysed by mixing with 200 μl of ice-cold 2 N NaOH and 8% β-mercaptoethanol (Yaffe and Schatz, 1984). After 10 min, 200 μl of ice-cold 50% trichloroacetic acid was added to precipitate the proteins. After 10 min, the samples were centrifuged for 5 min at 10,000 × *g*. The pellets were rinsed (not resuspended) with 0.5 M Tris base, washed with ice-cold acetone, and air dried. The pellets were resuspended in sample buffer (100 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.1% β-mercaptoethanol, 0.5 mM Na_3VO_4, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride) and boiled for 5 min.

Mig1 Detection

Samples were fractionated by SDS-PAGE on 8% gels. Proteins were electroeluted to an Immobilon polyvinylidene difluoride membrane for 10 h at 12 V by using an Idea Scientific Genie apparatus. Membranes were blocked with 5% nonfat milk powder in TTBS (100 mM Tris-HCl, pH 7.5, 0.9% NaCl, 0.1% Tween 20). Mig1 was detected with 9E10 monoclonal antibody (Berkley Antibody Company, Richmond, CA) and then with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Amersham, Arlington Heights, IL) and visualized using an ECL kit (Amersham).

RESULTS

Nuclear Localization of Mig1 Is Regulated by Glucose

The subcellular localization of Mig1 was monitored by fusing it to the GFP of *Aqueora victoria* (Chalfie *et al.*, 1994). The resulting chimeric protein, which was expressed from the *MIG1* promoter on a *CEN*-containing plasmid, is fully functional as determined by its ability to restore glucose repression of *GAL1* expression in a *mig1*Δ yeast strain (our unpublished observation). In cells grown overnight in 2% glucose, Mig1 is localized in the nucleus, as expected for a glucose-dependent transcriptional repressor (Figure 1A). Identical results are seen when cells are grown in fructose, another repressing carbon source (our unpublished result). In cells grown overnight on glycerol, conditions under which it does not repress transcription, Mig1 is found in the cytoplasm, apparently excluded from the nucleus (Figure 1B). Mig1 is also predominantly localized to the cytoplasm during growth on other nonrepressing carbon sources, such as raffinose, sucrose, and ethanol (our unpublished results). These results suggest that the cell regulates Mig1 function by controlling the transport of the protein into and out of the nucleus.

Mig1 Moves into and out of the Nucleus Rapidly in Response to Glucose Availability

Glucose repression and derepression of gene expression are rapid, occurring within minutes of the addition or removal of glucose (Flick and Johnston, 1990; Sierkstra *et al.*, 1992; Johnston *et al.*, 1994). If nuclear transport is the regulated function of Mig1, then the rates of its appearance in and exit from the nucleus should be at least as fast as the rates of establishment and relief of repression. When glucose is added to glycerol-grown cells, fluorescence is first detectable in the nucleus within 30 s and appears to be entirely nuclear within 2 min (Figure 2A). When glucose-grown cells are rapidly washed to remove glucose, all fluorescence disappears from the nucleus and appears in the cytoplasm in 2–3 min (Figure 2B). The disappearance of Mig1 from the nucleus is induced simply by the removal of glucose or fructose: if any other carbon source, or water alone, is used to wash the cells, Mig1 quickly disappears from the nucleus (our unpublished observation). The same result was obtained when cycloheximide was added 10 min before removing glucose (our unpublished observation), demonstrating that the appearance of fluorescence in the cytoplasm is not due to synthesis of new GFP-Mig1 protein. Readdition of glucose to cells washed in the presence of cycloheximide causes fluorescence to rapidly reappear in the nucleus, suggesting that the disappearance of Mig1 from the nucleus is not due to its degradation. The rapid rate of nuclear translocation of Mig1 is thus consistent with its proposed role in regulating Mig1 function and glucose repression.

To determine the level of glucose that induces nuclear entry of Mig1, cells were grown overnight on glycerol then shifted to various concentrations of glucose for 2 min before examination (Figure 3). All detectable Mig1 is nuclear on 1% glucose (60 mM, Figure 3A) and cytoplasmic on 0.005% (0.3 mM, Figure 3F). At intermediate concentrations (0.01–0.5%), the ratio of nuclear to cytoplasmic Mig1 increases with the amount of glucose (Figure 3, B–E).

Figure 2 (facing page). Time course of appearance (A) and disappearance (B) of GFP-Mig1 in the nucleus. (A) Yeast strain YM4342 was grown on YM-uracil + 5% glycerol and fluorescence was imaged after addition of glucose to 2% to induce nuclear import. Frames from the 20-s intervals are shown. (B) The same strain was grown on YM-uracil + 2% glucose, the glucose was diluted to induce nuclear export, and fluorescence was imaged at indicated times after glucose removal. Bar, 5 μm.

