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Identification of a Member of a DNA-Dependent ATPase Family That Causes Interference with Silencing

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DNA in eukaryotic cells is packed in tandem repeats of nucleosomes or higher-order chromatin structures, which present obstacles to many cellular processes that require protein-DNA interactions, such as transcription, DNA repair, and recombination. To find proteins that are involved in increasing the accessibility of specific DNA regions in yeast, we used a genetic approach that exploited transcriptional silencing normally occurring at *HML* and *HMR* loci. The silencing is mediated by *cis*-acting silencer elements and is thought to require the formation of a special chromatin structure that prevents accessibility to the silenced DNA. A previously uncharacterized gene, termed *DIS1*, was isolated from a screen for genes that interfere with silencing when overexpressed. *DIS1* encodes a protein with conserved motifs that are present in a family of DNA-dependent ATPases, the SWI2/SNF2-like proteins. Overproduction of N-terminal half of DIS1 protein interfered specifically with ectopic silencing used in the screen as well as *HMR* E silencing. Two-hybrid studies revealed a specific interaction between the N terminus of DIS1 and the C-terminal half of SIR4, a protein essential for silencing. Cells with a *dis1* knockout mutation had significantly lower mating-type switching rate. These results suggest that DIS1 may contribute to making the silenced DNA template at *HM* loci more accessible during the mating-type switching process.

In eukaryotes, almost all DNA is packed in tandem repeats of nucleosomes or higher-order chromatin structures, which create an impediment to DNA access (79). DNA accessibility, therefore, could provide an important regulatory step in a number of cellular processes that require protein-DNA interactions. Recent studies have started to illustrate the dynamic interactions between transcriptional machinery and nucleosomal DNA, providing insights into how transcription factors and activators interact with promoters and upstream regulatory elements embedded in chromatin structures. A combination of genetic and biochemical approaches has led to the identification of a conserved multisubunit complex, the SWI/SNF complex, that appears to function as a chromatin remodeling machine to promote the binding of transcription factors to nucleosomal targets (12, 20, 45, 47, 58–60). One of the key subunits is SWI2 (SNF2) protein, the prototype of an expanding SWI/SNF protein family (reviewed in reference 13). All members in this family contain the sequence motifs common to DNA-stimulated ATPases, and some proteins, including SWI2, have been shown biochemically to have DNA-dependent ATPase activity (41, 48). The ATPase activity in SWI2 subunit appears to provide the energy source to the SWI/SNF complex for remodeling chromatin (20, 36, 45). The purified SWI/SNF protein complex can alter the nucleosomal sensitivity to DNase I (45) and stimulate binding of GAL4 to its target site on nucleosomal DNA in vitro in the presence of ATP (20). These activities are lost in the absence of ATP or when one of the conserved motifs in SWI2 is mutated (20, 45). It appears that the SWI/SNF complex increases the accessibility of the binding sites for GAL4 by destabilizing the histone octamer (20). Studies of yeast SWI/SNF complex have provided important insights into how DNA accessibility is achieved during transcription in yeast and higher eukaryotes, as the SWI2 homologs in *Drosophila* and human cells appear to have similar functions (reviewed in reference 60).

Other cellular processes such as DNA repair and recombination face a nucleosomal barrier similar to that for transcription. Proteins involved in these processes may require assistance from proteins like the SWI/SNF complex to gain access to specific DNA target regions. Interestingly, some of the members in the SWI/SNF family are involved in processes other than transcription activation. Genetic analysis has linked these family members to diverse DNA-related activities including transcriptional repression (e.g., yeast MOT1 [21]), mitotic chromosome segregation (e.g., *Drosophila* lodestar [28]), and DNA repair (e.g., human ERCC6 [78]). Although the biochemical details of these actions are not well understood, it is possible that these SWI/SNF family members function by promoting the DNA accessibility during a variety of processes in a fashion similar to that seen with the yeast SWI/SNF complex.

To understand how accessibility is achieved in specific DNA regions, we attempted to identify proteins that may promote DNA accessibility in a region where it is transcriptionally inactive normally. The model that we chose to study was associated with yeast transcriptional silencing (reviewed in reference 46), which is required for controlling yeast mating types. The mating types of the haploid yeast *Saccharomyces cerevisiae* are determined by the actively transcribed genes at the *MAT* locus, which contains **a***1* and **a***2* genes in **a** cells or $\alpha 1$ and $\alpha 2$ genes in α cells (reviewed in reference 56). Intact but inert copies of these genes are present at the *HMR* and *HML* loci. These extra copies of mating-type genes serve as the DNA templates for a nonreciprocal gene conversion event during mating-type switching (reviewed in reference 30). The tight repression or silencing of genes at the *HM* loci is mediated by a combination of *cis*-acting silencer elements and a series of *trans*-acting proteins including those encoded by four genetically identified *SIR*

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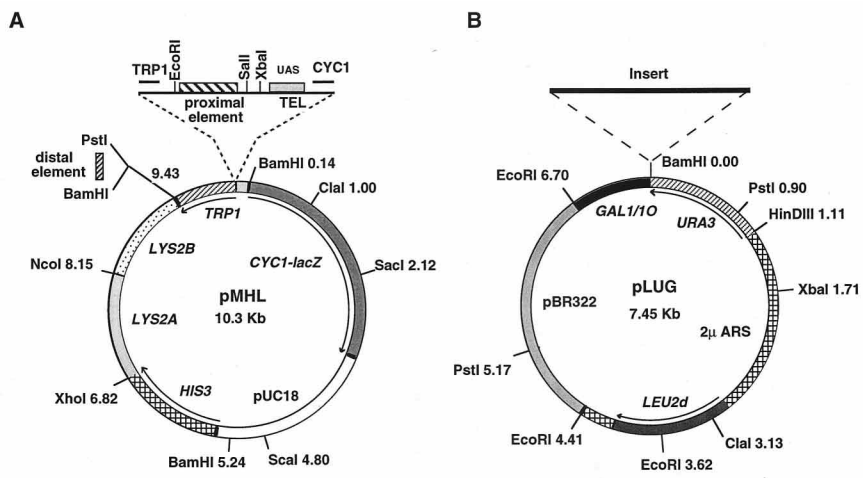


FIG. 1. Structures of yeast shuttle vectors pMHL and pLUG. (A) Plasmid map of the integration vector pMHL, showing the recognition sites of representative restriction enzymes. *LYS2A* contains the *Nco*I (1835)-*Xho*I (3161) fragment, and *LYS2B* contains the *Bam*HI (3543)-*Nco*I (4826) fragment, from *LYS2. HIS3* is used as the selectable marker. The *Nco*I site is unique in this plasmid. (B) pLUG is used to construct the high-copy-number genomic DNA library with *URA3* and *LEU2* as selectable markers. Genomic yeast DNA fragments prepared by partial *Sau*IIIA digestion were inserted at the unique *Bam*HI site.

genes. The silencer consensus sequence contains the binding sites for three multifunctional proteins, origin recognition complex (ORC) (5), repression activation protein 1 (RAP1) (10, 67), and autonomously replicating sequence (ARS)-binding factor 1 (ABF1) (10). Accumulating evidence suggests that these three proteins serve as a landing platform for recruiting other proteins that play more direct roles in gene repression (18, 54, 77). A recent model proposed that ORC and RAP1 at a silencer recruit SIR1 and SIR3, respectively, which both interact with SIR4. SIR3 and SIR4 both homodimerize and heterodimerize with each other (17, 54), thus acting to seed an array of SIR3/SIR4 complexes that spreads from the silencer (77). Histone H3 and H4 have been shown to be required for full gene repression at *HMR* and *HML* (42, 57, 76). The ability of SIR3 and SIR4 to directly interact with the N-terminal tails of histone H3 and H4 (33, 39) probably enables the formation of a higher-order chromatin structure nucleated from the silencer.

