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Genetic Redundancy between *SPT23* and *MGA2*: Regulators of Ty-induced Mutations and Ty1 Transcription in *Saccharomyces cerevisiae*

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***SPT23* was isolated as a dosage-dependent suppressor of Ty-induced mutations in *Saccharomyces cerevisiae*. *SPT23* shows considerable sequence homology with *MGA2*, a gene identified as a dosage-dependent suppressor of a *snf2*-imposed block on *STA1* transcription in *S. cerevisiae* var. *diastaticus*. Although single mutations in either of these genes have only modest effects on cell growth, *spt23 mga2* double mutants are inviable. Unlike *SPT23*, multicopy expression of a truncated form of *MGA2* suppresses a narrow subset of Ty-induced mutations. *SPT23*/*MGA2* and the *SNF*/*SWI* genes affect transcription of certain target genes in similar ways. Spt23p appears to be a rate-limiting component required for functional *HIS4* expression of *his4-912*δ, a promoter insertion mutation induced by the Ty1-912 long terminal repeat. Furthermore, both Spt23p and Mga2p can activate transcription when fused to the Gal4p DNA-binding domain, as previously observed with Snf2p and Snf5p. A 50-amino-acid region in the N terminus of the predicted Spt23p protein is necessary and sufficient for the transactivation and necessary for suppression of Ty1-induced mutations and the essential function of Spt23p. Cell fractionation and cytological experiments suggest that Spt23p is associated with the nucleus. Our results suggest that *SPT23*/*MGA2* affects transcription of a subset of genes in yeast, perhaps by changing chromatin accessibility.**

The Ty elements of *Saccharomyces cerevisiae* are a group of retrotransposons that transpose via an RNA intermediate and are similar to retroviruses (for a review, see reference 23). Ty1 and Ty2 elements are approximately 5.9 kb long and contain long terminal repeat sequences (LTRs) (δ) flanking an internal coding region (ε). The δ region contains the TATA box and transcription initiation site. Once inserted in the 5′ noncoding region of a gene, Ty1 and Ty2 elements can abolish or otherwise alter the transcription of the adjacent gene. The internal coding region of Ty1 and Ty2 can be lost by recombination between the homologous δ segments and results in a solo δ insertion. The most-studied Ty1 and Ty2 insertion mutations are those with a Ty element or solo δ inserted in the 5′ noncoding region of *HIS4* or *LYS2* (56). These Ty- or δ-mediated promoter mutations confer a conditional or unconditional His^- or Lys^- phenotype, respectively. Many genes that are important for general RNA polymerase II transcription have been isolated as extragenic suppressors of these Ty insertion mutations and are called *SPT* genes (suppressor of Ty; for a review, see reference 56). For example, *SPT15* encodes the TATA-binding protein TBP, a key component of TFIID, and *SPT11* and *SPT12* encode the histone proteins H2A and H2B, two of the core nucleosome subunits. Most *SPT* genes have been found to be functionally related to either TFIID or histones and therefore have been classified into two major groups: the TFIID-related group and the histone-related group. Mutations in the TFIID-related *SPT* genes (*spt3*, *spt7*, *spt8*, and *spt15*) confer the same pattern of suppression for a set of Ty and δ insertion mutations. They abolish normal Ty1 transcription and cause mating and sporulation defects (19, 20, 58). Mutations in the histone-related *SPT* genes (*spt4*, *spt5*, *spt6*, *spt11*, and *spt12*) strongly suppress δ-induced mutations, only weakly suppress Ty insertion mutations, suppress *snf2*, *snf5*, and *snf6* mutations, and suppress the deficiency in the *SUC2* upstream activation sequence (UAS) region that lacks a UAS (11, 12, 41, 55). In addition, multicopy expression of *SPT5*, *SPT6*, *SPT11*, or *SPT12* confers an Spt^- phenotype. The gene products of *SPT4*, *SPT5*, and *SPT6* have been implicated in the establishment and maintenance of repressive chromatin (13, 39, 55). Recent studies demonstrate that Spt6p directly interacts with histones to enhance nucleosome formation (5).

In addition to the histone-related *SPT* genes, another group of rigorously studied genes, *SNF*/*SWI*, have been shown to regulate chromatin structure and genetically interact with the histone-related *SPT* genes. The connection between the *SPT* and *SNF*/*SWI* genes was first demonstrated by the finding that *SPT6* and *SSN20* are allelic (11). *ssn20* was isolated as a suppressor for the sucrose-nonfermenting phenotype conferred by the *snf2* and *snf5* mutations (40a, 41). This result prompted the findings that mutations in several other *SPT* genes in the histone-related group can also suppress *snf2* and *snf5* mutations (57). The *SNF2* and *SNF5* gene products function in a large multisubunit complex called *SNF*/*SWI* (7). This complex has been demonstrated to regulate the transcription of many genes (for a review, see reference 8). Both Snf2p and Snf5p can activate transcription when bound to DNA (36, 37). Further genetic and molecular studies have suggested that the *SNF*/*SWI* complex activates transcription by antagonizing transcription repression mediated by chromatin and facilitating the binding of activators to nucleosomal DNA (15, 27, 42).

Previously, we have reported the identification and initial molecular characterization of *SPT23*, a multicopy suppressor of Ty-induced mutations (6). Here, we continue our studies of

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TABLE 1. Yeast strains[a]

Strain	Genotype
DG418	*MAT***a**/α *his4-912*δ/*his4-912*δ *lys2-128*δ/*lys2-128*δ *ura3-52*/*ura3-52 leu2*Δ*-hisG*/*leu2*Δ*-hisG spt23-*Δ*1::URA3hisG*/+ *mga2*Δ*::LEU2*/+
DG421	*MAT*α *lys2-61 his4-912 ura3-52*
DG422	*MAT***a** *his4-912*δ *lys2-128R2*δ *ura3-52*
DG1251	*MAT*α *his3-*Δ*200 ura3-176 spt3-101 trp1*Δ*-hisG*
DG1557	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG mga2*Δ*::LEU2*
DG1587	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*-hisG trp1*Δ*-hisG spt23*Δ*-hisG*
DG1613	*MAT*α *his4-912*δ *lys2-61 ura3-52 leu2*Δ*-hisG trp1*Δ*-hisG spt23*Δ*-hisG mga2*Δ*::LEU2* (p*CEN-URA3-SPT23*)
DG1630	*MAT***a** *his4-917(480) lys2-173R2 ura3-52 trp1-289 leu2-1 ste12-*Δ*XbaI*
DG1667	*MAT*α *his4-912*δ *lys2-61 ura3-52 leu2*Δ*-hisG trp1*Δ*-hisG spt23*Δ*-hisG mga2*Δ*::LEU2* (p*CEN-TRP1-spt23-ts*)
JC364	*MAT***a** *ura3-167 his3-*Δ*200 leu2*Δ*-hisG trp1*Δ*-hisG* Ty588::*neo* Ty146(*tyb::lacZ*) Ty1-270*his3-AI*
L592	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 snf2*Δ*1::HIS3*
L896	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2-1 snf6*Δ*2*
S288C	*MAT*α *mal1 gal2*
TB432	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 trp1*Δ*-hisG snf5*Δ*1::TRP1*
SZ40	*MAT***a**/α *his4-917(480)*/*his4-*Δ*29 lys2-173R2*/*lys2-*Δ*201 ura3-52*/*ura3-52 trp1-289*/*trp1-289 leu2-1*/+ *cyh1*/+
SZ41	*MAT***a** *ura3-167 his3-*Δ*200 leu2*Δ*-hisG* Ty1-588::*neo* Ty1-146(*tyb::lacZ*) Ty1-270*his3-AI snf2::LEU2*
SZ42	*MAT***a** *ura3-167 his3-*Δ*200 trp1*Δ*-hisG* Ty588::*neo* Ty146(*tyb::lacZ*) Ty1-270*his3-AI SPT23::(SPT23-HA URA3)*
Y190	*MAT***a** *leu2-3,112 ura3-52 trp1-901 his3-*Δ*200 ade2-101 gal4*Δ *gal80*Δ *URA3::GAL1-lacZ LYS2::GAL1-HIS3 cyh1*

[a] All strains are from this study except for strains L592 and L896, which were obtained from F. Winston, and strain Y190, which was provided by D. Liu.

SPT23 by demonstrating that multicopy suppression of *his4-912*δ, a Ty1-induced promoter mutation, occurs at the transcriptional level. *spt23*Δ cells are viable due to a functionally redundant gene, *MGA2*. An *spt23 mga2* double deletion results in lethality, and Ty1 transcription is significantly reduced. When fused to the *GAL4* DNA-binding domain, both Spt23p and Mga2p activate transcription. Cell fractionation studies suggest that Spt23p is associated with the nucleus. Both *SPT23* and *MGA2* can suppress *snf2* mutations in some but not all genetic contexts. Implications for the involvement of Spt23p and Mga2p in gene activation will be discussed below.