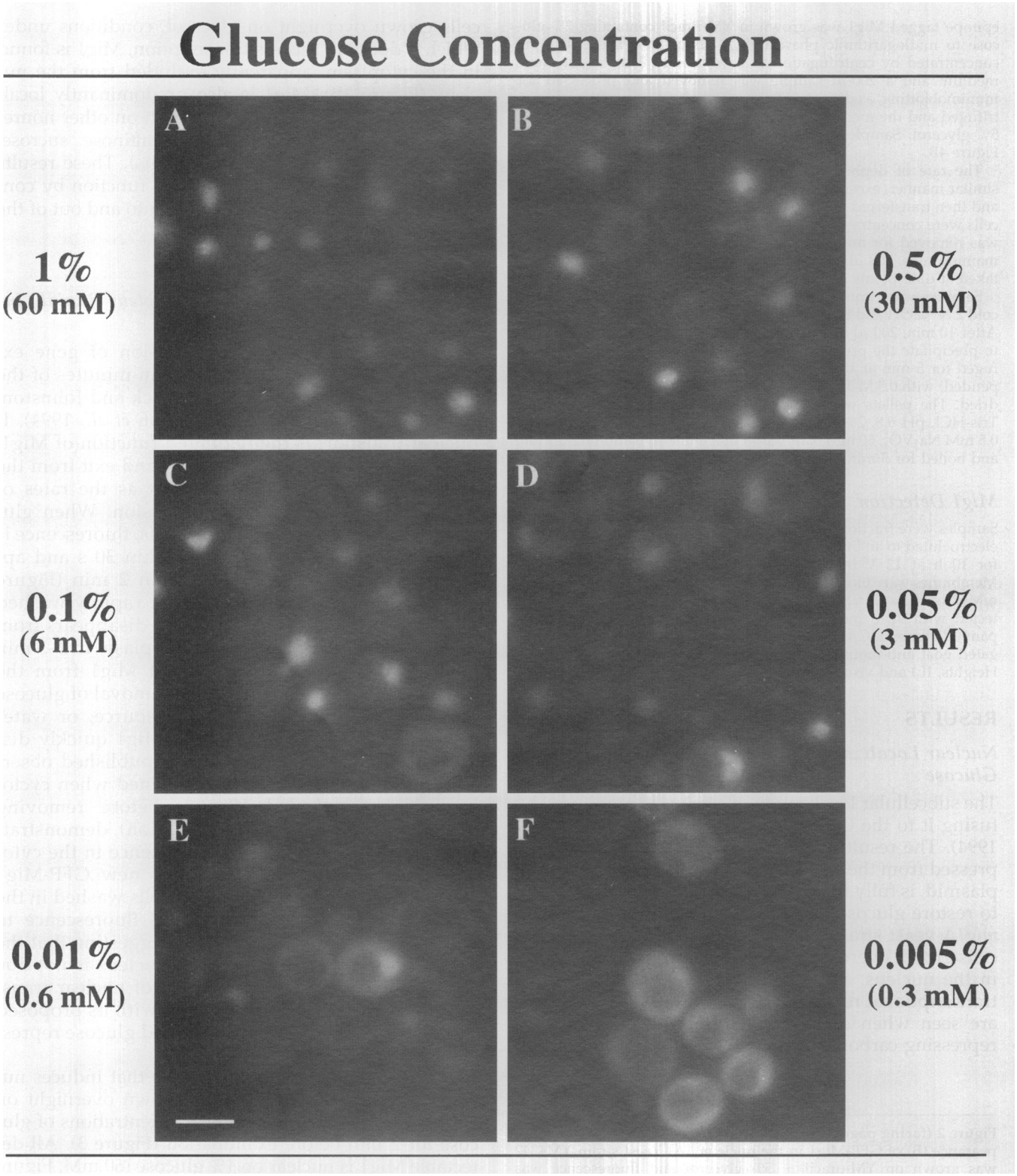


Figure 3. Effect of glucose concentration on the subcellular localization of GFP-Mig1. Strain YM4342 grown overnight on YM-uracil + 5% glycerol was shifted to the indicated concentrations of glucose for 2 min before imaging. Bar, 5 μm.

Mig1 Is Rapidly Dephosphorylated upon Addition of Glucose and Rapidly Phosphorylated upon Its Removal

Mig1 is more heavily phosphorylated in the absence of glucose than it is in the presence of glucose (Treitel and Carlson, 1995; DeVit, unpublished results). This glucose-dependent change in the phosphorylation status of Mig1, and the requirement for a kinase (Snf1) and a phosphatase (Reg1-Glc7) for glucose repression, raises the possibility that Mig1 activity may be regulated by phosphorylation. Phosphorylation would promote nuclear export and/or block import and dephosphorylation would promote nuclear import and/or prevent export. If this is the mechanism of regulation of Mig1 function, then dephosphorylation of Mig1 should occur at least as fast as its appearance in the nucleus, and its rate of phosphorylation should correlate with its rate of nuclear export. Cells grown on glucose were shifted to glycerol, and Mig1 in extracts prepared at various times was fractionated by SDS-PAGE and detected by immunoblotting. A decrease in the mobility of Mig1 on SDS-PAGE occurs within 2 min after removal of glucose (Figure 4B). This decrease in mobility appears to be due to phosphorylation since phosphatase treatment of the extracts erases the slower migrating forms of Mig1 (our unpublished result; also demonstrated by Treitel and Carlson, 1995). Addition of glucose to cells grown on glycerol caused dephosphorylation of Mig1 (increased mobility on SDS-PAGE), which also occurs in about 2 min (Figure 4A). Thus, changes in the phosphorylation state of Mig1 occur as fast as changes in its subcellular localization.

Regulation of Nuclear Localization of Mig1 Depends on an Intact Glucose Repression Pathway

Glucose repression requires several gene products that probably regulate (directly or indirectly) Mig1 activity and therefore might regulate its nuclear translocation. Reg1 and Hxk2 are required for glucose repression of *GAL1* and other glucose repressed genes and are also required for the glucose-induced nuclear localization of Mig1 (Figure 5, A–D). A small amount of fluorescence is visible in the nuclei of some cells, and this correlates well with the small amount of repression remaining in these mutants. Similar results are seen in *GAL82* and *GAL83* mutants grown on glucose, which exhibit only a partial repression defect (our unpublished observation). Ssn6 (Figure 5, E and F) and Tup1 (our unpublished observation), on the other hand, are not required for glucose-induced nuclear localization of Mig1. These proteins, which are recruited to glucose-repressed promoters by Mig1 and are thought to be responsible for inhibiting transcription (Keleher *et al.*, 1992; Tzamarias and Struhl, 1994, 1995; Treitel and Carlson, 1995), presumably act after or at the same time that Mig1 is activated for repression, so we would predict that they are not required for regulating Mig1 localization.

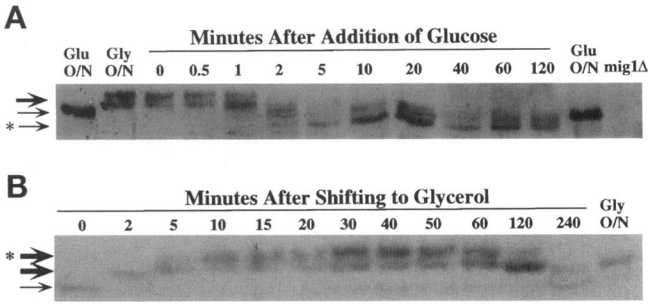


Figure 4. Rates of phosphorylation and dephosphorylation of Mig1. Yeast strain YM4580 expressing a triple-*c-myc* epitope-tagged Mig1 from plasmid pBM3076 was grown on YM-uracil + 5% glycerol and then glucose was added to 2% (A) or was grown on YM-uracil + 2% glucose and then shifted to YM-uracil + 5% glycerol (B). A sample was taken before and then at the times indicated after shifting carbon source. The samples were lysed and fractionated by SDS-PAGE, and Mig1 was detected by immunoblotting using anti-*c-myc* antibody. The thin arrows indicate the less-phosphorylated form associated with the presence of glucose; the thick arrows indicate the more heavily phosphorylated forms associated with the absence of glucose. The arrow marked with an asterisk indicates a transient apparently hypophosphorylated from in A; and a transient apparently hyperphosphorylated form in B. Due to unavoidable difficulties in accurately determining the protein concentration of the extracts, the actual amount of protein loaded in each lane varied somewhat. O/N signifies overnight growth on indicated carbon source. mig1Δ indicates a sample of YM4580 carrying empty vector (pRS316).