The presence of a heterochromatin-like structure in the silenced regions is evidenced by the regional inactivation near the silenced regions. The gene repression at *HML* and *HMR* is not gene specific or polymerase specific (7, 66). The regional inactivation is not limited to transcriptional repression either, as a variety of other DNA-interacting proteins cannot easily gain access to the silenced DNA. These include the HO endonuclease (34, 50), DNA repair enzymes (75), and *Escherichia coli dam* methyltransferase produced in yeast (70). The histones in the silenced regions tend to be hypoacetylated (9) and inaccessible (15, 16), indicating the presence of special chromatin structures. There are, however, processes that occur within yeast cells that must be able to efficiently access DNA at the silent mating-type loci, including DNA replication, DNA repair, and recombination during mating-type switching. Questions arise then. How do other activities gain access to the DNA? Are there proteins that can unravel the compact structure? It is conceivable that there are yeast proteins that are specialized for promoting access to the DNA in silent chromatin during replication, repair, and/or recombination. Such proteins might function, at least in part, through associations that antagonize components of the silencing machinery. To search for such proteins, we designed a genetic approach to identify genes that could interfere with silencing when overproduced. We found in the screen a previously uncharacterized gene, termed *DIS1*, which encodes a protein that appeared to be a member of the SWI2/SNF2 DNA-stimulated ATPase family. Our studies point to a role for DIS1 in facilitating mating-type switching through a specific interaction with the SIR4 protein.

MATERIALS AND METHODS

Plasmid constructions. A yeast high-copy-number vector, pLUG (Fig. 1B), was derived from pG12 (64) by ligation of a 1.1-kb *Hin*dIII-*Sma*I fragment of *URA3*, modified with a *Bam*HI linker on the *Sma*I end, to the 6.3-kb *Hin*dIII-*Bam*HI fragment of pG12. In addition to the *URA3* gene, pLUG contains the yeast selection marker *LEU2*, ARS from plasmid 2μm, bacterial β-lactamase gene, and oriC (pLUG also contains the yeast *GAL1/10* promoter, but this feature was not used in the present study). Southern blot analysis of yeast carrying pLUG derivatives revealed that there were roughly 100 copies of pLUG per cell when strains were grown in the absence of uracil (data not shown).

The integrative plasmid vector pMHL (Fig. 1A) was designed to stably insert reporter genes into the *LYS2* locus of chromosome II. Derivatives of this plasmid were constructed in multiple steps, which were equivalent to joining the following seven DNA fragments in sequential order: (i) the 4.8-kb *Xba*I-*Sca*I segment from pCX-TEL (11) containing the *CYC1-lacZ* gene and elements allowing propagation in *E. coli*, (ii) the 2.02-kb *Sca*I-*Xho*I fragment from pUN90 (25) with the *Nco*I site downstream of the *HIS3* gene removed by Klenow treatment, (iii) the 1.33-kb *Xho*I-*Nco*I *LYS2* fragment, (iv) the 1.28-kb *Nco*I-*Bam*HI *LYS2* fragment, (v) various *Bam*HI-*Pst*I segments from pCZ plasmids (11) containing desired distal elements, (vi) the 0.83-kb *Pst*I-*Eco*RI *TRP1* gene from pSE266 (obtained from S. Elledge, Baylor College of Medicine), and (vii) various *Eco*RI-*Xba*I segments containing desired proximal regulatory elements from pCX plasmids (11).

Yeast strains constructed using pMHL differed in the regulatory elements inserted at three positions in pMHL: (i) the UAS (upstream activating sequence) position located immediately next to the *CYC1* promoter, (ii) the proximal regulator position located immediately adjacent to the *TRP1* promoter, and (iii) the distal regulator position located beyond the transcription termination region of the *TRP1* gene. All constructs contained a 38-bp synthetic oligonucleotide, TEL (11), with two tandem RAP1-binding sites inserted at the UAS position. pMHL-60, used to generate strain YZZ60, contains an E69 element at the distal position, a TEL oligonucleotide, and an E52 oligonucleotide at the proximal position (Fig. 2). E52 is a 52-bp sequence derived from *HMR* E, 5′-TCGACAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAT-3′, that contains the ARS consensus element (underlined) and surrounding sequence. E69 is a 69-bp sequence derived from *HMR* E that extends the E52 sequence to include the RAP1-binding site (ARS and the RAP1-binding site are underlined): 5′-TCGACAAATCGCAATTTAATACCTAAATATAAAAAATGTTATTATATTGCAAAAACCCATCAACCTTGT-3′. pMHL-SR10 and pMHL-SR16, were made by Sue K. Reimer (Pennsylvania State University) and used to generate YSR10 and YSR16 (62a). pMHL-SR16 does not contain

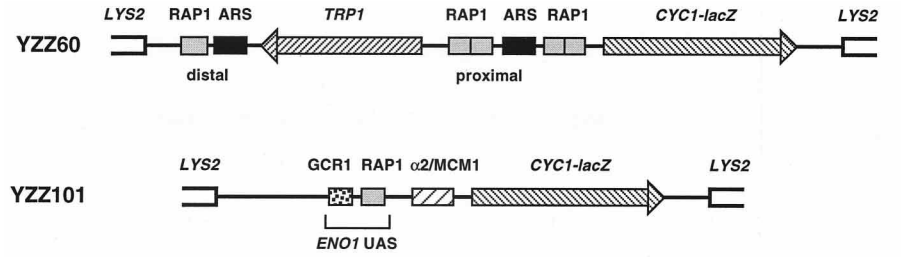


FIG. 2. Structures of reporter genes and regulatory elements. The schematic representation is not drawn to scale. The GCR1 and RAP1 sites in YZZ101 are from the UAS of the *ENO1* gene. The distal elements (RAP1 and ARS) in YZZ60 are from the E69 oligonucleotide derived from *HMR* E. In the common promoter region between two reporter genes, the tandem RAP1 sites are from the TEL oligonucleotide and ARS is from the E52 oligonucleotide derived from *HMR* E (see Materials and Methods).

any proximal or distal elements, while pMHL-SR10 contains an α2 operator derived from the *STE6* gene (38), inserted at the proximal regulator position. pZHL-101, used to generate YZZ101 and YZZ102 (Fig. 2), does not contain the *TRP1* reporter gene. pZHL-101 contains the UAS derived from the *ENO1* gene containing the binding sites for GCR1 and RAP1 (51) and an *STE6* gene α2 operator inserted between UAS and the *CYC1* promoter.

The plasmid vectors used to generate most of the constructs used for two-hybrid studies in this work were provided by Steve Elledge: pAS2 (32) for expressing fusion proteins with the GAL4 DNA-binding domain (amino acids 1 to 147) [GAL4(DB)] and pACTII (72) for expressing fusion proteins with the GAL4 activation domain (amino acids 768 to 881) [GAL4(AD)]. Derivatives of two-hybrid vectors constructed for this study are all designated with numbers in parentheses that indicate the amino acid residues of the test protein that are present in each fusion construct. Gene fusions were engineered by using either naturally occurring restriction enzyme cleavage sites or restriction sites generated by site-directed mutagenesis (43). Details of each construct are available upon request.

A set of plasmids expressing GAL4(768-881)-SIR fusions, using the pGAD vector (17), was provided by Rolf Sternglanz (State University of New York, Stony Brook). pCTC18 expresses a GAL4-SIR4(839-1358) fusion, pCTC24 expresses a GAL4-SIR4(1262-1358) fusion, pCTC49 expresses a GAL4-SIR4(839-1149) fusion, pKL13 expresses a GAL4-SIR3(17-978) fusion, pCTC88 expresses a GAL4-SIR1 fusion, and pCTC85 expresses a GAL4-SIR2 fusion. Control plasmid pSE1111 expressing a GAL4-SNF1 fusion was obtained from Steve Elledge, and control plasmid pACT-p34 was obtained from Suresh Shenoy (Pennsylvania State University).

Yeast strains. Genetic modifications of yeast strains were performed by a single-step gene disruption procedure (63). Most of the yeast strains created for this work were derivatives of YPH500 (α *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 his3-Δ200 leu2-Δ1*) or YPH499 (**a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 his3-Δ200 leu2-Δ1*) (69), and the plasmid vector routinely used in DNA integration was pMHL (Fig. 1A). YPH500 and YPH499 were first converted into YZZ1 and YBB499, respectively, by replacing the mutant *lys2* gene with the wild-type *LYS2* gene, using a 4.6-kb *Eco*RI-*Hin*dIII fragment containing the complete *LYS2* gene. *LYS*$^+$ transformants were further tested for sensitivity to 0.2% DL-α-aminoadipic acid (80). To construct yeast strains carrying reporter genes, pMHL plasmids described above were linearized by *Nco*I digestion and transformed into yeast strain YZZ1 (α) or YBB499 (**a**), with selection for histidine prototrophy (*HIS*$^+$). Transformants were confirmed by testing their resistance to DL-α-aminoadipic acid (Lys2$^-$).