MATERIALS AND METHODS

Yeast strains. The yeast strains used in this study are listed in Table 1. With the exception of Y190 (26), all strains were derived from S288C. The Ty- or δ-induced mutations *his4-912*δ, *lys2-61*, *his4-917(480)*, and *lys2-173R2* have been described previously (9, 21, 45, 54). The marked Ty1-270*his3-AI* element in strains JC364 and SZ41 has been previously described (16). *SPT23-HA(URA3)* was made by integrating the appropriate yeast strain with plasmid pSRZ75 (see below) linearized at a unique *Sal*I site. The *spt23-*Δ*1::URA3hisG* allele was introduced by using the one-step gene replacement method (48) with pBTB134 digested with *Xba*I and *Pvu*II. *spt23*Δ*::GB* was derived from *spt23-*Δ*1::URA3hisG* by loss of the *URA3* marker through homologous recombination (1). The *mga2*Δ*::LEU2* allele, which contains a deletion from 161 nucleotides upstream of the *MGA2* ATG to codon 578, was constructed by using the one-step gene replacement method (48) with the *Sal*I-*Aat*II fragment of pHW29. The *ste12*Δ*XbaI* allele, in which the first 214 codons were deleted from the N terminus of *STE12*, was created by using the two-step gene replacement method (48) with the yeast integrating plasmid pBTB80 linearized at a unique *Sac*I site. The *snf2::LEU2* allele, in which the entire *SNF2* coding region was deleted, was constructed by using microhomologous recombination (40) with primers AACATACCACAGCGTCAATTTAGCAACGAAGAGGTCACTCGAGGAGAACTTC and CTATACACTCGCTTCTGTCATGCTCGAGTCCGCTTCATCTCGTCGTTAAGGCCG (sequences homologous to *SNF2* are underlined) to generate a *snf2::LEU2* PCR fragment. All gene replacements were verified by either Southern hybridization or PCR, combined with phenotypic analyses.

Media and genetic methods. Rich medium (yeast extract-peptone-dextrose [YEPD]), synthetic complete (SC) medium lacking a specific nutrient (e.g., SC-Ura lacks uracil), minimal medium (SD) supplemented with a specific nutrient (e.g., SD + His is supplemented with histidine), and sporulation medium were used as described previously (47). Medium containing 820 mg of 5-fluoroorotic acid (5-FOA) liter^{-1} was used to select cells that had lost the *URA3* gene (3). Standard procedures were used for mating, sporulation, and tetrad dissection (47). Lithium acetate transformation was used to introduce DNA into yeast cells (24). Suppression of Ty-mediated promoter mutations was determined by growth on SC-His or SC-Lys plates at 20, 30, and 37°C.

Plasmids. Plasmids were constructed by standard procedures (49). Generally, restriction fragments were purified from agarose gels by using glass milk (Bio 101, Inc.). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (NEB) and Boehringer Mannheim, respectively. *Taq* DNA polymerase was purchased from Amersham. Plasmids were purified from *Escherichia coli* by a boiling lysis method (30). pBTB134, which contains *spt23-*Δ*1::URA3hisG*, was constructed with a 3.3-kb *Bgl*II-*Bam*HI fragment containing 815 codons from the N terminus of *SPT23* cloned into pBR322. The internal region of *SPT23* between codons 82 and 732 was deleted by PCR mutagenesis (29). A *Bgl*II site was inserted between the deleted sequences. A 3.8-kb *Bgl*II-*Bam*HI fragment containing the *URA3hisG* gene blaster (1) was inserted into the *Bgl*II site. An *Xba*I-*Pvu*II fragment of the resulting plasmid, pBTB134, was used to transform appropriate yeast strains to disrupt the chromosomal *SPT23* locus. pHW29, which contains *mga2-*Δ*1::LEU2*, was constructed by first subcloning the 5.3-kb *Ban*III-*Bgl*II fragment containing *MGA2* into the *Ban*III and *Bam*HI sites of pBR322, resulting in pHW23. The 2-kb *Sac*I-*Pvu*I fragment was then replaced with the 2.2-kb *Sal*I-*Xho*I fragment containing *LEU2*. pBTB80 was created by subcloning a 5.5-kb *Cla*I fragment containing *STE12* from pSC4 (22) into YIp5 and deleting the *Xba*I fragment that contains 214 codons from the N terminus of the *STE12* coding sequence. A 4.3-kb *Bgl*II-*Kpn*I fragment containing full-length *SPT23* from pAB14 (6) was subcloned into pRS vectors (52) pRS416 to generate pSRZ46, pRS414 to generate pSRZ47, and pRS426 to generate pBDG769. pYK2 is a *URA3*-based centromeric plasmid containing a 9.0-kb yeast genomic DNA fragment spanning *MGA2*. pRSZ65 was constructed by subcloning a 5.1-kb *Hin*dIII fragment that contains *MGA2* into the *URA3*-based multicopy vector pRS426. pSRZ112 and pSRZ67 were constructed by subcloning a 4.2-kb *Hin*dIII-*Bgl*II fragment containing a truncated form of *MGA2* into pRS416 and pRS426, respectively. pSRZ94, which contains a *GAL4* DNA-binding domain–*SPT23* coding sequence hybrid, was constructed by digestion of pSRZ46 with *Bsp*EI, fill-in synthesis of the ends with Klenow polymerase, and ligation to an unphosphorylated *Bam*HI 10-mer linker (NEB). A 2.4-kb *Bam*HI fragment containing codons 25 to 815 of *SPT23* from the resulting plasmid was then cloned into the *Bam*HI site of pGBT9 (Clontech), which contains the *GAL4* DNA-binding domain coding sequence. pSRZ97 was constructed by deleting sequences between the *Sal*I site in the vector and the *Sal*I site in *SPT23*. pSRZ98 was constructed by digestion of pSRZ94 with *Sma*I and *Sac*I, fill-in synthesis of the DNA ends with Klenow polymerase, and religation. pSRZ103 was constructed by replacing the small *Sac*I-*Nde*I fragment of pSRZ94 with that of pSRZ95, which is an in-frame deletion between the *Eco*RI and the *Cla*I sites of pSRZ46. pSRZ106, which contains the intact 3′ coding sequence of *SPT23*, was constructed by inserting the *Sal*I-*Xho*I fragment of pSRZ72 into the *Sal*I site of pSRZ94. pSRZ72 was constructed by inserting an unphosphorylated *Xho*I 8-mer linker (NEB) into the *Not*I site of pSRZ54 (see below). pSRZ110 was constructed by digestion of pSRZ94 with *Sac*I and *Cla*I, fill-in synthesis of the DNA ends with Klenow polymerase, and ligation to an unphosphorylated *Xho*I 10-mer linker (NEB). pSRZ111 and pSRZ116 were created by digestion of pSRZ94 with *Sal*I and either *Cla*I or *Sac*I, respectively, fill-in synthesis of the DNA ends with Klenow polymerase, and religation. pSRZ105, which contains a *GAL4* DNA-binding domain–*MGA2* coding sequence hybrid, was created by inserting a PCR-amplified *MGA2* coding region into pGBT9. A segment containing *MGA2* (codons 2 to 1113) was PCR amplified from pSRZ65 with primers 5′-CGCGGATCCAGCAGAACAGTGAGTTC-3′ and 5′-CGCGGATCCTAACTGACAATTAAATCGTTC-3′, which added a *Bam*HI site to both the 5′ and the 3′ ends of *MGA2*, and was used to create an in-frame fusion of *MGA2* to the *GAL4* DNA-binding domain coding sequence. The *Bam*HI fragment of the resulting PCR product was inserted into the *Bam*HI site of pGBT9, pSRZ105Δ173, in which 173 codons were deleted from the C terminus of *MGA2* in pSRZ105, was created by diges-

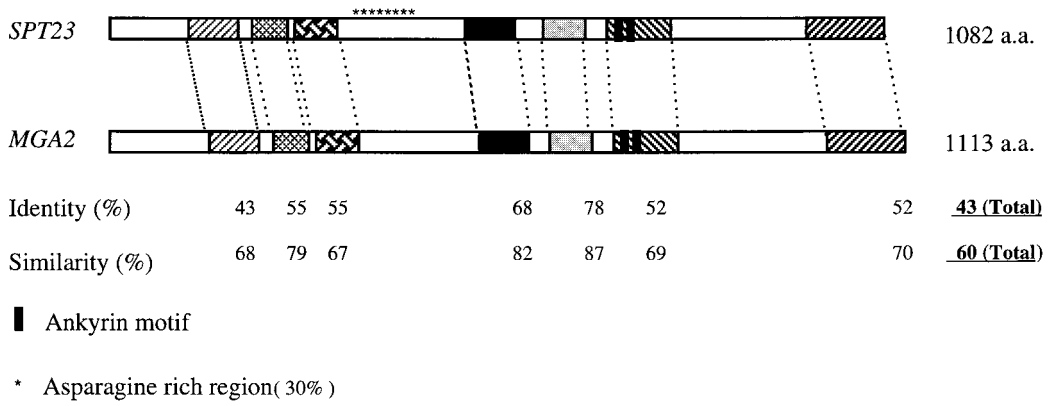

FIG. 1. *SPT23* and *MGA2* are homologs. The ORFs predicted from the nucleotide sequences of *SPT23* and *MGA2* were aligned by using BLAST and FASTA, and the resulting regions of protein homology are highlighted (shaded rectangles). The alignment is presented from the N terminus (left) to the C terminus (right) of the ORFs. a.a., amino acids.

tion of pSRZ105 with *Bgl*II and *Sal*I, filling in of the DNA ends with Klenow polymerase, and religation. To tag *SPT23* with the influenza hemagglutinin (HA) epitope, PCR-based mutagenesis was used to add a *Not*I-containing sequence (GCGGCCGCG) just before the stop codon TAA of *SPT23* in pSRZ46, generating pSRZ54. Two 111-bp *Not*I fragments containing triple HA1 epitope tags from plasmid GTEP1 (generously provided by M. Rose) were cloned into the *Not*I site of pSRZ46 in the correct orientation. Subsequently, the 4.4-kb *Bss*HII fragment was used to replace the small *Bss*HII fragment of yeast-integrating plasmid pRS406 (52). To construct the *GAL1*-promoted *GFP-lacZ-SPT23* fusion, a 1.5-kb *Sac*I-*Hin*dIII fragment of pPS816 (kindly provided by P. Silver) was replaced by a 1.5-kb *Sac*I-*Hin*dIII fragment of pPS817 (kindly provided by P. Silver), resulting in pSRZ76. Then a 3.3-kb *Xho*I PCR fragment containing *SPT23* (codons 1 to 1082), which was amplified with primers 5′-GGGGGCTC GAGATGATGAGTGGCACAGG-3′ and 5′-GGGGGCTCGAGTTAATTGA CTCGCATGTC-3′ from pSRZ46, was cloned into the *Sal*I site of pSRZ76, generating pSRZ79.