The Snf1 protein kinase inhibits Mig1 function (Johnston *et al.*, 1994; Vallier and Carlson, 1994), and so we expected Mig1 to be always localized in the nucleus in a Snf1 mutant. We were not able to detect any fluorescence in a *snf1* mutant carrying the GFP-Mig1 fusion, possibly because Mig1 represses its own expression. To prevent autorepression, we expressed GFP-Mig1 in a *snf1* mutant that is also deleted for *SSN6*, thereby relieving Mig1-mediated repression of GFP-Mig1 expression. The *ssn6* mutation itself does not affect nuclear localization of Mig1 (Figure 5, E and F). In this double mutant, Mig1 is localized to the nucleus even in cells shifted to glycerol for 2 h (Figure 5, G and H). Thus the *snf1* mutation, which causes constitutive repression by Mig1, also results in constitutive nuclear localization of Mig1.

An Internal Region of Mig1 Contains Sequences Required for Regulated Nuclear Localization

Two domains of Mig1 are required for repression: the zinc-finger DNA-binding domain at the amino terminus (amino acids 40–94), and a transcriptional repres-

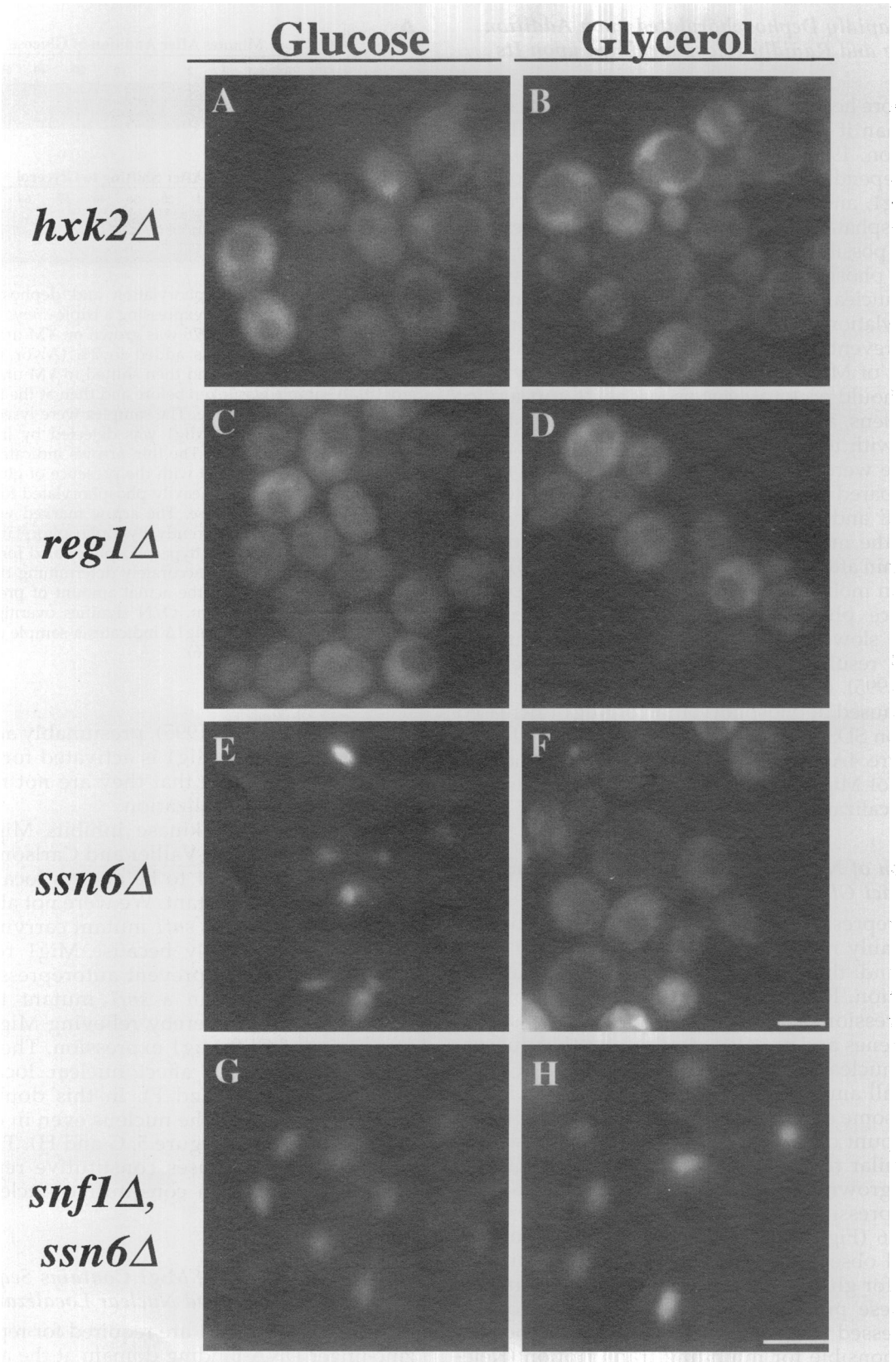


Figure 5.