To test if ectopic silencing mediated by synthetic silencers was dependent on SIR proteins, four derivatives of strain YZZ60 were made, each with one of the four *SIR* genes disrupted. Plasmids used to create *SIR* gene disruptions were obtained from Rolf Sternglanz and Sue Reimer. Each plasmid replaced most or all of a *SIR* coding region with the *URA3* gene. The DNAs used in *SIR1*, *SIR2*, *SIR3*, and *SIR4* disruptions were pES17 (*sir1::URA3*) (73), pES28 (*sir2::URA3*) (18), pCTC73 (*sir3::URA3*) (18), and p*SIR4::URA3* (62), respectively.

Strains YDS36 and YDS39 (44) were obtained from David Shore (Columbia University). The reporter yeast strain used in two-hybrid studies, Y190 (22), was provided by Steve Elledge.

Yeast DNA library construction and screen. To investigate dosage interference with silencing, a library of yeast genomic DNA was constructed in the high-copy-number vector pLUG. High-molecular-weight genomic DNA was isolated from yeast strain YPH500 (61, 69). *Sau*3AI partially digested DNA fragments, ranging from 2 to 10 kb, were fractionated by agarose gel electrophoresis and ligated with *Bam*HI-digested pLUG. Ligations were introduced into *E. coli* DH5α (31), and approximately 120,000 ampicillin-resistant transformants were obtained and pooled to generate the genomic DNA library. Restriction enzyme mapping of randomly chosen *E. coli* transformants revealed that the majority of the plasmids contained inserts, and the average size was 4 kb.

The reporter strain YZZ60 was transformed with the pLUG genomic DNA library and plated on minimal synthetic medium lacking tryptophan and uracil to select for interference with silencing and presence of pLUG, respectively. A total of 540 *TRP*$^+$ colonies were obtained from approximately 500,000 *URA3*$^+$ transformants, representing over four times the library's complexity. To eliminate *TRP*$^+$ colonies caused by spontaneous genomic mutations in the yeast host strain, these 540 colonies were tested for tryptophan prototrophy while selecting against the presence of the pLUG vector by growth in the presence of 5-fluoroorotic acid (68). Colonies that grew on 5-fluoroorotic acid-containing medium were eliminated from further consideration. The remaining 56 *TRP*$^+$ clones were examined for increased expression of the second reporter gene present in the YZZ60 host strain, *CYC1-lacZ*, using X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) indicator plates (29); 45 clones showed evidence of increased β-galactosidase expression. pLUG plasmid DNA was retrieved from each of these yeast transformants and used to transform *E. coli* (35). The pLUG derivatives that contained inserts of significant sizes were reintroduced into the host strain YZZ60 to find those that could reproducibly increase expression of both *TRP1* and *CYC1-lacZ* reporter genes. Fourteen pLUG derivatives that had the ability to reproducibly interfere with silencing were identified.

β-Galactosidase enzyme assays. Yeast protein extracts were prepared as described previously (11), and β-galactosidase enzyme assays were performed with *o*-nitrophenyl-β-D-galactoside (ONPG) (53). Protein concentration was determined with Bradford reagent (6), and the specific activity expressed as nanomoles of ONPG hydrolyzed per minute per milligram of total yeast protein at 28°C. All β-galactosidase enzyme assays were done multiple times, and only the average activities are presented. The error for any given sample was less than ±15%.

Southern and Northern blot analysis. For Southern blot analysis, yeast genomic DNA was isolated as described previously (4). DNA was digested with restriction enzymes, electrophoresed in an agarose gel, and transferred to a GeneScreen Plus hybridization membrane (NEN Research Products, Boston, Mass.), using a vacuum device (TE 80 TransVac; Hoefer Scientific Instruments, San Francisco, Calif.) as specified by the manufacturer. DNA on the membrane was hybridized to ^{32}P-labeled DNA probes prepared by using a DECAprime II DNA labeling kit (Ambion, Austin, Tex.) and Bio-Spin 6 columns (Bio-Rad, Hercules, Calif.). For Southern blot analysis used in mating-type switching studies, DNA was extracted as described elsewhere (19).

For Northern blot analysis, yeast total RNA was extracted as previously described (4). Equal amounts of total RNA, as quantified by spectrophotometer, were electrophoresed on a 1.2% agarose gel and transferred to a GeneScreen Plus membrane by using the Hoefer TE 80 TransVac device. RNA was detected with ^{32}P-labeled DNA probes derived from the 285-bp *Hin*fI fragment from the *HMR* **a***1* gene (2).

DNA sequencing. Since the original *DIS1* clone did not contain the complete *DIS1* coding region, a 0.47-kb *Pst*I-*Eco*RI fragment from pLUG-DIS$_{207E}$ was ^{32}P labeled and used as a hybridization probe to screen (4) a yeast genomic DNA λ_{YES}-P library (24) provided by Steve Elledge. Positive single plaques were picked and allowed to undergo automatic conversion from phage λ to the plasmid form by infecting bacterial strain BNN132 (24). Seven overlapping clones were obtained, mapped by restriction enzyme digestion, and subcloned into pUC19 for DNA sequencing and further analysis. DNA sequencing was performed with a Sequenase version 2.0 kit (U.S. Biochemical, Cleveland, Ohio) following standard protocols. For the right portion of the *DIS1* gene, where restriction sites were limited, the 1.7-kb *Sac*I-*Eco*RI fragment of *DIS1* DNA was cloned into pUC19, and nested deletions at each end of this fragment were constructed by exonuclease III digestion (4) for further sequencing analysis. After this work was performed, the complete sequence of the *S. cerevisiae* genome was reported (*Saccharomyces* genome database at Stanford University). The sequence of *DIS1* determined in the present study is in agreement with that deposited as YOR191w in the yeast genome database.

Functional and structural analysis of the *DIS1* gene. From the original pLUG-DIS$_{207}$ clone with a 7.8-kb insert, a series of subclones was constructed in the same vector in order to map the region that caused silencing interference. In a few cases, *DIS1* DNA fragments were first inserted into the multiple cloning site

of pUC19 and then liberated by using different restriction enzymes to modify the ends for cloning into vector pLUG. Details of each construct are available upon request. Constructs containing subclones of the *DIS1* region were then introduced into strain YZZ60 to examine their ability to interfere with silencing.

To study the in vivo functions of *DIS1*, the chromosomal *DIS1* gene was disrupted by the insertion of *LYS2*. Plasmid pUC-*dis1*Δ*::LYS2* was created by multiple steps equivalent to joining the following fragments: a 1.7-kb *Sac*I-*Eco*RI fragment of *DIS1*, a 4.8-kb *Eco*RI-*Hin*dIII fragment containing intact *LYS2*, a 0.67-kb *Hin*dIII-*Xho*I fragment downstream of *DIS1*, and *Sac*I-*Eco*RI-cut pUC19. This plasmid was linearized with *Pst*I and introduced into yeast strains, and *LYS*$^+$ cells were selected. Southern blot analysis confirmed that an internal 3.47-kb *Eco*RI-*Hin*dIII fragment of *DIS1* was replaced by the *LYS2* gene (data not shown). YZZ109 is the *dis1::LYS2* isogenic strain of YPH499, and YZZ110 is that of YPH500.

Mating-type switching assays. A plasmid expressing the HO endonuclease under the control of the inducible *GAL10* promoter, pGAL-HO (33), was introduced into test strains YZZ109, YZZ110, YPH499, and YPH500. These cells (*URA*$^+$ *his*) were grown in synthetic minimal medium containing sucrose (2%), adenine, lysine, tryptophan, leucine, and histidine until the optical density at 600 nm reached 0.1. Galactose was added to a final concentration of 2%. At various times after galactose addition, 100 μl of cell culture was removed and mixed with a 10-fold excess of a tester yeast strain of the same mating type, either YSR20 (α *ura HIS*$^+$) or YSR21 (**a** *ura HIS*$^+$) (provided by Sue Reimer), in YPD medium. Cell mixtures were incubated without shaking at room temperature for 3 h, washed, diluted, and spread onto plates (containing dextrose, adenine, lysine, tryptophan, and leucine), selecting for diploid cells (α/**a** *URA*$^+$ *HIS*$^+$). Plates were incubated 2 days at 30°C, and the resulting colonies were counted.