Isolation of the *spt23-ts* allele. A standard plasmid shuffle protocol was used to isolate *spt23* temperature-sensitive (*ts*) mutants. pSRZ47, a *TRP1*-based centromeric vector carrying *SPT23*, was mutagenized with hydroxylamine as described elsewhere (51). Strain DG1613, which contains the *spt23*Δ *mga2*Δ double mutation and the *SPT23 URA3* plasmid pSRZ46, was transformed with the mutagenized pSRZ47. Trp^+ transformants were replica plated on 5-FOA plates followed by incubation at 20 and 37°C to detect mutants that could survive the loss of pSRZ46 at 20°C but not at 37°C.

RNA isolation and Northern (RNA) hybridization. For analysis of *HIS4* RNA, strains were grown in supplemented SD medium at the indicated temperature. For analysis of Ty1 transcripts, cells were grown in YEPD or SC medium at 30°C, unless otherwise noted. Total yeast RNA was isolated as previously described (50). Northern hybridization was performed as previously described by Burkett and Garfinkel (6). For analysis of *HIS4* RNA, 5 μg of total yeast RNA was separated on a 1% agarose gel for 1,200 V · h and blotted onto a Hybond-N membrane (Amersham). RNA was UV cross-linked to the membrane according to the supplier's recommendations. Riboprobes for *HIS4* and Ty1, made by in vitro transcription with T7 or SP6 polymerase, were hybridized with RNA at 57°C in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.05 M sodium phosphate (pH 7.0)–5× Denhardt's solution (49). The amount of RNA transferred was normalized by hybridization with ^{32}P-labeled probes for *PYK1* mRNA or 18S rRNA.

β-Galactosidase assay. Yeast strain Y190 transformed with plasmids for two-hybrid analysis (vector pGAD424 [Clontech], which contains the *GAL4* activation domain coding sequence, and *GAL4* DNA-binding domain–*SPT23* or –*MGA2* coding sequence hybrids) was grown in SC-Leu-Trp liquid medium at 30°C to log phase. Cells were harvested, and total cell extracts were used to assay β-galactosidase activity (46). Typically, four independent transformants of each plasmid pair were assayed.

Ty1 transposition assay. The assay for Ty1 transposition was done essentially as described by Curcio and Garfinkel (16, 17). Briefly, cells containing the Ty1-270*his3-AI* transposition reporter were transformed with pRS426 or pBDG769. The resulting transformants were grown in SC-Ura liquid medium at 20°C to saturation. The titers of the cultures were determined on SC-Ura medium, and the cultures were plated on SC-Ura-His medium to determine the number of His^+ prototrophs. The His^+ frequencies from four independent transformants were averaged to determine the transposition efficiency.

Cell fractionation and immunoblot analysis. Fractionation of yeast strain SZY42 was carried out by differential centrifugation essentially as described by Amati and Gasser (2). Briefly, cells were grown at 30°C in YEPD liquid medium to mid-log phase, and the cell walls were digested with Zymolyase-100T (ICN). The resulting spheroplasts were disrupted by Dounce homogenization on ice. Cell debris and unlysed cells were pelleted by centrifugation in a Sorvall HB4 rotor at 5,000 × *g*. A portion of the supernatant was reserved as total cellular protein extract, and the rest of the supernatant was centrifuged in the HB4 rotor at 23,500 × *g*. The resulting supernatant was designated the cytoplasmic fraction. The crude nuclear pellet was washed twice with buffer containing 10 mM Tris-HCl (pH 7.4), 2 mM EDTA-KOH, 0.125 mM spermidine, 0.05 mM spermine, 20 mM KCl, 1% thiodiglycol (Pierce), 1% Trasylol (Bayer), 0.5 mM phenylmethylsulfonyl fluoride (Sigma), and 2 of pepstatin A μg ml^{-1} and designated the nuclear fraction. Protein concentration was determined with a Bio-Rad protein assay kit. A 10-μg aliquot of protein from each fraction was resolved by electrophoresis through sodium dodecyl sulfate–8% polyacrylamide gels (NOVEX). The separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore) and analyzed with the ECL Western blotting analysis system (Amersham). Antibodies against nuclear Snf2p (kindly provided by B. Laurent [7]), cytoplasmic Hts1p (kindly provided by T. Mason), and HA (12CA5; Boehringer Mannheim) were used to detect these proteins in each fraction.

***GFP-lacZ-SPT23* fusion protein visualization.** To visualize the green fluorescent protein (GFP) autofluorescence of *GAL1*-promoted *GFP-lacZ-SPT23* fusion protein, wild-type strain JC364 transformed with either pSRZ76 (*GFP-lacZ*) or pSRZ79 (*GFP-lacZ-SPT23*) was pregrown in SC-Ura raffinose medium to early log phase. Galactose (2% final concentration) was added to the culture to induce the expression of the fusion gene. The cultures were incubated for another 2 h, and then the cells were washed with water, resuspended with SC-Ura glucose medium, and incubated for another 3 h. Samples were taken at 1-h intervals after the cultures were shifted to SC-Ura glucose medium. The cells were fixed with 5% formadehyde, and cell wall was digested with 1 mg of Zymolyase-100T ml^{-1} for 30 min at 30°C. Nuclei were stained with 400 ng of 4′,6-diamidino-2-phenylindole (DAPI) ml^{-1}. The GFP autofluorescence and DAPI staining were examined with a Zeiss Axiophot microscope. The images were processed by using Adobe Photoshop (Adobe).

RESULTS

***SPT23* and *MGA2* are functionally redundant.** We previously reported isolating *SPT23* as a dosage-dependent suppressor of the Ty-induced promoter mutations *his4-912*δ and *lys2-61* (6). Cells with an *spt23* deletion were viable, suggesting that *SPT23* is not essential. Another *S. cerevisiae* open reading frame (ORF) on chromosome IX, YIR033w, shows considerable sequence homology with *SPT23* (Fig. 1). This ORF was also identified as a multicopy suppressor of a transcription defect of *STA1* conferred by a *snf2* mutation in *S. cerevisiae* var. *diastaticus* and designated *MGA2* (multicopy suppressor of *gam1* [*snf2*]) (61). *SNF2* is required for transcription of *STA1*, which encodes an extracellular glucoamylase in *S. cerevisiae* var. *diastaticus* (62). *snf2* cells are unable to utilize starch (Sta^-), but overexpression of *MGA2* on a multicopy plasmid can suppress the Sta^- defect (61). Like *SPT23*, *MGA2* is not

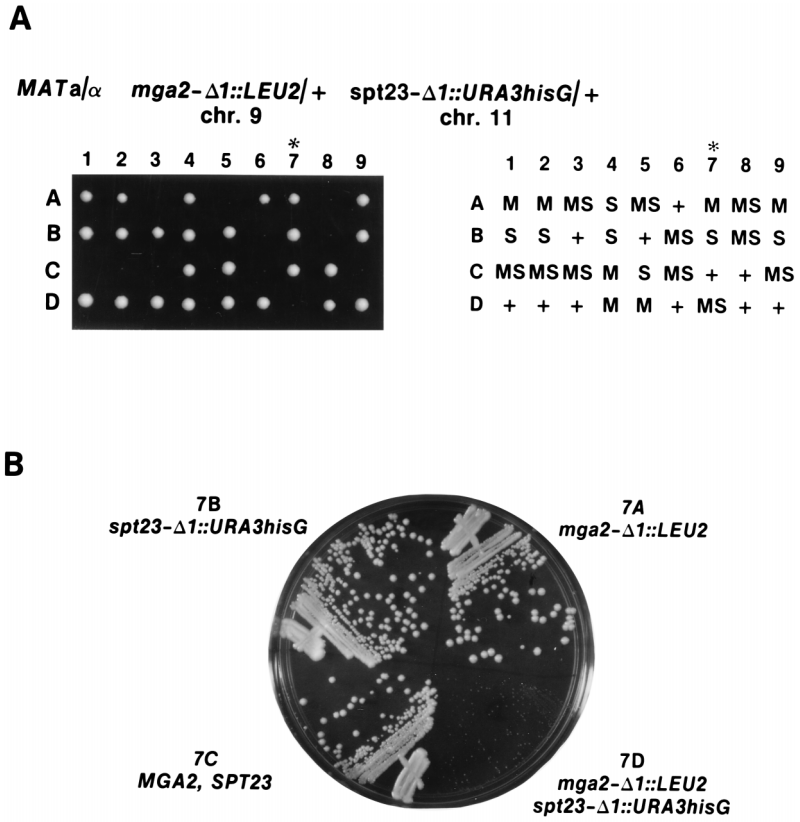

FIG. 2. An *spt23 mga2* null mutant is inviable. (A) Tetrad analysis of a compound heterozygote containing the deletion substitution mutations *mga2-Δ1::LEU2* and *spt23-Δ1::URA3hisG*. Ascosporal colonies (A to D) from nine tetrads (1 to 9) are shown on the left. The relevant genotypes for wild-type (+), *mga2-Δ1::LEU2* (M), *spt23-Δ1::URA3hisG* (S), and *mga2-Δ1::LEU2 spt23-Δ1::URA3hisG* (MS) spores are shown on the right. (B) Clonal purification of ascosporal colonies from tetrad 7* in panel A. Photographs were taken after a 4-day incubation on YEPD medium at 30°C.

essential for cell viability. Detailed characterization of *MGA2* will be published elsewhere.