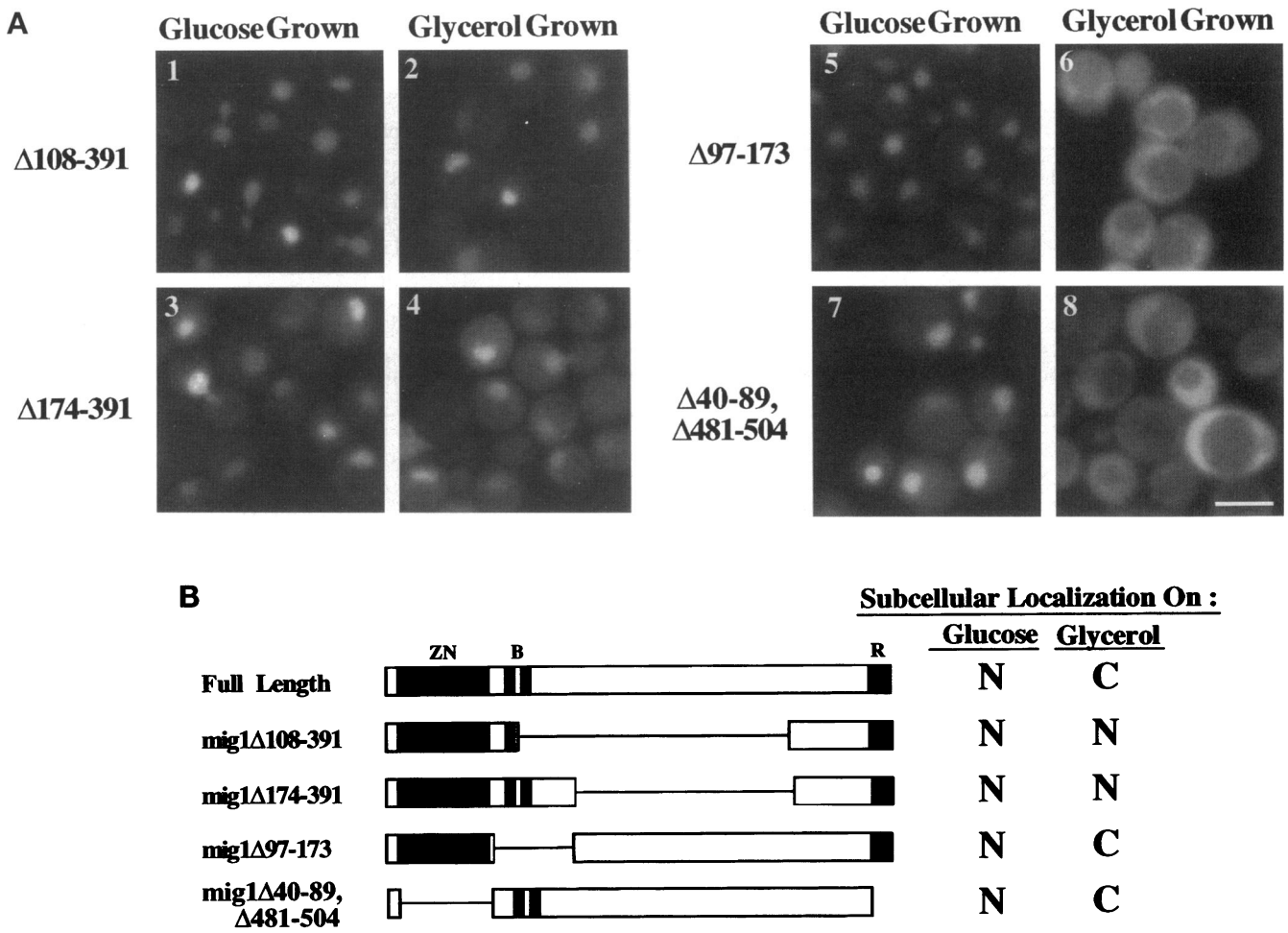


Figure 6. Internal region of Mig1 regulates its nuclear localization. (A) Wild-type (YM4342) yeast strains carrying plasmid-borne internal deletions of Mig1 fused to GFP were grown on YM-uracil + 2% glucose or YM-uracil + 5% glycerol and then imaged for GFP fluorescence. Deletions remove the amino acids indicated. Bar, 5 μm. (B) Schematic representation of each deletion and summary of subcellular localization. ZN, Zinc fingers; B, region rich in basic amino acids; R, domain required for repression; N, nuclear localized; C, cytoplasmic localization.

sion domain within the carboxyl-terminal 25 amino acids that probably mediates interaction with Ssn6 and Tup1 (Ostling *et al.*, 1996). Neither of these domains is required for regulated nuclear translocation (Figure 6A, parts 7 and 8). Thus, accumulation of Mig1 in the nucleus is not simply a consequence of its interaction with DNA or Ssn6/Tup1.

Figure 5 (facing page). Subcellular localization of GFP-Mig1 in glucose repression mutants. Yeast strains lacking *hxk2* (YM2175, A and B), *reg1* (YM4902, C and D), *ssn6* (YM4901, E and F), or *snf1* and *ssn6* (YM3564, G and H) were grown on YM-uracil + 2% glucose (A, C, and E), YM-uracil + 5% glycerol (B, D, and F), YP + 2% glucose (G), or YP + 2% glucose, then switched to YP + 5% glycerol for 2 h (F), and then imaged for GFP fluorescence. All strains carry a plasmid-borne GFP-Mig fusion (pBM3315). Bar, 5 μm.

Additional deletions identify an internal region of the protein required for inactivation of Mig1 and its removal from the nucleus. A large internal deletion that removes amino acids 108–391 converts Mig1 into a repressor that cannot be completely inactivated by removal of glucose (Table 4, pBM3220) and which is predominantly localized in the nucleus even in glycerol-grown cells (Figure 6A, parts 1 and 2). Two smaller deletions define the domain further: deletion of amino acids 174–391 also results in a partially constitutive repressor (Table 4, pBM3255) that is constitutively in the nucleus (Figure 6A, parts 3 and 4); deletion of amino acids 97–173 has little effect on glucose regulation of transcriptional repression (Table 4, pBM3350) and no apparent effect on regulation of nuclear localization (Figure 6A, parts 5 and 6). This

Table 4. Effect of Mig1 deletions on glucose repression of *GAL1*

Mig1 construct[a]	β-gal activity[b]		Fold constitutive repression[c]
	Glucose	Glycerol	
pBM2433 full-length Mig1	30 ± 6	1034 ± 83	1.0
pBM3220 mig1Δ108–391	16 ± 6	336 ± 54	3.1
pBM3255 mig1Δ173–391	19 ± 6	469 ± 28	2.2
pBM3350 mig1Δ96–173	26 ± 3	1420 ± 156	0.7

[a] All are *CEN*-based plasmids (pRS316).

[b] Assays were carried out on duplicate cultures of three independent transformants grown to early logarithmic phase on YM-uracil containing 2% glucose (repressing) or 5% glycerol (nonrepressing). Data are the mean ± SD.