RESULTS

A genetic screen for factors that cause dosage interference with silencing. To search for proteins that specialize in promoting access to DNA in silent chromatin during replication, repair, and/or recombination, a genetic approach was designed to identify genes that could interfere with silencing when overexpressed. The rationale was that overproduction of certain proteins might disrupt a balanced relationship between components that direct silencing and those that create transient accessibility. We first established ectopic silencing controlling reporter genes that closely mimic the silencing occurring at the *HM* loci. A yeast integration vector, pMHL (Fig. 1A), was constructed to allow stable integration of reporter genes and silencers into the *LYS2* locus on chromosome II when linearized at a unique *Nco*I site. We constructed a reporter strain (YZZ60) containing two integrated reporter genes, yeast *TRP1* and the fusion gene *CYC1-lacZ*. Both genes are under the control of two synthetic silencers, one at the common promoter region of these two genes and the other at the downstream region of the *CYC1-lacZ* gene (Fig. 2). The synthetic silencers contained the ARS elements from the *HMR* E silencer and RAP1-binding sites from either a telomere or an *HMR* E silencer. These two silencers were able to repress *TRP1* and *CYC1-lacZ* genes at the *LYS2* locus, as evidenced by the Trp$^-$ phenotype and white growth on X-Gal indicator plates of this strain. To examine if this ectopic silencing was SIR dependent, YZZ60 derivatives that contained a disruption of either *SIR1*, *SIR2*, *SIR3*, or *SIR4* were generated. The removal of any of the *SIR* genes increased the *lacZ* expression at least 35-fold (Table 1), showing that the complete repression required all four SIR proteins. Unlike the silencing occurring at the *HMR* and *HML* loci, however, synthetic silencing in YZZ60 did not have significant redundancy, as point mutations in the ARS element or RAP1-binding sites disrupted silencing (81).

A yeast genomic DNA library was constructed in a high-copy-number *URA3 LEU2d* vector, pLUG (Fig. 1B). Selection for *LEU2d* maintains a high copy number, while the initial selection for *URA3* ensured high yeast transformation efficiency. This library was used to select YZZ60 transformants that expressed from the ectopically silenced locus, as evidenced by tryptophan prototrophy and blue color on the X-Gal indicator plates. From approximately 500,000 *URA3* transformants, 14 clones were found to interfere with silencing reproducibly and were named DIS (for dosage interference of silencing) clones. Southern blot analysis and DNA sequencing analysis showed that 8 of the 14 clones contained the *HMR* E silencer elements, and 3 contained either complete or truncated *SIR4* genes. We expected to obtain these pLUG-DIS_{HMRE} and pLUG-DIS_{SIR4} clones because multiple copies of *HMR* E could disrupt silencing simply by titrating away limiting proteins required for silencing, and SIR4 is a known dosage interference factor in silencing (37, 52). Multiple appearance of *HMR* E silencers and the *SIR4* gene demonstrated that the screen was capable of isolating specific clones interfering with silencing. Of the 14 clones, 2 contained genes which did not appear to be directly related to silencing and will not be discussed here. The focus of this paper is a clone, pLUG-$DIS1_{207}$, that contained a portion of a previously unidentified gene.

TABLE 1. *SIR* dependence of ectopic silencing of *TRP1* and *CYC1-lacZ* reporter genes

Strain	β-Galactosidase activity (U[a])
YZZ60 (*SIR*$^+$)	0.26
YZZ601 (*sir1*)[b]	27
YZZ602 (*sir2*)[b]	10
YZZ603 (*sir3*)[b]	9.2
YZZ605 (*sir4*)[b]	13

[a] Nanomoles of ONPG hydrolyzed per minute per milligram of protein at 28°C.
[b] *sir* isogenic derivative of YZZ60.

pLUG-$DIS1_{207}$ specifically interferes with silencing. When clone pLUG-$DIS1_{207}$ was present in the original reporter strain (YZZ60), β-galactosidase activity increased 20-fold (Table 2). A similar effect was seen with strains overexpressing *SIR4*. To determine whether the derepression caused by pLUG-$DIS1_{207}$ resulted from a specific antagonism of silencing and not a general derepressive mechanism, we examined if pLUG-$DIS1_{207}$ could also interfere with repression mediated by the repressor α2. Yeast strains (YZZ101 and YZZ102) were constructed with an α2 operator inserted between the CYC1 promoter and a strong UAS element derived from the *ENO1* gene (Fig. 2). The *CYC1-lacZ* reporter gene in this test construct was actively transcribed in **a** cells, which do not produce the α2 repressor (YZZ102 [Table 2]) but tightly re-

TABLE 2. Effect of DIS1 overproduction on *CYC1-lacZ* reporter gene in various yeast strains

pLUG insert	β-Galactosidase activity (U[a])			
	YZZ60 (silencing)	YZZ101 (α2 repression)	YZZ102[b] (**a** cell)	YZZ62 (Δ*dis1*)
None	0.23	0.25	180	0.14
$DIS1_{207}$[c]	4.6	0.23	140	0.79
SIR4[d]	4.3	ND	ND	5.0
HMR E[e]	35	ND	ND	ND
$DIS1_{207M}$[f]	0.39			

[a] Nanomoles of ONPG hydrolyzed per minute per milligram of protein. ND, not determined.
[b] The *MAT***a** isogenic derivative of YZZ101.
[c] Overexpresses the N terminus (1 to 894) of DIS1.
[d] Overexpresses full-length SIR4.
[e] Contains the entire *HMR* locus, including *HMR* E and *HMR* I silencers.
[f] Overexpresses the full-length DIS1.

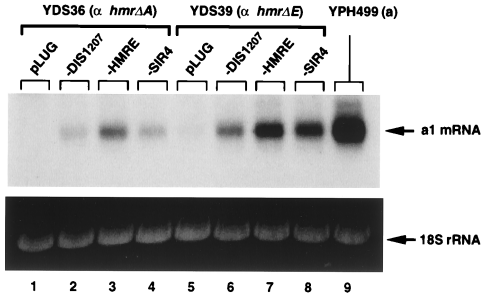


FIG. 3. DIS1 overproduction interferes with silencing at *HMR*. Total RNA isolated from α cells (YDS36 and YDS39) carrying various pLUG derivatives or from **a** cells (YPH499) was analyzed by Northern blotting. Equal amounts of total RNA, as evidenced by the ethidium bromide staining of the 18S rRNA in the lower panel, were loaded in each lane. The **a***1* mRNA-specific probe was a ^{32}P-labeled 285-bp *Hin*fI DNA fragment derived from the **a***1* gene at *HMR*. pLUG-DIS1$_{207}$ expresses a truncated form of DIS1(1-894). pLUG-DIS$_{HMRE}$ carries *HMR* E silencer DNA. pLUG-DIS$_{SIR4}$ expresses full-length SIR4.

pressed in α cells, which produce the α2 repressor (YZZ101). When plasmid pLUG-DIS1$_{207}$ was introduced into the α2 repression test strain (YZZ101), there was no change in β-galactosidase production (Table 2). This result showed that pLUG-DIS1$_{207}$ does not have a general effect of causing gene activation or disruption of repression. Rather, the effect of pLUG-DIS1$_{207}$ was specific for repression of the test genes by silencer elements.