Both *SPT23* and *MGA2* encode proteins of over 120 kDa that have several regions of homology. These homology domains are colinear and distributed over most of the coding sequence (Fig. 1). Overall, Spt23p and Mga2p are 43% identical and 60% similar as aligned by using BLAST or FASTA alignment programs. Both proteins contain at least two ankyrin repeats, which have been implicated in protein-protein and protein-membrane interactions (4). *SPT23* also contains an asparagine-rich region, which may have functional significance.

To further understand the functional relatedness of *SPT23* and *MGA2*, we determined the phenotype of an *spt23 mga2* double mutant. A diploid strain heterozygous for both *SPT23* and *MGA2* was constructed, and tetrad analysis was performed (Fig. 2). The recessive null mutations *spt23-Δ1::URA3hisG* and *mga2-Δ1::LEU2*, containing the *URA3* and *LEU2* genes, respectively, provide tightly linked markers that unambiguously identify these mutations. Tetrad analysis clearly shows that *spt23-Δ1::URA3hisG mga2-Δ1::LEU2* double mutants are inviable (Fig. 2), whereas the single mutants grow as well as wild-type spores from the same tetrad (Fig. 2B). We isolated an *spt23-ts* allele which complemented the *spt23*Δ *mga2*Δ double deletion lethality at 20°C but not at 37°C. When the *spt23*Δ *mga2*Δ cells were transformed with a centromeric (CEN) plasmid carrying the *spt23-ts* allele, cells stopped dividing at all stages of the cell cycle, as judged by microscopic analysis after 5 h at 37°C, and gradually lost viability (63). This indicates that the *spt23 mga2* double mutant does not have a cell cycle defect.

Multicopy suppression of Ty insertion mutations by *SPT23* and *MGA2*. At least 17 *SPT* genes have been isolated to date. According to their genetic and molecular suppression patterns, they have been classified into two major groups (56). Mutations in the histone group of *SPT* genes strongly suppress only δ-induced promoter mutations, a minor group consisting of *spt13* and *spt14* suppress only Ty-induced promoter mutations, and mutations in the TFIID group of *SPT* genes strongly suppress both types of promoter mutations. Therefore, studying the suppression pattern of *SPT23* may help assign it to one of the two major groups of *SPT* genes.

To understand further the functional relatedness between *SPT23* and *MGA2*, we determined whether *MGA2* is a dosage-dependent suppressor of Ty insertion mutations by comparing its activity with that of *SPT23* (Table 2). *SPT23* and *MGA2* were subcloned into CEN or 2μm plasmids to modulate the plasmid copy number (44). The resulting plasmids were introduced into strains containing different Ty or δ insertion mutations. In some cases, null mutations in other transcriptional regulatory genes were introduced to help determine the suppression activity of *SPT23* and *MGA2*. Unlike for *SPT23*, no suppression of any Ty insertion mutations analyzed was observed with *2*μm-*MGA2* (pSRZ65). However, if the C-terminal 173 codons were deleted from *MGA2* (*mga2-Δ173*), the truncated gene was able to suppress the Ty-induced mutation *lys2-61*. The low-copy-number *CEN-mga2-Δ173* (pSRZ112) also suppressed *lys2-61*. Therefore, the suppression by *mga2-Δ173* was not always gene dosage dependent (Table 2). These results suggest that *SPT23* and *MGA2* are functionally and genetically

TABLE 2. Suppression of Ty-induced promoter mutations by *SPT23* and *MGA2*

Strain	Relative genotype	Suppression with a plasmid containing[a]:						
		Vector	*CEN-SPT23*	2μm-*SPT23*	*CEN-MGA2*	2μm-*MGA2*	*CEN-mga2-Δ173*	2μm-*mga2-Δ173*
DG421	*his4-912*	−	−	+	−	−	−	−
DG422	*his4-912δ*	CS[b]	CS	+	CS	CS	CS	CS
L592	*his4-912δ snf2*	−	+/−	+	−	−	−	−
DG421	*lys2-61*	CS	+/−	+	CS	CS	+	+
DG1630	*lys2-173R2 ste12*	−	−	+	−	−	−	+
DG1630	*his4-917(480) ste12*	−	−	+	−	−	+	+
SZ40	*lys2-173R2* **a**/α	−	−/+	+	−	−	−	+
SZ40	*his4-917(480)* **a**/α	−	−/+	+	−	−	−	+

[a] Suppression strength: + > +/− > −/+ > −. Plasmids carrying *CEN-SPT23* (pSRZ46), 2μm-*SPT23* (pBDG769), *CEN-MGA2* (pKY2), 2μm-*MGA2* (pSRZ65), *CEN-mga2-Δ173* (pSRZ112), 2μm-*mga2-Δ173* (pRSZ67), and vector (pRS426) were transformed into the indicated strains and assayed for histidine or lysine requirement.

[b] CS, cold sensitive.

related but that their functions do not completely overlap. The C terminus of *MGA2* may encode an inhibitory function that affects its activity or stability.

Key genes in the *SNF/SWI* complex, such as *SNF2*, *SNF5*, and *SNF6*, also affect the expression of some Ty and δ insertion mutations (25). The *SNF/SWI* complex is involved in controlling the expression of many genes. Several lines of evidence suggest that transcriptional activation by the *SNF/SWI* complex is mediated by changes in chromatin structure (27, 42). Normally, the *his4-912δ* and *lys2-61* mutations confer cold-sensitive His^- and Lys^- auxotrophies, respectively. Mutations in *SNF2* and *SNF5* render *his4-912δ* and *lys2-61* mutants unconditionally His^- and Lys^- (25). In addition, mutations in the histone group of *SPT* genes, including *spt4*, *spt5*, and *spt6*, suppress the unconditional auxotrophies of *his4-912δ* and *lys2-61* caused by *snf2* and *snf5*. If multicopy *SPT23* suppresses Ty insertion mutations like the histone group of *SPT* genes, then *SPT23* should suppress the transcriptional defect of *his4-912δ* conferred by *snf2*, *snf5*, or *snf6*. Therefore, we introduced a 2μm-*SPT23* plasmid (pBDG769) into a *his4-912δ* yeast strain carrying *snf2*, *snf5*, or *snf6* and determined whether the transformants became His^+. 2μm-*SPT23* strongly suppressed the His^- phenotype conferred by *snf2* (Fig. 3; Table 2) and *snf5* but not by *snf6* (Fig. 3). When 2μm-*MGA2* (pSRZ65) or 2μm-*mga2-Δ173* (pSRZ67) was introduced into *snf his4-912δ* strains, suppression was not observed.

We previously reported that 2μm-*SPT23* suppresses Ty-induced promoter mutations *his4-917(480) and lys2-173R2*, which are under mating-type control (6). The expression of many Ty insertion mutations under *MAT* control requires *STE7* and *STE12* in haploid cells (14). *STE12* is a gene-specific activator necessary for Ty1 transcription. *STE12* has been shown to exert its regulatory effect on the Ty1 insertion mutation *CYC7-H2* through a binding site within Ty1 (14). To determine whether this is true for *his4-917(480)* and *lys2-173R2*, we introduced *ste12Δ* into a strain containing the Ty-activating