[c] Fold constitutive repression is the relative amount of repression seen under nonrepressing conditions (5% glycerol) compared to full-length Mig1.

region of the protein contains two stretches very rich in the basic amino acids characteristic of nuclear localization signals, one of which is conserved in the Mig1 homologs of *Kluyveromyces lactis* and *Kluyveromyces marxianus* and is very similar to the well-characterized nuclear localization signal of the yeast transcription factor Swi5 (Cassart *et al.*, 1995; Ostling *et al.*, 1996). However, these sequences are not required for the nuclear localization of Mig1 (Figure 6A, parts 5 and 6) and thus are unlikely to be involved in nuclear localization. These results suggest that sequences required for nuclear export and/or its regulation lie between amino acids 174 and 391 and that there is a nuclear localization signal within amino acids 1–96 and/or 392–504.

Sequences between Amino Acids 261 and 400 Confer Glucose-regulated Nuclear Transport on a GFP-β-gal Chimeric Protein

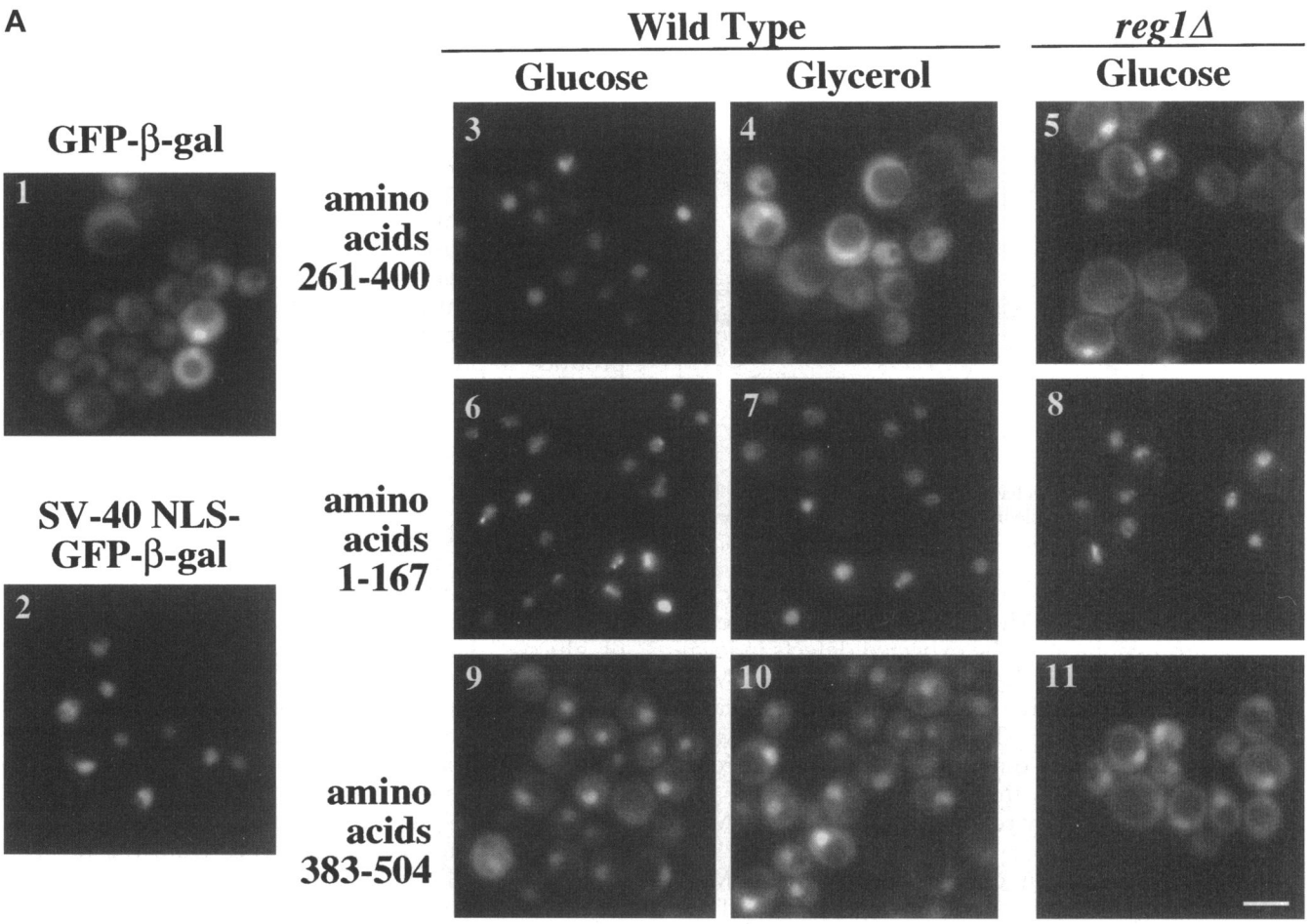
To determine the contribution of various parts of the protein to regulated nuclear translocation, we fused portions of Mig1 to a GFP-β-gal chimeric protein. When expressed in yeast, the GFP-β-gal chimera is localized throughout the cytoplasm and the nucleus (Figure 7A, part 1). Addition of the SV-40 nuclear localization signal causes it to be localized exclusively in the nucleus (Figure 7A, part 2). Addition of Mig1 amino acids 261–400 to the GFP-β-gal chimera causes its nuclear localization to be glucose-regulated, in a manner similar to full-length Mig1 (Figure 7A, parts 3 and 4). This regulation is dependent on the glucose repression signaling pathway: in either a *reg1* (Figure 7A, part 5) or *hxk2* (our unpublished results) mutant, the chimera is not effectively transported into the nucleus in glucose-grown cells. The signals for glucose-regulated nuclear import and export, therefore, appear to lie between amino acids 261 and 400.