We also examined if pLUG-DIS1$_{207}$ could interfere with silencing at *HMR*. Previous studies of *HMR* had revealed that repression at this locus is exceptionally strong due to the contribution of redundant silencer elements (8). Consequently, to enhance the sensitivity of detecting interference with silencing, we used two yeast strains that contained partially mutated but functional silencers (YDS36 and YDS39) (44). Northern blot analysis of **a***1* mRNA expressed from the *HMR* locus in an α cell was used as a means of monitoring repression at *HMR* (Fig. 3). Normally, **a***1* expression at the *HMR* locus is silenced, as seen when pLUG alone was transformed into the α strains. pLUG-DIS1$_{207}$ significantly increased **a***1* gene expression in both strains YDS36 (α *hmr* Δ*A*) and YDS39 (α *hmr* Δ*E*) (lanes 2 and 6) to levels comparable to those observed with pLUG-DIS$_{SIR4}$ (lanes 4 and 8), a SIR4-overproducing plasmid obtained in our screen. Increased copies of *HMR* E caused the strongest interference with silencing at *HMR* (lanes 3 and 7), in keeping with the results observed in the original test strain YZZ60. Thus, the results with the reporter genes in the original test strain YZZ60 are comparable to results at the silenced locus *HMR*, demonstrating that the effect of pLUG-DIS1$_{207}$ is not limited to the test genes *TRP1* and *CYC1-lacZ*.

pLUG-DIS1$_{207}$ expresses a truncated form of a protein homologous to the SWI/SNF family of DNA-dependent ATPases. Deletion analysis was used to map the boundaries of the functional region within the pLUG-DIS1$_{207}$ insert (7.8 kb) that conferred dosage interference with silencing (Fig. 4). The smallest subclone that caused dosage interference as measured by a tryptophan prototrophy test was a 3.9-kb fragment that contained the right half of the original pLUG-DIS1$_{207}$ insert (subclone 207E [Fig. 4]), where the left boundary was formed by cleavage of a *Hin*dIII site. DNA sequencing of the entire 207E fragment uncovered the presence of two incomplete open reading frames that extended beyond each edge of the insert. One open reading frame contained sequences that were identical to those of a previously characterized yeast gene, *SPR1*, which encodes a secreted β-glucanase involved in yeast sporulation (55). The second open reading frame exhibited no homology to any known yeast genes. To test the significance of the second open reading frame for the function of 207E, the continuity of the open reading frame was disrupted by insertion of 4 bp at an *Xho*I restriction site (207Eins [Fig. 4]). The

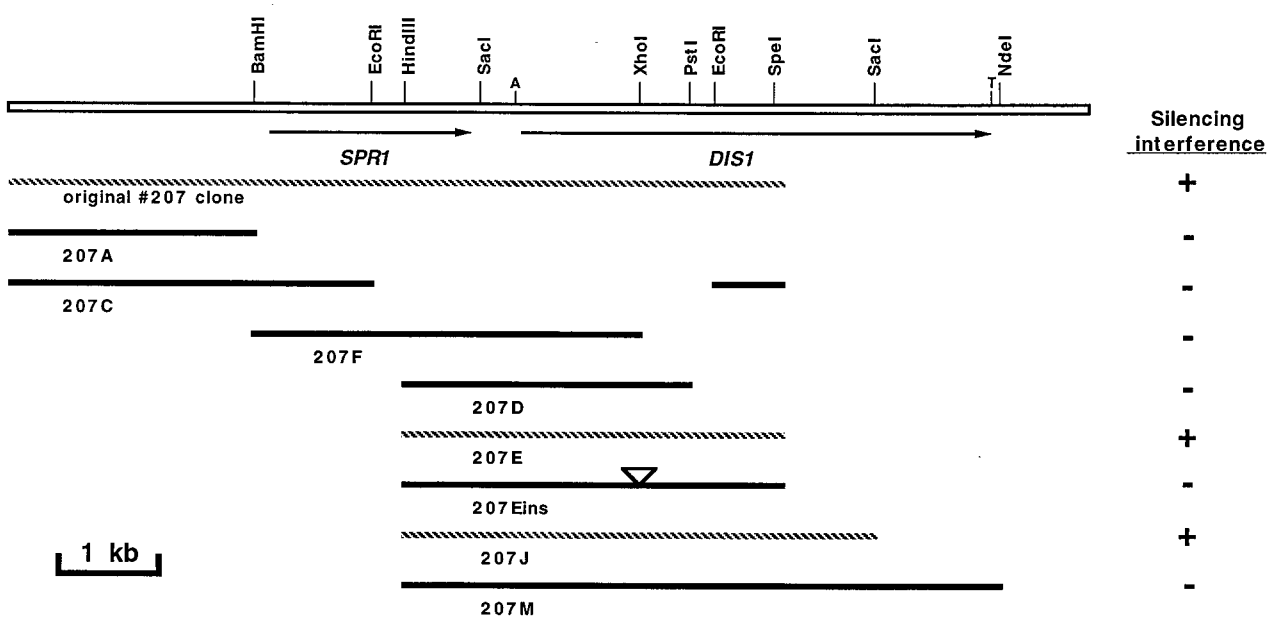


FIG. 4. Structural and functional map of the *DIS1* region. The drawing at the top shows a restriction map of the *SPR1* and *DIS1* loci on chromosome XV. The restriction sites marked represent only those used in generating subclones. Rightward pointing arrows below the map depict the coding regions for *SPR1* and *DIS1*. "A" and "T" mark the initiation and termination codons, respectively, of *DIS1*. Shown below the gene map are various subclones that were tested for silencing interference activity when inserted in pLUG and introduced into the test strain YZZ60. 207Eins contains a 4-bp insertion at the *Xho*I site to disrupt the *DIS1* coding region. The subclones which exhibited silencing interference activity are represented as cross-hatched lines and are also marked + in the column at the right.

FIG. 5. Regional amino acid sequence comparison of selected members of SNF2/SWI2 DNA-dependent ATPase family: DIS1, RAD5, RAD16, lodestar (LoDE), SNF2, and MOT1 proteins. Alignment of amino acid sequences from nine regions of these proteins is shown. Numbers flanking each region indicate the positions of the first and last amino acids in each peptide segment. Sequences that are similar to previously described seven helicase motifs and RING finger motif are underlined. Residues boxed in black are identical in at least three of the sequences compared. Other homologous residues are boxed in gray. Sequence alignments were generated by the Genetics Computer Group software package (University of Wisconsin) and BLAST programs, optimized by visual inspection, and further modified by the Boxshade software.

4-bp insertion completely eliminated silencing interference, suggesting that the product of the novel open reading frame was responsible for the functional activity of the 207E insert.

Since the original pLUG-DIS1$_{207}$ isolate contained an incomplete open reading frame, an intact form of this gene was isolated from a different yeast genomic DNA library propagated in a λ phage vector (24). Restriction mapping and DNA sequencing of an overlapping clone obtained from the phage library revealed that the intact *DIS1* gene contained an open reading frame capable of encoding a protein (Fig. 5) of 1,619 amino acids (~184 kDa). The *DIS1* gene corresponds to the YOR191w open reading frame in the *Saccharomyces* genome database at Stanford University. The truncated fragment in the original pLUG-DIS1$_{207}$ isolate potentially encodes an N-terminal fragment of 894 amino acids (~102 kDa).

While the DIS1 N-terminal half did not have significant

homology to any known proteins, a computerized search (BLAST) (1) of peptide sequence databases revealed that the C-terminal half of the intact DIS1 exhibited strong sequence similarity to the SWI/SNF family of DNA-dependent ATPases (Fig. 5). The predicted DIS1 protein sequence not only has all seven conserved motifs that are present in the superfamily of nucleoside triphosphate-binding proteins that include DNA and RNA helicases but also contains additional homologies found only in the SWI2/SNF2 DNA-dependent ATPase family (Fig. 5). Biochemical studies showed that DIS1 expressed in *E. coli* had DNA-stimulated ATPase activity (81). In addition, DIS1 appears to contain a RING finger motif (27), inserted between motifs IV and V of the conserved ATPase domain (Fig. 5). The RING finger motif, a zinc-binding C_3HC_4 motif, has been implicated in protein-DNA or protein-protein interactions (27). Two other yeast proteins in the SWI/SNF family, RAD5 (40) and RAD16 (65), also contain RING finger structure. Both are involved in the repair of DNA damage, and RAD5 protein has been shown to possess single stranded DNA-dependent ATPase activity (41).

DIS1 participates in mating-type switching. To understand the normal function of DIS1, we constructed yeast strains that contained disruptions of the *DIS1* gene. An internal 3,437-bp *Hin*dIII-*Eco*RI fragment of *DIS1* was excised and replaced with *LYS2* DNA by one-step gene disruption. The altered strains were extensively analyzed for deviations from normal physiological behavior. The Δ*dis1* strains behaved no differently from isogenic *DIS1*$^+$ strains with respect to growth rate in rich media, minimal media, alternative carbon sources (glycerol, galactose), high temperature (37°C), or low temperature (22°C). Thus, *DIS1* is not an essential gene, nor does it contribute in any obvious way to the general growth properties of yeast cells. We next examined the effect of *DIS1* disruption on transcriptional silencing. If the role of DIS1 is to antagonize silencing, a *dis1* knockout should improve silencing. Isogenic *DIS1*$^+$ and Δ*dis1* strains that contained two reporter genes, *TRP1* and *CYC1-lacZ*, controlled by synthetic silencers, were tested for β-galactosidase activities. The Δ*dis1* mutation caused a slight but reproducible increase in silencing of the *CYC1-lacZ* gene (1.5- to 2-fold) (81) (also see Table 2 and Fig. 7).