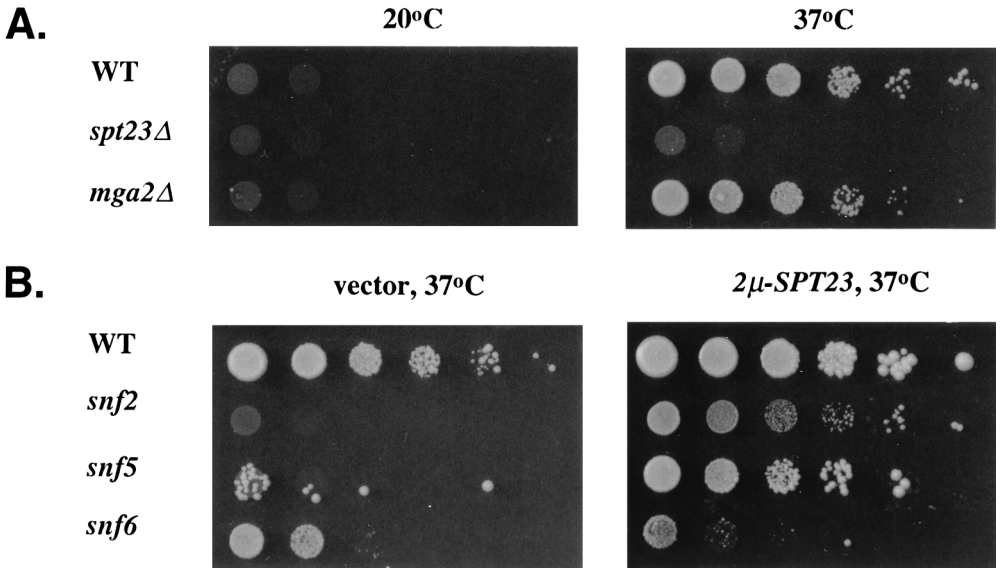

FIG. 3. Different strain backgrounds cause different *his4-912δ* expression. (A) The *spt23* mutation renders *his4-912δ* an unconditional His^- phenotype. Yeast strains DG422 (wild type [WT]; top row), DG1587 (*spt23Δ*; middle row), and DG1557 (*mga2Δ*; bottom row) were grown in YEPD liquid medium at 30°C to early log phase. The cultures were spotted on SC-His plates with 10-fold serial dilutions from left to right. The resulting plates were incubated at 20 or 37°C for 4 days before photographs were taken. (B) Suppression of *his4-912δ* by overexpressing *SPT23* on a multicopy 2μm-*SPT23* plasmid. Strains DG422 (WT), L592 (*snf2*), TB432 (*snf5*), and L896 (*snf6*) were transformed with either pBDG769 (2μ-*SPT23*) or the vector plasmid alone, as indicated above the panels, and were grown in SC-Ura liquid medium at 30°C to early log phase. The cultures were then spotted onto SC-His plates as described above. The resulting plates were incubated at 37°C for 4 days before photographs were taken.

mutations *his4-917(480)* and *lys2-173R2* and then determined whether the resulting strain was His^+ and Lys^+. Cells containing *STE12* [*his4-917(480)* and *lys2-173R2*] were His^+ Lys^+ (6); however, when *STE12* was deleted, the cells became His^- and Lys^- (Table 2). Overexpression of *SPT23* on a multicopy plasmid, pBDG769, restored the His^+ and Lys^+ phenotypes of *his4-917(480)* and *lys2-173R2* in the *ste12*Δ strain. In addition, multicopy expression of *mga2-*Δ*173* (pSRZ67) also restored the His^+ Lys^+ phenotypes of these mutations. Our data suggest that multicopy *SPT23* or *MGA2* can replace the *STE12* activator in the context of the *his4-917(480)* and *lys2-173R2* mutations, implying that *SPT23*, *MGA2*, and *STE12* functionally overlap. *STE12* is required for cell mating. However, a mating defect has not been observed in *spt23* or *mga2* mutants, indicating that the functional overlap between *SPT23*, *MGA2*, and *STE12* is limited to specific target genes.

Expression of *his4-912*δ in an *spt23* mutant. As mentioned above, *spt23* null mutants are viable and appear to grow normally. To determine the effect of an *spt23* null mutation on *his4-912*δ expression, we disrupted wild-type *SPT23* in a *his4-912*δ strain and assayed the resulting *spt23* mutant for histidine prototrophy. Cells became His^- at all temperatures when *SPT23* was disrupted (Fig. 3). Therefore, unconditional His^- is another phenotype that the *spt23* null mutant shares with *snf2*, *snf5*, and *snf6* in the context of *his4-912*δ. The *mga2* null mutation, however, had no effect on *his4-912*δ; cells remained His^- at 20°C and His^+ at 37°C.

Suppression of *his4-912*δ by multicopy *SPT23* occurs at the level of transcription. The *his4-912*δ mutation contains a solo LTR (δ) inserted at the *HIS4* promoter and arose by homologous recombination between the LTRs of the complete Ty1 insertion mutation *his4-912* (53). The 334-nucleotide LTR sequence is inserted between the *HIS4* enhancer (UAS) and TATA box. Two transcripts are produced from *his4-912*δ: a longer, nonfunctional transcript (*his4-912*δ) that begins in δ at the normal initiation site for Ty1 mRNA (28, 53), and a shorter, wild-type *HIS4* transcript (*HIS4*) that begins at the normal initiation site for *HIS4* mRNA. Mutational data support the hypothesis that promoter competition leads to the conditional His^- phenotype of *his4-912*δ (28). Since promoter preference can be influenced by numerous *cis*- and *trans*-acting factors, *his4-912*δ is a sensitive reporter gene for detecting subtle changes in the transcriptional apparatus and chromatin structure (12, 33).

As shown above, *2*μm-*SPT23* strongly suppresses the cold-sensitive His^- phenotype of *his4-912*δ. To determine whether this suppression occurs at the transcriptional level, we examined the *his4-912*δ transcripts from wild-type and various mutant strains containing *2*μm-*SPT23* by Northern hybridization analysis (Fig. 4B). The relative amounts of the *his4-912*δ and *HIS4* transcripts were readily observed when cells containing the plasmid vector alone were grown at different temperatures. At the permissive temperature of 30 or 37°C, more of the short wild-type *HIS4* transcript was observed (Fig. 4B, lanes 4 and 6) than at the nonpermissive temperature (20°C). At 20°C, more of the longer *his4-912*δ transcript than of the *HIS4* transcript was observed (Fig. 4B, lane 2). For cells containing *2*μm-*SPT23*, the *HIS4* transcript appeared concomitantly with the *his4-912*δ transcript at all temperatures (Fig. 4B, lanes 3, 5, and 7); *2*μm-*SPT23* apparently did not have a significant effect on the amount of the long *his4-912*δ transcript except at 30°C. At 30°C, the amount of *his4-912*δ transcript decreased with *2*μm-*SPT23* (Fig. 4B, lane 5).

We also examined the *his4-912*δ transcripts in *snf2* strains with or without *2*μm-*SPT23* (Fig. 4B, lanes 10 and 11). In the absence of *2*μm-*SPT23*, an aberrant internally initiated *HIS4* transcript predominated (25). In addition, a low level of the long *his4-912*δ transcript was also observed. In the presence of *2*μm-*SPT23*, the wild-type *HIS4* transcript was restored and the other two nonfunctional transcripts were maintained. The *spt23*Δ mutant, on the other hand, showed a different *his4-912*δ transcription profile from the *snf2* mutant even though they had the same phenotype (unconditional His^-). Cells containing an *spt23* null mutation produced 70-fold more of the long *his4-912*δ transcript than the wild-type cells (Fig. 4B, lane 6) and very little, if any, of the wild-type *HIS4* transcript (lane 8), as determined by scanning densitometry. Interestingly, our results indicate that Spt23p apparently is limiting for the expression of *his4-912*δ at the *HIS4* transcription initiation site: cells become His^+ when Spt23p is present at higher levels, cells are conditionally His^+ when Spt23p is present at normal levels, and cells are unconditionally His^- when Spt23p is absent.

***SPT23* and *MGA2* affect Ty1 transcription and transposition.** To determine whether *SPT23* or *MGA2* affects Ty1 transcription, we performed Northern hybridization analysis of Ty1 transcripts in a variety of strain backgrounds (Fig. 5). Generally, neither *spt23* nor *mga2* single mutants affected Ty1 transcription. Multicopy expression of *SPT23* or *MGA2*, however, slightly increased the amount of Ty1 transcripts. There was also a shorter RNA present, which is approximately 1.8 kb (Fig. 5, bottom arrowhead). Loss of function of both *SPT23* and *MGA2* caused by shifting of strain DG1667 (*spt23*Δ *mga2*Δ carrying an *spt23-ts* allele on a centromeric plasmid) to the nonpermissive temperature (37°C) decreased the amount of full-length 5.7-kb Ty1 transcript and maintained the amount of a shorter, 4.9-kb Ty1 transcript (Fig. 5, top arrowhead) that comigrated with a 4.9-kb transcript present in an *spt3* strain (59). Relative to the level of 18S rRNA (63), no Ty1 transcripts were detected after DG1667 was shifted to the nonpermissive temperature for 24 h. *snf2* mutants contained very little full-length Ty1 transcript but instead had a shorter Ty1 transcript, which migrated to a position similar to that of the short Ty1 transcript observed in the *spt3* mutant (Fig. 5) (25). We determined whether *2*μm-*SPT23* had any effect on Ty1 transcription in *snf2* mutants. Northern analysis suggests that *2*μm-*SPT23* can restore the full-length Ty1 transcript in a *snf2* mutant. Since there was little full-length Ty1 transcript in the *snf2* mutant and *2*μm-*SPT23* restored it, Ty1 transposition should decrease in a *snf2* strain, and *2*μm-*SPT23* expression might also suppress this phenotype. We replaced the *SNF2* chromosomal locus with a *LEU2* marker in strain JC364, which contains Ty1-270*his3-AI*, a marked genomic Ty1 element containing the *his3-AI* retrotransposition indicator gene (16, 17). The *2*μm-*SPT23* plasmid was introduced into the resulting *snf2::LEU2* strain, SZY41, and the transformants were assayed for Ty1 transposition. As expected, Ty1 transposition was reduced 24-fold in the *snf2::LEU2* mutant, as monitored by the level of His^+ prototroph formation (Table 3). However, multicopy *SPT23* expression increased the Ty1 transposition frequency in the *snf2::LEU2* mutant to the wild-type level. In addition, *2*μm-*SPT23* expression stimulated Ty1 transposition in wild-type cells. Northern blot analysis showed that the levels of both the Ty1 and the Ty1-270*his3-AI* transcripts increased in wild-type cells as well as in the *snf2* mutant carrying *2*μm-*SPT23* (63). This increase in Ty1 transcript is probably responsible for the increase in Ty1 transposition in these strains. Other factors, such as more-efficient utilization of Ty1 RNA by the excess amount of Spt23p, may also contribute to the increase in Ty1 transposition. However, *2*μm-*SPT23* expression did not suppress other defects conferred by *snf2*, such as the inability to catabolize raffinose, galactose, or sucrose (63).