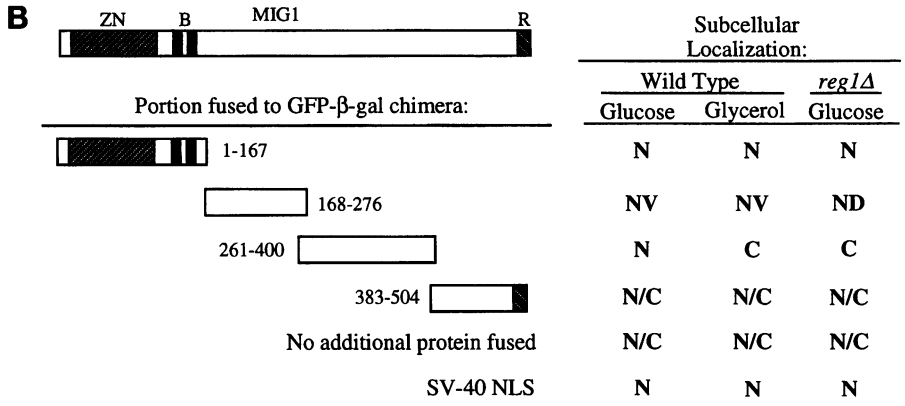
Fusion of the amino-terminal portion of Mig1 (amino acids 1–167), which includes the zinc fingers and the basic region, localizes the GFP-β-gal chimera to the nucleus in a glucose-independent manner (Figure 7A, parts 6 and 7) that is not affected by deletion of *reg1* (Figure 7A, part 8). The basic region alone (amino acids 92–167) does not contain the import signal, since it does not direct the chimera to the nucleus (our unpublished result). Thus, there appears to be an unregulated nuclear localization signal within amino acids 1–92, which includes the zinc fingers. It is probably this domain that causes the internal deletions described above to be constitutively localized to the nucleus. The carboxyl terminus, which is thought to interact with the Ssn6–Tup1 complex (Ostling *et al.*, 1996), does not confer nuclear localization to the chimera: it is found in both the cytoplasm and the nucleus, as though it has no localization signal (Figure 7A, parts 9 and 10; though it is also possible that the Mig1 sequences are cleaved from this chimeric protein). The same results are seen in *reg1* (Figure 7A, part 11) and *snf1* mutants (our unpublished observation). Thus, neither the amino-terminal 167 amino acids nor the carboxyl-terminal 121 amino acids seem to contribute to regulated nuclear transport. (No fluorescence is detectable in a strain carrying a fusion of Mig1 amino acids 168–275 to GFP-β-gal, so the contribution of this region of Mig1 to regulated transport could not be determined).

DISCUSSION

Mig1 appears to be the major glucose repressor, but little is known about how its function is regulated. We have found that the nuclear localization of Mig1 is regulated by glucose: addition of glucose causes a rapid translocation to the nucleus; removal of glucose results in an equally rapid movement back into the cytoplasm.

The nuclear localization of Mig1 correlates with the occurrence of glucose repression under a variety of conditions: 1) Growth on the repressing carbon sources glucose and fructose result in translocation of all detectable Mig1 to the nucleus, whereas growth on





Portion fused to GFP-β-gal chimera:	Subcellular Localization: Wild Type Glucose	Wild Type Glycerol	reg1Δ Glucose
1-167	N	N	N
168-276	NV	NV	ND
261-400	N	C	C
383-504	N/C	N/C	N/C
No additional protein fused	N/C	N/C	N/C
SV-40 NLS	N	N	N

Figure 7. Amino acids 261–400 of Mig1 confer glucose-regulated nuclear localization. (A) Wild-type (YM4342) and *reg1*Δ (YM4902) yeast strains were grown on YM-uracil + 2% glucose or YM-uracil + 5% glycerol and then imaged for GFP fluorescence. The yeast carry the following versions of a GFP-β-gal chimera: no additional protein sequences (pBM3098, part 1), a fusion with SV-40 NLS (pBM3099, part 2), or fusions with the indicated portions of Mig1 (pBM3403, parts 3–5; pBM3401, parts 6–8; pBM3404, parts 9–11). Bar, 5 μm. (B) Schematic representations of the sequences fused to the GFP-β-gal chimera and a summary of the subcellular locations. N, nuclear localization; C, cytoplasmic localization; N/C, both nuclear and cytoplasmic localization; ND, not determined; NV, not able to visualize.

nonrepressing carbon sources causes Mig1 to be predominantly localized to the cytoplasm. 2) The rate of movement of Mig1 into and out of the nucleus is similar to the rate at which repression and derepres-

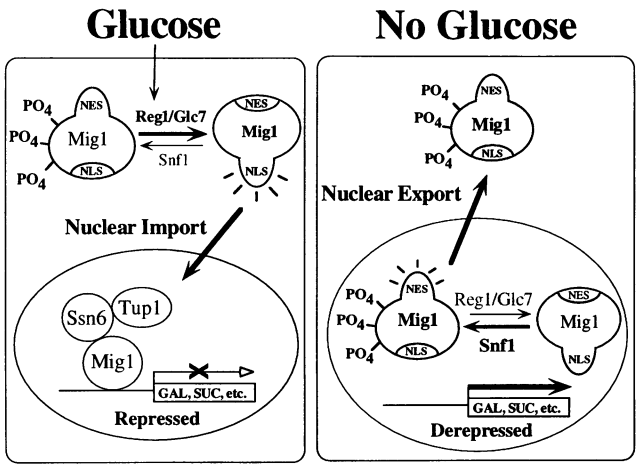


Figure 8. Hypothetical mechanism for glucose-control of Mig1 nuclear localization and its role in regulating glucose repression. See text for details.

sion are established. 3) Mutations in genes involved in glucose repression cause the expected defects in localization of Mig1. 4) Internal deletions that cause Mig1 to constitutively localize to the nucleus also cause constitutive repression. These correlations between the presence of Mig1 in the nucleus and the occurrence of repression suggests that yeast may regulate glucose-repressed genes by regulating the ability of Mig1 to enter the nucleus.

Glucose-induced changes in the localization of Mig1 occur independently of DNA binding and Ssn6–Tup1 interaction and thus are not a consequence of either of these interactions. The appearance of Mig1 in the nucleus upon addition of glucose can only be explained by induction of nuclear import. The disappearance of Mig1 from the nucleus upon glucose removal could also be due to its degradation in the nucleus and resynthesis in the cytoplasm, but two results argue against this idea. First, the disappearance of nuclear fluorescence from the nucleus upon glucose removal correlates with the reappearance of fluorescence in the cytoplasm even when protein synthesis is inhibited with cycloheximide. Second, when cells are shifted from glucose to glycerol in the presence of cycloheximide and then back to glucose, the fluorescence moves from the nucleus to the cytoplasm and back into the nucleus. In the absence of protein synthesis, this could only occur if the protein was exported to the cytoplasm when glucose was removed and reimported into the nucleus when glucose was added back. Thus, we believe the removal of glucose induces export of Mig1 from the nucleus.