Since the C-terminal half of DIS1 exhibited significant homology to several yeast proteins involved in the repair of DNA damage (RAD5, RAD16, and RAD54), the Δ*dis1* strains were examined for sensitivity to several DNA-damaging agents: UV irradiation, 4-nitroquinoline-1-oxide, ethidium bromide, and hydroxylamine. Strains that carried mutations known to affect DNA repair (*rad3*, *rad5*, *rad16*, *rad17*, and *rad54*) exhibited high sensitivity to these agents; however, isogenic *DIS1*$^+$ and Δ*dis1* strains exhibited no difference in sensitivity and were relatively resistant to the damaging agents compared to the *rad* strains (data not shown). Thus, DIS1 does not appear to contribute in a significant fashion to the general pathways of DNA repair in yeast.

Since the N terminus of DIS1 specifically interfered with silencing and not other forms of gene repression (i.e., α2 repression), we suspected that DIS1 participated in processes that operate primarily at silenced regions, such as providing access to the silenced loci in the natural recombinational process of mating-type switching. We therefore developed an assay for the rate of switching in cells deficient for DIS1. A plasmid carrying a *GAL-HO* fusion gene was introduced into isogenic *DIS1*$^+$ and Δ*dis1* yeast strains. *GAL-HO*-containing cells were grown in medium containing sucrose, and then galactose was added to induce *HO* expression. Cleavage and recombination events associated with mating-type switching

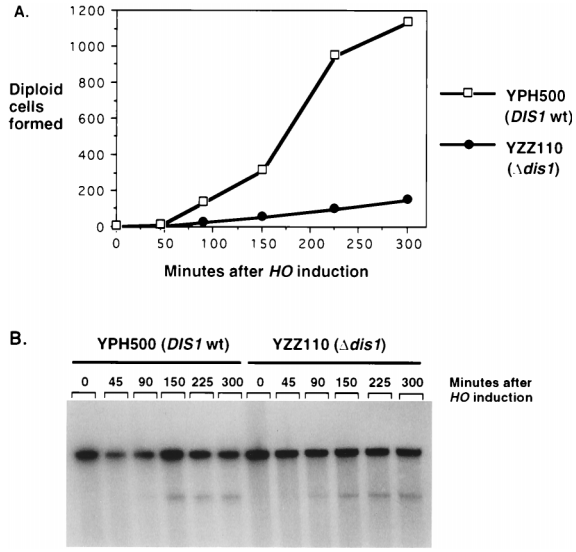


FIG. 6. Disruption of the *DIS1* gene reduces the rate of mating-type switching. (A) Mating-type switching curve. A mating assay was used to measure the efficiency of mating-type switching in isogenic *DIS1*$^+$ (YPH500) and Δ*dis1* (YZZ110) strains carrying plasmid pGAL-HO. Cells were isolated at various times after *HO* induction, indicated on the abscissa, and mixed with an appropriate partner strain to measure the rate of switching as determined by the yield of diploid cells. wt, wild type. (B) Southern blot analysis showing HO cleavage at the *MAT* locus at various time points after *HO* gene induction. Genomic DNA isolated at various time points from cells used for panel A was digested by restriction enzyme *Hin*dIII before electrophoresis and Southern blot transfer, followed by probing with a ^{32}P-labeled *MAT*-specific DNA (520-bp *Eco*RI-*Hin*dIII fragment). HO-cut *MAT* DNA began to be visible on the blot 90 min after galactose induction and reached the steady state at the 150-min point.

are initiated by the HO endonuclease. At various times after the addition of galactose, cells that switched mating type were detected in a mating assay with an appropriately marked partner strain of the same mating type (see Materials and Methods). This analysis revealed that a Δ*dis1* mutation caused an approximately fivefold decrease in the efficiency of mating-type switching at various time points after the induction of *HO* expression in both **a** and α mating-type strains (Fig. 6A and data not shown). Control experiments confirmed that there was no difference in growth rate or viability of *DIS1*$^+$ and *dis1*Δ cells under HO induction (data not shown). Also, no intrinsic difference was detected in mating efficiencies of *DIS1*$^+$ and Δ*dis1* cells in quantitative assays (data not shown). Furthermore, Northern blot analysis of *HO* mRNA demonstrated that there was no significant difference in the rate of *HO* induction between *DIS1*$^+$ and Δ*dis1* cells (data not shown). Also, Southern blot analysis revealed that there was no detectable difference in the rates of HO cleavage of *MAT* DNA in *DIS1*$^+$ and Δ*dis1* cells (Fig. 6B). Thus, our results are consistent with the possibility that in a wild-type cell, DIS1 enhances the efficiency of gene conversion at a step(s) subsequent to HO cleavage of MAT DNA.

The C-terminal catalytic domain of DIS1 is required for silencing interference. The sequence features of the DIS1 protein implied that DIS1 may function to remodel protein-DNA structures, since it has the C-terminal conserved catalytic domain also present in MOT1 and the SWI/SNF complex (3, 45). However, this model creates a paradox since the region of DIS1 that caused silencing interference does not contain the C-terminal catalytic domain. This paradox could be resolved by the following explanation: since wild-type DIS1 was present in the reporter strain YZZ60, if full-length DIS1 normally exists

TABLE 3. Two-hybrid analysis of DIS1-DIS1 interactions

pACTII insert[b]	β-Galactosidase activity (U[a])	
	Alone	With pAS2-DIS1[c]
None	0.04	0.06
p34	0.04	0.05
SNF4	0.04	0.04
DIS1(43-1619)	0.61	5.20

[a] Nanomoles of ONPG hydrolyzed per minute per milligram of protein by extracts of reporter strain Y190 carrying indicated plasmids.
[b] pACTII plasmids express various GAL4(AD) fusions. p34 is the product of mouse *cdc2* gene. SNF4 is a yeast protein expressed on pSE1111.
[c] Expresses the GAL4(DB)-DIS1(43-1619) fusion protein.

in homodimers (or other multimeric forms) that are normally inactive, the presence of excess DIS1 N-terminal domain might induce the formation of full-length/truncated DIS1 mixed dimers which are catalytically active. To test this model, plasmid pLUG-DIS1$_{207E}$ was introduced into strain YZZ62, a Δ*dis1* isogenic derivative of YZZ60, to examine if DIS1 overproduction could still interfere with silencing in absence of full-length *DIS1* gene. As shown in Table 2, silencing interference by pLUG-DIS1$_{207E}$ was greatly reduced in the Δ*dis1* strain, whereas Δ*dis1* did not affect interference by SIR4 overproduction. This genetic interaction between full-length DIS1 and the DIS1 N terminus suggested that the C-terminal catalytic domain was at least partly responsible for silencing interference. To test DIS1-DIS1 interaction further, we took advantage of the two-hybrid system in yeast (26), which uses reporter gene activation as an indirect assay of protein-protein interactions. A GAL4(DB)-DIS1(43-1619) fusion gene was made in vector pAS2 (32). This fusion alone did not activate the *lacZ* gene in the two-hybrid reporter strain Y190. However, when the GAL4(AD)-DIS1(43-1619) fusion was also present in the cells, β-galactosidase activity was increased ~80-fold (Table 3), demonstrating interaction between the two DIS1 fusion proteins. Other GAL4(AD) control fusions (pACTII, pACTII-p34, and pSE1111) did not activate the reporter gene. This evidence supports the model that DIS1 forms a homocomplex.

The reporter gene was weakly activated in the presence of GAL4(AD)-DIS1(43-1619) fusion alone (Table 3). Although the exact cause of activation in the absence of the DNA-binding domain is not known, a DIS1 C-terminal fragment expressed in *E. coli* was found to bind DNA nonspecifically (data not shown), which could cause the random delivery of the GAL4 activation domain.