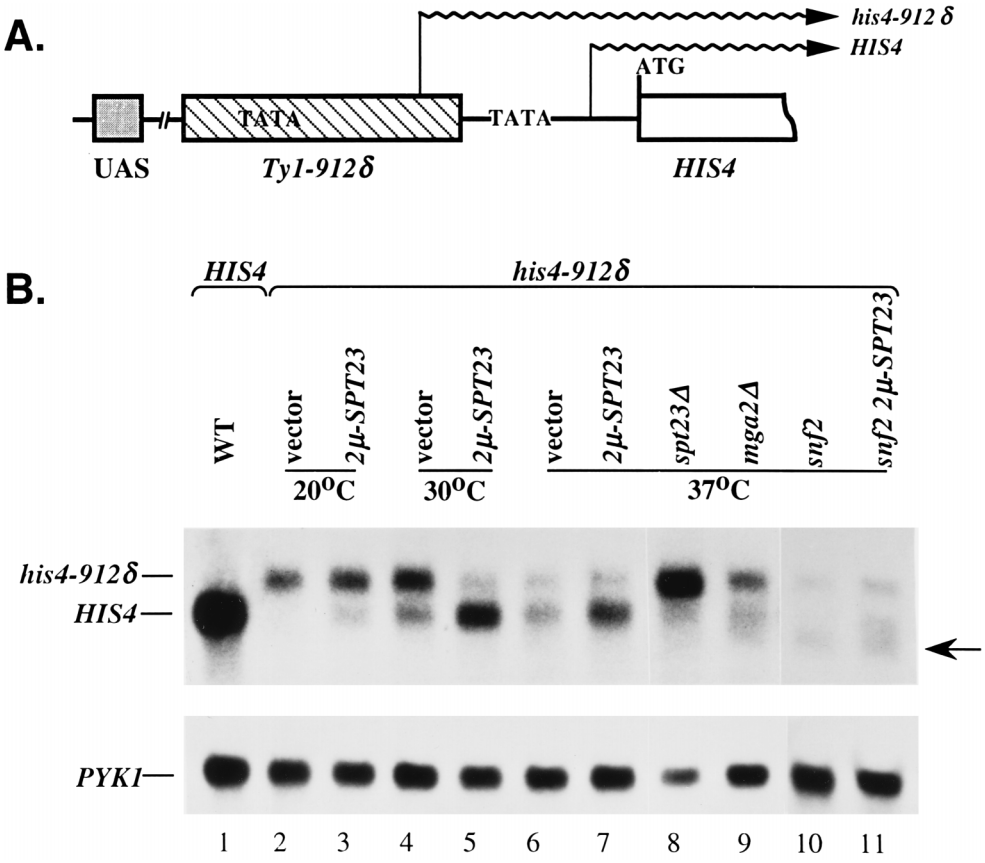

FIG. 4. 2μm-*SPT23* restores the wild-type *HIS4* transcript in cells containing *his4-912*δ. (A) Ty1 LTR (δ)-induced mutation *his4-912*δ and its transcription. The *HIS4* enhancer (UAS), ORF, and Ty1-912δ LTR (rectangles), TATA boxes and the translation start codon (ATG) for *HIS4*, and the initiation and direction of transcription (wavy lines) for the functional *HIS4* and nonfunctional *his4-912*δ transcripts are indicated. (B) Northern hybridization analysis of *his4-912*δ transcripts. Total RNA was prepared from the following strains and analyzed by Northern hybridization: S288C (wild type [WT]; lane 1), DG422 transformed with vector plasmid (lanes 2, 4, and 6), DG422 transformed with pBDG769 (lanes 3, 5, and 7), DG1587 (lane 8), DG1557 (lane 9), L592 transformed with vector (lane 10), and L592 transformed with pBDG769 (lane 11). The temperatures at which cells were grown are as indicated; S288C was grown at 30°C. The same filter was hybridized sequentially with ^{32}P-labeled riboprobes specific for transcripts from *HIS4* and *PYK1*. The *his4-912*δ and *HIS4* transcripts are indicated on the left. The aberrant *his4* transcript found in *snf2* mutants is also indicated (arrow).

Spt23p and Mga2p are transcriptional activators. It is not known whether Spt23p and Mga2p are classical transcriptional activators. Neither of the genes encoding these proteins contains any recognized DNA-binding motifs. To determine whether Spt23p and Mga2p can function as sequence-specific transcriptional activators, we fused the coding regions of both *SPT23* and *MGA2* to the DNA-binding domain coding region of *GAL4* and assayed the hybrid genes for transactivation activity in strain Y190 (26), which contains an integrated *GAL1-lacZ* reporter construct. To construct the hybrid genes, we fused codons 25 to 1082 of *SPT23* or codons 2 to 1113 of *MGA2* to the DNA-binding domain coding region of *GAL4*. The hybrid genes were under the control of the *ADH1* promoter. Both of the hybrids were functional, as revealed by their ability to rescue the lethality associated with an *spt23 mga2* double mutant. As shown in Table 4, both the DNA-bound *SPT23* and *MGA2* activated transcription of *GAL1-lacZ*, with *SPT23* being more active than *MGA2*. Interestingly, deletion of 173 amino acids from the C terminus of *MGA2*, which was found to suppress some Ty insertion mutations, increased its transactivation activity. Therefore, the net increase in *MGA2* activity as an activator in this assay may also be responsible for its suppression activity.

In addition to its ability to activate transcription of *GAL1-lacZ* and provide an essential function, Gal4-Spt23p suppressed the unconditional His$^-$ defect of *his4-912*δ conferred by *spt23*Δ. We hypothesized that both the essential function and the suppression activity of Gal4-Spt23p are dependent on its transactivation activity. To determine the relatedness of these three activities and to identify the sequence in Spt23p responsible for transactivation, we analyzed a series of deletion derivatives of the Gal4-Spt23p hybrid. Coding sequences from either the N terminus, the C terminus, or the internal region of *SPT23* were deleted. All the N-terminal deletions remained fused in frame to the *GAL4* DNA-binding domain coding sequence. The transactivation activity, suppression activity, and essential function were then determined (Fig. 6). Unexpectedly, the conserved C terminus of *SPT23* had only a slight effect on all three activities. In contrast, the N terminus of *SPT23* was necessary for the three activities. Deletion of only 50 codons from the N terminus completely eliminated all three activities (pSRZ98). The transcriptional activation of *GAL-lacZ* remained when these 50 codons alone were fused to the *GAL4* DNA-binding domain coding sequence (pSRZ116), but this small region could not provide the essential function of *SPT23* or suppress *his4-912*δ. These results demonstrate that codons 25 to 75 of *SPT23* contain a transcriptional activation domain (Fig. 6, I), which is both necessary and sufficient for

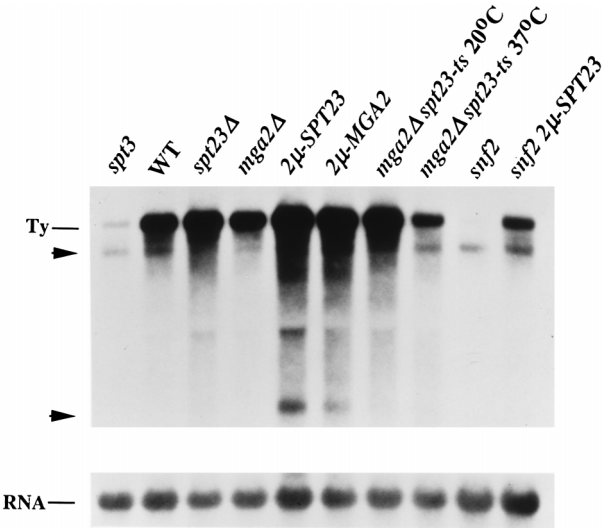

FIG. 5. *SPT23* and *MGA2* alter Ty1 transcription. Total RNA was prepared from the following strains and analyzed by Northern hybridization: DG1251 (*spt3*), DG422 (wild type [WT]), DG1587 (*spt23*Δ), DG1557 (*mga2*Δ), DG422 transformed with either pBDG769 (2μm-*SPT23*) or pSRZ65 (2μm-*MGA2*), DG1667 (*mga2*Δ *spt23-ts*) grown at 20°C or shifted to 37°C for 6 h, L592 (*snf2*), and L592 transformed with pBDG769 (*snf2* 2μm-*SPT23*). The same filter was hybridized sequentially with ^{32}P-labeled riboprobes specific for transcripts from Ty1 and 18S rRNA. Two internally initiated transcripts (arrowheads) and the full-length Ty1 transcript (Ty) are indicated.

transactivation and necessary for the essential function of Spt23p and suppression activity of Ty1 insertion mutations. Closer examination of this sequence shows that it is enriched in negatively charged amino acids (9 net negatively charged amino acids of 50 with a pI of 3.55, as predicted from the amino acid sequence) and leucine rich (11 of 50 amino acids). Hydrophobic residues in the transactivation domain of Gcn4p have been reported to play an important role in transcriptional activation (18). To determine the relative strength of the Spt23p activation domain, we determined the level of β-galactosidase activity in the cells containing a plasmid (pCL1; Clontech) with the complete *GAL4* gene expressed from the same promoter as *GAL4-SPT23* (Table 4). *GAL4* activated transcription about 13-fold more than *GAL4-SPT23*. Taken together, the results showed that Spt23p could possibly exert its transcriptional activation through these hydrophobic residues (leucines) and the negative charges within codons 26 to 75, similar to the transactivation domain of Gcn4p (18, 31, 32).