An internal domain of Mig1 appears to be responsible for these events: Fusion of amino acids 261–400 confers glucose-regulated import and export to a GFP-β-gal chimera in a manner similar to full-length Mig1. A short stretch of basic amino acids (amino acids 364–368) similar to known nuclear localization sequences (NLS) and a leucine/valine-rich region (amino acids 313–319) characteristic of nuclear export sequences (Gorlich and Mattaj, 1996; Moore, 1996; Murphy and Wente, 1996) reside within the region between amino acids 261 and 400. The rate of export conferred by amino acids 261–400 is somewhat slower than for full-length Mig1, possibly because a second potential nuclear export sequence (amino acids 224–232) is not included in this portion. Internal deletions that remove the region including amino acids 261–400 produce a protein that is no longer responsive to glucose (it is a constitutive repressor) and is always in the nucleus. Ostling *et al.* (1996) also showed that removal of regions of Mig1 including amino acids 261–400 convert it into a constitutive repressor. Internally truncated proteins are targeted to the nucleus by an unregulated nuclear localization signal in the amino-terminal 92 amino acids of the protein.

It has previously been shown that Mig1 is more heavily phosphorylated in cells grown in the absence of glucose than in cells grown in the presence of glucose, suggesting that the activity of Mig1 might be regulated by phosphorylation (Treitel and Carlson, 1995; DeVit, unpublished results). We found that dephosphorylation of Mig1 upon addition of glucose is coincident with its appearance in the nucleus and that its rate of phosphorylation upon removal of glucose matches its rate of disappearance from the nucleus. Although it still remains to be proven that phosphorylation has an affect on Mig1 activity, these results are consistent with a role for phosphorylation in regulating the nuclear translocation of Mig1.

Snf1 protein kinase activity varies in response to glucose in a manner that parallels the localization of Mig1: Snf1 activity increases within 5 min after glucose removal and is inactivated within 5 min of glucose addition (Woods *et al.*, 1994; Wilson *et al.*, 1996). In addition, Snf1 kinase activity, like the ratio of cytoplasmic to nuclear Mig1, varies over a range of 10–60 mM glucose (Wilson *et al.*, 1996). There is no evidence yet that Snf1 directly phosphorylates Mig1, but it is worth noting that there are three putative Snf1 kinase sites (Dale *et al.*, 1995) within the region (amino acids 261–400) that confers regulated nuclear transport.

Our results are consistent with the results of previous experiments aimed at understanding Mig1 regulation by using chimeric molecules. A LexA DNA-binding domain-Mig1 chimera represses transcription through *lexA* operators (Treitel and Carlson, 1995; DeVit, unpublished results), and repression is regulated by glucose. This suggests that a function other than DNA-binding ability is glucose-regulated (Treitel and Carlson, 1995). Replacement of the repression domain of Mig1 with the transcriptional activation domain of VP16 results in a chimera whose ability to activate

transcription is inhibited by Snf1 (Ostling *et al.*, 1996). This suggests that a function other than repression is regulated. We believe that regulation of both chimeras is due to glucose regulation of their nuclear localization. Furthermore, the LexA-Mig1 (DeVit, unpublished results) and the Mig1-VP16 (Ostling *et al.*, 1996) chimeras become constitutively active (for repression and activation, respectively) if the region including amino acids 261–400 is removed. Because these molecules have lost the domain required to regulate subcellular localization, they are likely to be constitutively localized in the nucleus.

On the basis of our results, we propose that addition of glucose, which inactivates Snf1, permits a rapid dephosphorylation of Mig1, probably by the Reg1–Glc7 phosphatase complex (Figure 8). Dephosphorylation induces nuclear import, either by activating a nuclear localization signal or by inactivating an nuclear export mechanism. Once access to the nucleus has been gained Mig1 binds to its target genes and represses transcription of genes not required for growth on glucose. Upon glucose depletion, Snf1 protein kinase is activated and rapidly phosphorylates Mig1. Phosphorylation induces nuclear export and/or inhibits import, thus sequestering the protein in the cytoplasm. Genes needed for growth on nonglucose carbon sources are thus derepressed. This model predicts that Snf1 has access to Mig1 and is, therefore, in the nucleus. Snf1 has indeed been found throughout the cell (Celenza and Carlson, 1986). Alternatively, it is possible that unphosphorylated (and, therefore, nuclear) Mig1 is constantly moving between the nucleus and cytoplasm but is phosphorylated by Snf1 in the cytoplasm upon removal of glucose and, therefore, trapped there.

We do not know how the nuclear transport of Mig1 is regulated; import, export, or both could be regulated. There are several examples of regulated nuclear import. For example, the yeast transcriptional activator Swi5 is imported into the nucleus only when its NLS is dephosphorylated in the G_1 phase of the cell cycle (Moll *et al.*, 1991); the NLS of NF-κB is activated by its release from the inhibitor I-κB upon mitogen or cytokine treatment (Shirakawa and Mizel, 1989; Ganchi *et al.*, 1992). Although the active export of proteins from the nucleus is known to occur, in no case has this process been shown to be regulated. It will be necessary to identify the specific sequences of Mig1 required for nuclear import and export to determine which of these is regulated by glucose.

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REFERENCES

Cassart, J. P., Georis, I., Ostling, J., Ronne, H., and Vandenhaute, J. (1995). The Mig1 repressor from *Kluyveromyces lactis*: cloning, sequencing and functional analysis in *Saccharomyces cerevisiae*. FEBS Lett. *371*, 191–194.

Celenza, J.L., and Carlson, M. (1986). A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science *233*, 1175–1180.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science *263*, 802–805.

Dale, S., Wilson, W.A., Edelman, A.M., and Hardie, D.G. (1995). Similar substrate recognition motifs for mammalian AMP-activated protein kinase, higher plant HMG-CoA reductase kinase-A, yeast Snf1, and mammalian calmodulin-dependent protein kinase I. FEBS Lett. *361*, 191–195.

Entian, K.D., and Zimmermann, F.K. (1980). Glycolytic enzymes and intermediates in carbon catabolite repression mutants of *Saccharomyces cerevisiae*. Mol. Gen. Genet. *177*, 345–350.

Flick, J.S., and Johnston, M. (1990). Two systems of glucose repression of the *GAL1* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *10*, 4757–4769.