The N-terminal half of DIS1 protein specifically interacts with the C-terminal half of SIR4. It has been proposed that the nonconserved regions of SWI/SNF family members may be responsible for the unique protein-protein interactions for different functions (3). We suspected that the unique N-terminal half of DIS1 might direct the catalytic DNA-dependent ATPase domain to the silenced regions through specific interactions with components of the silencing machinery. The obvious targets for DIS1 action at silencers are SIR proteins, since these proteins are required for the establishment or maintenance of silencing (reviewed in reference 46). We therefore examined potential DIS1-SIR interactions through a series of two-hybrid studies.

DNA encoding the N-terminal region of DIS1 (amino acids 43 to 888) was fused in-frame to GAL4(DB) in plasmid pAS2 (32), and various segments of the *SIR1*, *SIR2*, *SIR3*, and *SIR4* coding regions were fused in-frame to GAL4(AD) in plasmid pACTII (72). Combinations of fusion constructs were tested

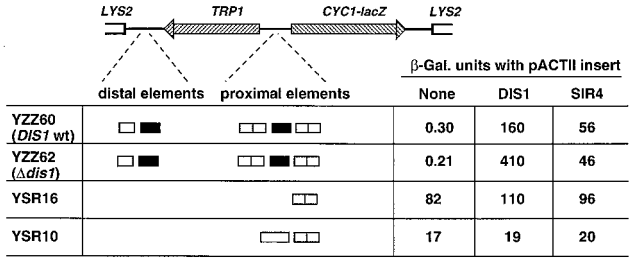
TABLE 4. Two-hybrid analysis reveals specific DIS1-SIR4 interaction

GAL4(AD) fusion	β-Galactosidase activity (U[a])	
	With pAS2-DIS1[e]	With pSE1112[f]
None[b]	0.030	ND
SNF4[d]	0.036	1.0
SIR1[c]	0.029	ND
SIR2[c]	0.027	ND
SIR3(17-978)[c]	0.035	ND
SIR4(58-839)[b]	0.63	ND
SIR4(732-1358)[b]	7.2	0.027
SIR4(839-1358)[c]	0.35	ND
SIR4(839-1149)[c]	0.035	ND
SIR4(1262-1358)[c]	0.038	ND

[a] Nanomoles of ONPG hydrolyzed per minute per milligram of protein by extracts of reporter strain Y190 carrying indicated plasmids. ND, not determined.
[b] Expressed on the pACTII vector.
[c] Expressed on the pGAD vector.
[d] Expressed on plasmid pSE1111.
[e] Expresses the GAL4(DB)-DIS1(43-888) fusion protein.
[f] Expresses the GAL4(DB)-SNF1 control fusion protein.

for the ability to activate transcription of *GAL-lacZ* and *GAL-HIS3* reporter genes (Table 4). Constructs containing GAL4(AD) alone and SNF4 fusion (14) served as controls for the specificity of interaction. Only low basal-level β-galactosidase activity (<0.05 U) was detected in the reporter strain Y190 (22) when the GAL4(DB)-DIS1(43-888) fusion was alone or combined with most of the pACTII derivatives. The GAL4(DB)-DIS1(43-888) in combination with the GAL4 (AD)-SIR4(732-1358) fusion resulted in a sharp activation of the *GAL-lacZ* reporter gene (~200-fold increase in β-galactosidase activity) (Table 4), suggesting a strong interaction between the DIS1 N-terminal domain and the SIR4 C-terminal region. A weaker interaction (~10-fold increase in β-galactosidase activity) was found between DIS1(43-888) and SIR4(839-1358). This weaker interaction may have one of two explanations. First, the GAL4(AD)-SIR4(839-1358) fusion was expressed on a different vector, pGAD (17), and this vector may produce less fusion protein than the pACTII vector. Second, the SIR4 region, 732 to 839, may be necessary for optimal DIS1 interaction but not sufficient for the interaction with DIS1, as SIR4(58-839) did not interact with DIS1 based on the two-hybrid studies. SIR4 is proposed to interact with other proteins through a long leucine zipper domain at its C terminus (17); however, it appeared that the DIS1-SIR4 interaction did not occur solely at this long coiled-coil region, since the GAL4(AD)-SIR4(1262-1358) fusion (pCTC24) did not show any detectable activation of the *GAL-lacZ* reporter gene. DIS1-SIR4 interaction seemed to be quite specific, as SIR1, SIR2, SIR3, and other control fusions failed to activate the reporter genes.

In an effort to map the domain in DIS1 that interacts with SIR4, two shorter fusions, GAL4(DB)-DIS1(43-444) and GAL4(DB)-DIS1(444-888), were tested in the two-hybrid studies. Neither of these two fusions was able to activate the *GAL-lacZ* reporter gene in strain Y190 (data not shown), showing that the SIR4 interaction probably required a fairly long region spanning residue 444 in DIS1. Further studies showed that DIS1(43-444) and DIS1(444-888) were not able to interfere with the synthetic ectopic silencing when they were overproduced on the pLUG vector in strain YZZ60, correlating with their inability to interact with SIR4 (data not shown). Curiously, when a longer fusion, GAL4(DB)-DIS1(43-1619)



	β-Gal. units with pACTII insert		
	None	DIS1	SIR4
YZZ60 (*DIS1* wt)	0.30	160	56
YZZ62 (Δ*dis1*)	0.21	410	46
YSR16	82	110	96
YSR10	17	19	20

FIG. 7. One-hybrid studies to probe DIS1 N-terminus interactions with the silencing machinery. pACTII plasmid vectors expressing GAL4(AD) alone, GAL4(AD)-DIS1(43-888), or GAL4(AD)-SIR4(732-1358) were introduced into indicated yeast strains, and the β-galactosidase (β-Gal.) activity in each cell extract was determined. All yeast strains carry reporter genes, *TRP1* and *lacZ*, under the control of various regulatory elements shown at the left. ▭, RAP1-binding site derived from *HMR* E; ▭▭, two RAP1-binding sites derived from telomere; ▬, ARS derived from *HMR* E; ▭, α2 operator. wt, wild type.

containing the DIS1 C terminus, was used to test the DIS1-SIR4 interaction in the two-hybrid system, only a ninefold increase in the expression of *GAL-lacZ* reporter gene was observed, indicating a weaker interaction. Perhaps the SIR4-interacting activity of DIS1 is normally masked, unless there is a conformational change in DIS1 induced by events such as DNA damage or strand invasion during mating-type switching. When the DIS1 N-terminal domain is overproduced, the formation of mixed dimers containing full-length and truncated DIS1 may adapt an active conformation that allow strong SIR4 interaction. In agreement with this model, overproduction of full-length DIS1 failed to cause the tryptophan prototrophy in YZZ60 by silencing interference (207M in Fig. 4), although the possibility that full-length DIS1 was not produced to an optimal amount in these experiments could not be ruled out.

Strong silencing interference by GAL4(AD)-DIS1 fusions. Since the DIS1 N-terminal domain interacted strongly with SIR4, one might argue that silencing interference by DIS1 simply resulted from titration of SIR4 from the silencers. To test if DIS1 could physically associate with silencers, we used an approach which has been called the one-hybrid system (49). The one-hybrid system devised here assayed whether a GAL4 activation domain can be delivered to the silencer when fused to DIS1.