We performed further deletion analyses on codons 75 to 816 of the *GAL4-SPT23* hybrid gene to locate other domains necessary for the essential function and suppression activity of Spt23p. Deletion of the C terminus of *SPT23* to the *Cla*I site

TABLE 3. Frequency of Ty1-270*his3-AI* transposition[a]

Strain	Plasmid	His$^+$ frequency	Relative efficiency
JC364 (*SNF2*)	2μm vector	3.47×10^{-7}	1
	2μm-*SPT23*	3.75×10^{-6}	10
SZY41 (*snf2*)	2μm vector	1.4×10^{-8}	0.04
	2μm-*SPT23*	7.5×10^{-7}	2.2

[a] Transformants were grown to saturation in SC-Ura liquid medium at 20°C and plated on SC-Ura plates to determine the titer and on SC-Ura-His plates to determine the number of His$^+$ prototrophs. The Ty1 transposition frequency is the number of Ura$^+$ His$^+$ prototrophs divided by the total number of Ura$^+$ colonies.

TABLE 4. Transcriptional activation by Spt23p and Mga2p

Gene(s)	β-Galactosidase activity[a]	Essential function[b]
$GAL4_{BD}$	1.17	−
GAL4[c]	582	−
$GAL4_{BD}$-*SPT23*	44.1	+
$GAL4_{BD}$-*MGA2*	3.7	+
$GAL4_{BD}$-*mga2-Δ173*	14.9	+

[a] The units of β-galactosidase activity (nanomoles per minute per microgram) were determined in crude cell extracts and normalized to the amount of total cellular protein assayed (46). Values are the averages of assays of four individual transformants. Standard errors were <20%.

[b] The essential function was determined by transforming each hybrid plasmid into strain DG1613. The transformants were replica plated to 5-FOA medium. + and −, positive and negative growth, respectively.

[c] The entire coding region of *GAL4* is carried on plasmid pCL1 (Clontech).

abolished its suppression activity but retained its essential function, implying that a component of the suppression domain (III, Fig. 6) resides within codons 488 to 576 (compare pSRZ97 with pSRZ111 [Fig. 6]). Deletion of a segment from the *Cla*I site to the *Sac*I site abolished the essential function of Spt23p. Since the sequence from the *Cla*I site to the *Eco*RI site is not needed for any Spt23p functions (pSRZ103), these results suggest that the essential function domain (Fig. 6, II) of Spt23p resides within codons 76 to 353. These deletion analyses suggest that Spt23p can be divided into three domains responsible for transcriptional activation, essential function, and suppression activity. Furthermore, both the essential function of *SPT23* and suppression of Ty1 insertion mutations require its transcriptional activation domain.

Spt23p is associated with the nucleus. Studies aimed at determining the subcellular localization of Spt23p are complicated by several factors. First, *SPT23* is expressed at a very low level, as demonstrated by the fact that *SPT23::lacZ* fusions produce β-galactosidase activity at a level only threefold above background. Second, several antisera raised against Spt23p fusion proteins, peptides, or epitope-tagged derivatives barely detect immunoblotted Spt23p from vegetative cell extracts and do not detect Spt23p by indirect immunofluorescence (63). Third, when *SPT23* is fused to the green fluorescent protein gene *GFP* (9a) on a multicopy plasmid, very little autofluorescence is observed. Fourth, *GAL1*-promoted expression of *SPT23* is toxic to cells.

To determine the cellular localization of Spt23p, we fused six repeats of HA epitope tag to the C terminus of *SPT23* and constructed a *GAL1*-promoted *lacZ-GFP-SPT23* fusion. The *SPT23-HA* fusion was used in cellular fractionation experiments along with marker proteins for the nucleus (Snf2p) and cytoplasm (Hts1p). The *SPT23-HA* fusion was functional, as revealed by its ability to rescue the synthetic lethality of the *spt23 mga2* double mutant (63). To obtain stable expression, *SPT23-HA* was integrated into the chromosome. A monoclonal antibody against HA, 12CA5, recognized the Spt23-HAp fusion protein in Western analysis; extracts from cells lacking the HA tag did not cross-react with any proteins in the same size range as Spt23p (63). Extracts from yeast cells containing Spt23-HAp were fractionated into crude nuclear and cytoplasmic fractions. Western analysis of these fractions showed that Spt23-HAp was predominantly in the nuclear fraction (Fig. 7), suggesting that Spt23p is a nuclear protein. The same fractionation pattern was observed for the nuclear protein Snf2p with an antibody specific for Snf2p, as was previously reported by indirect immunofluorescent microscopy (31). In contrast, the heat shock protein Hts1p was localized to the cytoplasmic fraction, as previously reported by Chiu et al. (10).

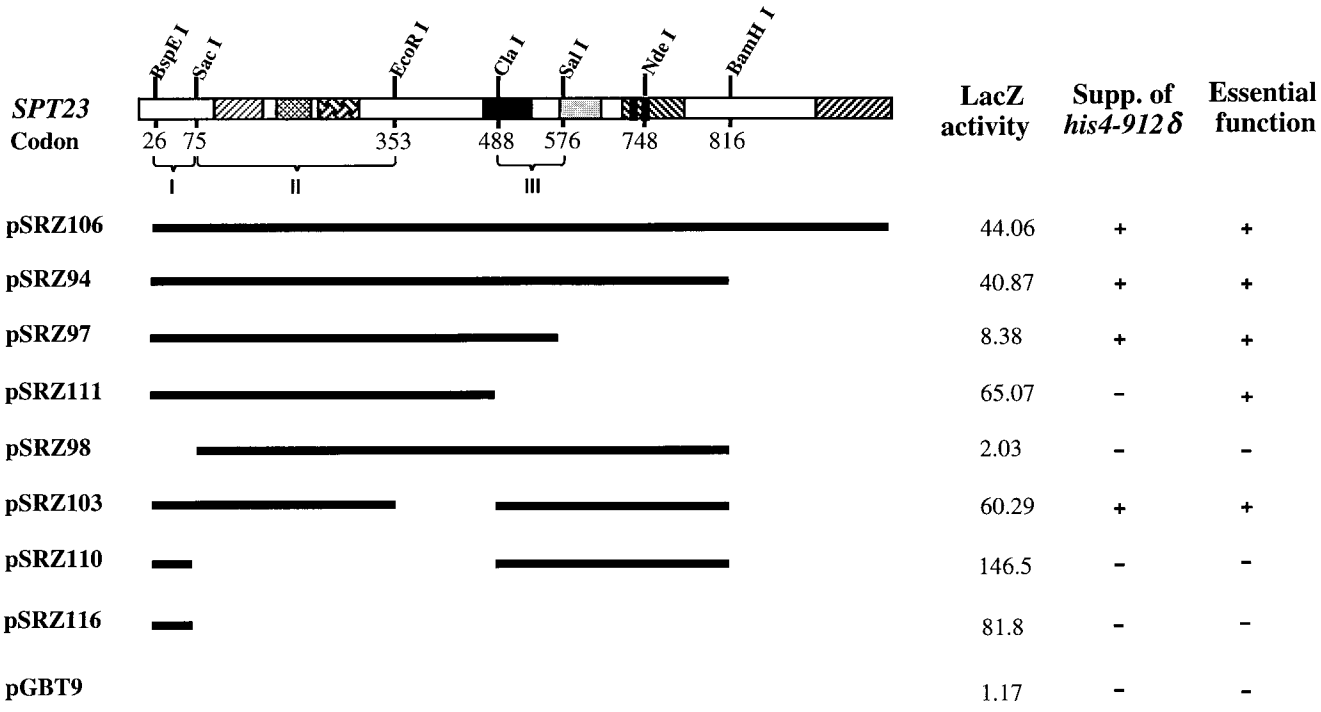

FIG. 6. Mutational analysis of the $GAL4_{BD}$-*SPT23* hybrid. The coding region of *SPT23* is shown as a rectangle (refer to Fig. 1) at the top, and the deduced restriction map is indicated below. The part of the coding region of *SPT23* that was fused to the Gal4p DNA-binding domain carried on vector pGBT9 (solid bars) is indicated. Deletions are shown as gaps, and end points are aligned with the *SPT23* restriction map. The resulting plasmids were transformed into a yeast strain that contains a *GAL1-lacZ* reporter construct and were assayed for β-galactosidase activity (46). Values are the averages of assays of four individual transformants. Standard errors were <20%. Suppression of *his4-912*δ was assayed in strain DG1587 (*spt23*Δ). The essential function of the hybrids indicates their ability to rescue the lethality of *spt23*Δ *mga2*Δ double deletions in strain DG1613 grown on 5-FOA plates. +, positive result; −, negative result; I to III, transactivation domain, the essential domain, and the suppression domain of Spt23p, respectively.