Flick, J.S., and Johnston, M. (1992). Analysis of URSG-mediated glucose repression of the *GAL1* promoter of *Saccharomyces cerevisiae*. Genetics *130*, 295–304.

Ganchi, P.A., Sun, S.C., Greene, W.C., and Ballard, D.W. (1992). I-κB/MAD-3 masks the nuclear localization signal of NF-κB p65 and requires the transactivation domain to inhibit NF-κB p65 DNA binding. Mol. Biol. Cell *3*, 1339–1352.

Gietz, D., St, J.A., Woods, R.A., and Schiestl, R.H. (1992). Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. *20*, 1425.

Gorlich, D., and Mattaj, I.W. (1996). Nucleocytoplasmic transport. Science *271*, 1513–1518.

Griggs, D.W., and Johnston, M. (1991). Regulated expression of the *GAL4* activator gene in yeast provides a sensitive genetic switch for glucose repression. Proc. Natl. Acad. Sci. USA *88*, 8597–8601.

Hu, Z., Nehlin, J.O., Ronne, H., and Michels, C.A. (1995). Mig1-dependent and Mig1-independent glucose regulation of *MAL* gene expression in *Saccharomyces cerevisiae*. Curr. Genet. *28*, 258–266.

Johnston, M., and Carlson, M. (1992). Regulation of carbon and phosphate utilization. In: The Molecular Biology of the Yeast Saccharomyces (Gene Expression), ed. J.R. Broach, J.R. Pringle, and E.W. Jones, Plainview, NY: Cold Spring Harbor Laboratory Press.

Johnston, M., Flick, J.S., and Pexton, T. (1994). Multiple mechanisms provide rapid and stringent glucose repression of *GAL* gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *14*, 3834–3841.

Keleher, C.A., Redd, M.J., Schultz, J., Carlson, M., and Johnson, A.D. (1992). Ssn6-Tup1 is a general repressor of transcription in yeast. Cell *68*, 709–719.

Lutfiyya, L.L., and Johnston, M. (1996). Two zinc-finger-containing repressors are responsible for glucose repression of *SUC2* expression. Mol. Cell. Biol. *16*, 4790–4797.

Moll, T., Tebb, G., Surana, U., Robitsch, H., and Nasmyth, K. (1991). The role of phosphorylation and the Cdc28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor Swi5. Cell *66*, 743–758.

Moore, M.S. (1996). Protein translocation: nuclear export—out of the dark. Curr. Biol. *6*, 137–140.

Murphy, R., and Wente, S.R. (1996). An RNA-export mediator with an essential nuclear export signal. Nature *383*, 357–360.

Nehlin, J.O., Carlberg, M., and Ronne, H. (1991). Control of yeast *GAL* genes by Mig1 repressor: a transcriptional cascade in the glucose response. EMBO J. *10*, 3373–3377.

Nehlin, J.O., and Ronne, H. (1990). Yeast Mig1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. EMBO J. *9*, 2891–2898.

Ostling, J., Carlberg, M., and Ronne, H. (1996). Functional domains in the Mig1 repressor. Mol. Cell. Biol. *16*, 753–761.

Ronne, H. (1995). Glucose repression in fungi. Trends Genet. *11*, 12–17.

Rose, M.D., Winston, F., and Hieter, P. (1990). Methods in Yeast Genetics: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Plainview, NY: Cold Spring Harbor Laboratory Press.

Schuller, H.J., and Entian, K.D. (1991). Extragenic suppressors of yeast glucose derepression mutants leading to constitutive synthesis of several glucose-repressible enzymes. J. Bacteriol. *173*, 2045–2052.

Schultz, J., and Carlson, M. (1987). Molecular analysis of *SSN6*, a gene functionally related to the Snf1 protein kinase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. *7*, 3637–3645.

Shirakawa, F., and Mizel, S.B. (1989). In vitro activation and nuclear translocation of NF-κB catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. Mol. Cell. Biol. *9*, 2424–2430.

Sierkstra, L.N., Nouwen, N.P., Verbakel, J.M., and Verrips, C.T. (1992). Analysis of glucose repression in *Saccharomyces cerevisiae* by pulsing glucose to a galactose-limited continuous culture. Yeast *8*, 1077–1087.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Treitel, M.A., and Carlson, M. (1995). Repression by Ssn6-Tup1 is directed by Mig1, a repressor/activator protein. Proc. Natl. Acad. Sci. USA *92*, 3132–3136.

Trumbly, R.J. (1992). Glucose repression in the yeast *Saccharomyces cerevisiae*. Mol. Microbiol. *6*, 15–21.

Tu, J., and Carlson, M. (1995). Reg1 binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae*. EMBO J. *14*, 5939–5946.

Tzamarias, D., and Struhl, K. (1994). Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. Nature *369*, 758–761.

Tzamarias, D., and Struhl, K. (1995). Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. *9*, 821–831.

Vallier, L.G., and Carlson, M. (1994). Synergistic release from glucose repression by *mig1* and *ssn* mutations in *Saccharomyces cerevisiae*. Genetics *137*, 49–54.

Vernet, T., Dignard, D., and Thomas, D.Y. (1987).A family of yeast expression vectors containing the phage f1 intergenic region. Gene *52*, 225–233.

Waddle, J.A., Karpova, T.S., Waterston, R.H., and Cooper, J.A. (1996). Movement of cortical actin patches in yeast. J. Cell Biol. *132*, 861–870.

Wang, J., and Needleman, R. (1996). Removal of Mig1p binding site converts a *MAL63* constitutive mutant derived by interchromosomal gene conversion to glucose insensitivity. Genetics *142*, 51–63.

Wilson, W.A., Hawley, S.A., and Hardie, D.G. (1996). Glucose repression/derepression in budding yeast: Snf1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. Curr. Biol. *6*, 1426–1434.

Woods, A., Munday, M.R., Scott, J., Yang, X., Carlson, M., and Carling, D. (1994). Yeast Snf1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase in vivo. J. Biol. Chem. *269*, 19509–19515.

Yaffe, M.P., and Schatz, G. (1984). Two nuclear mutations that block mitochondrial protein import in yeast. Proc. Natl. Acad. Sci. USA *81*, 4819–4823.

Yocum, R.R., Hanley, S., West, R., Jr., and Ptashne, M. (1984). Use of lacZ fusions to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *4*, 1985–1998.

Zimmermann, F.K., and Scheel, I. (1977). Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. Mol. Gen. Genet. *154*, 75–82.