A set of pACTII plasmid derivatives carrying the GAL4(AD) fusions used in the foregoing two-hybrid studies was introduced into the reporter strain YZZ60. GAL4(AD) alone was not able to activate the reporter gene, giving low basal β-galactosidase activity (0.3 U). When GAL4(AD) was fused to the DIS1 N-terminal half (43 to 888), there was a dramatic ~500-fold increase in β-galactosidase activity (160 U) (Fig. 7). In contrast, this fusion did not significantly affect α2-mediated repression occurring in strain YZZ101 (data not shown) or YSR10 (Fig. 7). This derepression also appeared to occur at the *HML* and *HMR* loci controlled by wild-type silencers, because the mating efficiency of these cells was reduced 30-fold, presumably due to expression of **a** genes at *HMR*. The 500-fold induction in β-galactosidase activity was particularly striking, since overproduction of DIS1 N terminus on the pLUG vector only gave a 20-fold increase in β-galactosidase activity in strain YZZ60 and a Δ*sir4* mutation had just a 50-fold effect (Table 1). Therefore, the complete disruption of ectopic silencing by GAL(AD)-DIS1(43-888) could not be attributed solely to the simple titration of SIR4. Rather, this result suggested that DIS1 directs GAL4(AD) to the silenced regions to overcome gene repression. The full-length DIS1 (amino acids 43 to 1619), when fused to GAL4(AD), caused a smaller but significant derepression of the *CYC-lacZ* reporter gene (~50-fold). This may reflect the lower affinity of normal DIS1 for its target proteins at the silencers or lower level of fusion protein made in the cells. When only the DIS1 C terminus (863 to 1619) fused to GAL4(AD) was used, a mere 10-fold activation of the *CYC-lacZ* reporter gene was observed, reflecting a rather weak or indirect interaction with proteins at the silencer. As expected, the SIR4 C-terminal domain fusion effectively disrupted silencing, probably by associating with normal SIR4 at the silencer, since SIR4 is known to interact with itself, SIR1, and SIR3 (18, 54, 77). The SIR4 N-terminal domain fusion, however, did not have a strong influence on silencing, indicating that SIR4 probably does not use its N-terminal region to anchor onto the silencing machinery.

Since wild-type *DIS1* gene was shown to be required for silencing interference when the DIS1 N-terminal domain was expressed on a pLUG high-copy-number vector, we examined if adding GAL4(AD) could bypass this requirement. When the GAL4(AD)-DIS1(43-888) fusion gene was expressed in the Δ*dis1* strain YZZ62, even stronger activation of the *CYC-lacZ* reporter gene was observed (Fig. 7). By contrast, the derepression caused by SIR4-GAL(AD) fusions was not significantly affected by *dis1* knockout. This result had two implications: first, an activating function was needed for silencing interference, either the DNA-dependent ATPase activity of DIS1 or the transcriptional activation domain of GAL4 specially targeted to the silencer; and second, it provided further support for the view that normal DIS1 has a built-in mechanism to regulate its antisilencing activity.

DISCUSSION

Studies in recent years have shown that eukaryotes have mechanisms that actively remodel protein-DNA interactions to regulate diverse cellular processes. Members in the SWI/SNF DNA-dependent ATPase family may be some of the key players in these mechanisms. We have identified a new protein member in the SWI/SNF family, DIS1, that may function as a silencing-antagonizing factor to facilitate yeast mating-type switching. Here we have explored the connections between DIS1, silencing, and mating-type switching, and as well as the functional implication and regulation of DIS1.

The initial evidence came from the observation that a DIS1 clone interfered with ectopic silencing mediated by synthetic silencers. The ectopic silencing established in strain YZZ60 had many similarities to natural silencing occurring at *HM* loci. First, all four *SIR* genes were required for the repression of the two reporter genes. Second, a compact nucleosomal structure highly resistant to micrococcal nuclease digestion was formed over the silenced reporter gene *TRP1*, and this structure disappeared in the *sir4* disruption background (62). Third, point mutations in DNA elements important for *HM* silencing, the ARS and RAP1-binding sites, caused derepression of the reporter genes, and this derepression could be suppressed by overproduction of SIR1, SIR2, or SIR3 (81). Indeed, the DIS1 clone interfered with both natural silencing and ectopic silencing but not other forms of repression such as α2 repression.

Functional studies suggest a role for DIS1 in mating-type switching. Normal mating-type switching occurs fairly efficiently, at the rate of almost once per generation. This rapid switching requires, at a minimum, the following steps: cell cycle-dependent expression of HO endonuclease, double-strand DNA breakage at the Y-Z junction at *MAT*, copying of the opposite information from a silenced gene cassette, and resolving of recombinational intermediates (reviewed in refer-

ence 30). A number of *SWI* genes that are required for mating-type switching have been identified (reviewed in reference 71). These are mostly genes involved in the regulation of *HO* expression. In our experiments, we used the *HO* gene under the control of a *GAL* promoter, bypassing the requirement for such *SWI* genes. Upon *HO* gene induction by galactose, *MAT* DNA was cleaved at the same efficiency in *DIS1* wild-type and *dis1* knockout strains; therefore, any difference in the rates of mating type switching must come from the steps after DNA cleavage by HO. A Δ*dis1* mutation greatly reduced but did not eliminate mating-type switching, suggesting an important role of DIS1 in this process. There is no obvious lethality caused by HO induction in Δ*dis1* cells, probably because mating-type switching can still occur albeit at a lower rate. While we still do not know the exact step at which DIS1 participates, we speculate that DIS1 might be critical for *MAT* DNA copying information from the silent gene cassettes, since there is a connection between DIS1 and silencing. To complete the nonreciprocal recombination in mating-type switching, *MAT* DNA with a double-strand break must efficiently find and copy the donor DNA at *HML* or *HMR*, which is presumably kept inaccessible by a special compact chromatin structure. DIS1 may interact with the silencing machinery and make the silenced DNA accessible for recombination. A number of genes involved in the DNA repair pathway, *RAD51*, *RAD54*, *RAD55*, and *RAD57*, have been implicated in facilitating strand invasion into otherwise inaccessible donor sequences (74). Despite extensive sequence homology between DIS1 and RAD54, DIS1 does not seem to be involved in DNA repair, although the possibility remains that DIS1 can interact with RAD proteins to regulate and direct strand invasion or recombination.

The C-terminal SNF2 domain (23) in DIS1 may enable it to modify the silencing machinery at *HML* and *HMR*. DIS1 belongs to the SWI/SNF family of DNA-dependent ATPases. The common mode of action of proteins in this family appears to be remodeling protein-DNA structure. For example, SWI2/SNF2, existing in a large complex, uses the energy of ATP hydrolysis to alter DNA-histone interaction, thus facilitating the binding of gene activators to DNA wrapped in nucleosomes (20). MOT1, on the other hand, apparently represses gene transcription by binding to the TATA-binding protein and removing it from promoters (3). Along the same lines, DIS1 might bind to the silencing machinery and modify its structure such that DNA becomes accessible to invasion of *MAT* DNA during mating-type switching. Although our genetic data support this model, it needs to be confirmed by further biochemical studies.

Outside of the conserved SNF2 domain in the SWI/SNF family members, there is little homology among these proteins (23). It has been proposed that different proteins have distinct functions because they are targeted to different protein-DNA complexes, and the nonconserved regions could be responsible for unique protein-protein interactions leading these molecules to specific targets (3). The specific interaction between the DIS1 N-terminal domain and SIR4 may be key for DIS1 targeting to the silencing machinery. The DIS1-silencing machinery interactions were underscored by the observation that GAL4 could be delivered to silencers when fused to DIS1. It is likely that DIS1 binds to SIR4 and/or other proteins in the silencing complex, and the DIS1 C-terminal catalytic domain transiently unravels the higher-order chromatin structure or dislodges components of the silencing apparatus (e.g., SIR4) to make DNA more accessible during mating-type switching.

If the role of DIS1 is restricted to mating-type switching, its silencing-antagonizing activity is expected to be properly regulated since genes at *HM* loci should be transcriptionally repressed at all times. We propose that DIS1 activity is regulated, at least in part, by self-dimerization. DIS1-DIS1 interaction was supported by two pieces of evidence. First, silencing interference by the DIS1 N terminus required a wild-type DIS1 background. Second, this interaction was seen in two-hybrid studies. However, until further biochemical evidence is obtained, we cannot distinguish if the DIS1-DIS1 interaction is direct or indirect. This interaction could be detected if DIS1 also exists in a large protein complex, as for SWI2/SNF2, and there are two DIS1 molecules in this complex. Nevertheless, the DIS1 dimer may usually exist in a latent form that interacts with SIR4 weakly and does not interfere with silencing. We speculate that such latent DIS1 dimers could be activated under certain conditions such as mating-type switching, perhaps signaled by the presence of cleaved *MAT* DNA. Upon completion of copying the template DNA at the *HM* loci, if DIS1 reverts to the inactive form, silencing would resume. Overproduction of DIS1 N-terminal domain may drive the formation of mixed dimers, composed of truncated and full-length DIS1, which adopt a conformation similar to the activated form. For example, the catalytic domain may be exposed in the heterodimer and therefore be able to constitutively antagonize silencing. Further biochemical studies may help us understand how DIS1 is activated and if and how it changes the chromatin structure at silenced regions.

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