To confirm and extend the results from cell fractionation experiments, we performed cytological analyses of cells expressing *GFP-lacZ-SPT23* from the *GAL1* promoter (Fig. 8). We attempted to minimize the toxic effects associated with high levels of Spt23p by inducing the cells with galactose for 2 h. The cells were washed, resuspended in SC-Ura glucose medium, and then incubated for up to 3 h before microscopic examination. At the end of the galactose induction, autofluorescence was present surrounding the nucleus and in cytoplasmic dots that did not strongly colocalize with DAPI-staining material. Cells were examined every hour after glucose addition for up to 3 h. During the glucose chase, GFP staining around the nuclear periphery was maintained and fewer cytoplasmic dots were visible. After 3 h, GFP staining was present surrounding most of the nucleus; lower levels of fluorescence were also present in the nucleus (Fig. 8). Cells producing GFP-LacZ showed uniform autofluorescence (63). Taken to-

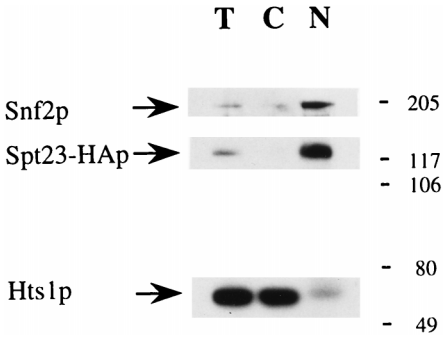

FIG. 7. Spt23p is associated with the nucleus as determined by cell fractionation. Strain SZ42 (*SPT23-HA*) was grown in YEPD to log phase. Cytoplasmic and nuclear fractions were isolated as described in Materials and Methods. Total cell extracts (T), cytoplasmic fraction (C), and nuclear fraction (N) were separated on a sodium dodecyl sulfate–8% polyacrylamide gel and blotted onto a polyvinylidene difluoride membrane. For Western immunoblotting analysis, three duplicate filters were probed with antibodies specific to Snf2p, Spt23-HAp, or Hts1p. Positive reactivity was visualized by using enhanced chemiluminescence. Identity of each band is indicated on the left. Positions of molecular mass standards (in kilodaltons) are indicated on the right.

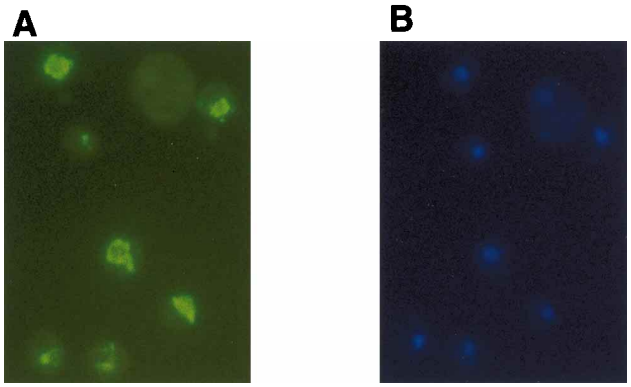

FIG. 8. Nuclear localization of *GFP-lacZ-SPT23* fusion protein. Cells containing *GFP-lacZ-SPT23* were fixed with formaldehyde and stained with DAPI. (A) Autofluorescence; (B) DAPI staining.

gether, our results suggest that Spt23p is associated with the nucleus and may interact with the nuclear membrane.

DISCUSSION

Here, we have presented the results of our studies of two functionally and genetically related genes, *SPT23* and *MGA2*. Because of their sequence similarity and functional redundancy, *SPT23* (chromosome XI) and *MGA2* (chromosome IX) might have arisen evolutionarily from a gene duplication, even though they are not in the currently recognized genome duplication region between chromosomes XI and IX (60). During evolution, they probably have diverged in some of their functions but kept their essential functions. Their essential functions are possibly involved in regulating the transcription of certain genes that are essential for cell viability. Because the functions of Spt23p and Mga2p are redundant, the genes under their regulation are still expressed when either of them is mutated. When neither is functional, however, the target genes under their regulation cannot be efficiently transcribed, resulting in cell death. Another interesting feature is that both *SPT23* and *MGA2* are functionally related to *SNF2*, a key gene of the *SNF*/*SWI* complex (7). Many studies, both in vivo and in vitro, demonstrate that the *SNF*/*SWI* complex can activate transcription by relieving chromatin-mediated repression (15, 27). Since both Spt23p and Mga2p can compensate for the loss of function of Snf2p in certain genetic contexts, Spt23p, Mga2p, and Snf2p might function in similar fashions.

Besides their overlapping functions, *SPT23* and *MGA2* show significant differences, especially in their suppression of Ty insertion mutations. Most of the known *SPT* genes can be placed into either the TFIID or the histone group. Mutations in the TFIID group strongly suppress both Ty- and δ-induced promoter mutations, affect Ty1 transcription, and cause mating and sporulation defects. Mutations, and sometimes multicopy expression, of the histone group strongly suppress only δ-mediated promoter mutations, as well as *snf2*, *snf5*, and *snf6* mutations (25, 27, 39, 56). Classification of *SPT23* is not straightforward. On the basis of the results showing that *2*μm-*SPT23* suppresses both Ty and δ insertion mutations and that the *spt23 mga2* double mutation affects Ty1 transcription, *SPT23* should belong to the TFIID group. The *his4-912*δ transcription profile with *2*μm-*SPT23*, however, does not support this conclusion. Cells with mutations in the TFIID-related *SPT* genes lack the long *his4-912*δ transcript, but cells containing *2*μm-*SPT23* retain the long *his4-912*δ transcript. In addition, cells with *spt23*Δ or *2*μm-*SPT23* lack the mating and sporulation defects associated with mutations in the TFIID group, suggesting that Spt23p functions by a different mechanism. The presence of both the long *his4-912*δ transcript and the short wild-type *HIS4* transcript in *his4-912*δ cells with *2*μm-*SPT23* implies that *SPT23* belongs to the histone group. Stoichiometry also seems to be crucial for the histone group of *SPT* genes. When too much or too little of these proteins is present, Ty insertion mutations are suppressed. Conversely, Spt23p is apparently a rate-limiting component required for functional *his4-912*δ expression: cells become His^+ when Spt23p is present at higher levels, cells are conditionally His^+ when Spt23p is present at normal levels, and cells are unconditionally His^- when Spt23p is absent. In summary, suppression of Ty insertion mutations by *2*μm-*SPT23* is apparently a gain-of-function phenotype. Without Spt23p activity, the phenotype becomes more severe.

Although *SPT23* does not strictly belong to the histone group, on the basis of our genetic analyses, we still favor the hypothesis that *2*μm-*SPT23* suppresses Ty1 and Ty2 insertion mutations by altering chromatin structure. First, *2*μm-*SPT23* suppresses a broad spectrum of Ty and δ insertion mutations. Such a broad effect could be caused by a general activation mechanism, such as remodeling of chromatin. When inserted in the promoter region of a gene, the whole Ty element or its LTR introduces a transcriptional signal (TATA) from the LTR that competes with the gene's own promoter (28). Simultaneously, a Ty or δ insertion might also disturb the local chromatin structure to repress gene expression. Identification of *SPT11*/H2A and *SPT12*/H2B as mutant suppressors of Ty and δ insertion mutations supports this hypothesis (12).

Second, *2*μm-*SPT23* suppresses the unconditional His^- phenotype of *his4-912*δ in the *snf2* or *snf5* background. In addition, *2*μm-*SPT23* restores Ty1 transcription and transposition in a *snf2* mutant. In both cases, overexpression of *SPT23* compensates for the loss of function of Snf2p, a key component in the *SNF*/*SWI* complex. A growing body of evidence suggests that the *SNF*/*SWI* complex activates transcription by disrupting nucleosomes to facilitate binding of transcriptional activator to chromosomal DNA (15, 42). Moreover, Spt23p, Snf2p, and Snf5p can activate transcription when allowed to bind DNA. All these similarities suggest that Spt23p and *SNF*/*SWI* activate transcription in similar fashions and that Spt23p is a member of an activation complex that can partially substitute for the *SNF*/*SWI* complex at particular promoters when *SPT23* is overexpressed. Spt23p is not likely to be a component of the *SNF*/*SWI* complex, however, because much narrower defects have been observed in *spt23* mutants than in relevant *snf* mutants. Certainly, *2*μm-*SPT23* could exist in the *SNF*/*SWI* complex and replace some roles of Snf2p and Snf5p by directing the complex to a specific target(s). We show that *2*μm-*SPT23* has different effects on *snf2* and *snf6* mutations in the context of *his4-912*δ. Our data suggest that Spt23p functions more like Snf2p and Snf5p than like Snf6p. Therefore, Snf2p and Snf5p may regulate Ty-induced promoter mutations by a mechanism different from that of Snf6p. Consistent with our results, other studies have demonstrated that transcriptional activation by Snf6p is fundamentally different from that by Snf2p and Snf5p (25, 35, 37). Transcriptional activation by Snf6p has been suggested to be due to a direct interaction with the basal transcription complex (35), whereas Snf2p and Snf5p interact directly with chromatin. The differential effects of *2*μm-*SPT23* on various *SNF* genes provide further evidence supporting the hypothesis that Spt23p functions by remodeling chromatin structure but not through a direct interaction with the basal transcription apparatus. The overlapping functions between Spt23p and the *SNF*/*SWI* complex are also limited to certain genetic contexts, because other defects caused by *snf* mutations, such as the Suc^- phenotype, are not suppressed by multicopy expression of *SPT23*. Therefore, more biochemical and genetic studies are needed to elucidate whether *SPT23*/*MGA2* is involved in chromatin accessibility or basal transcription.

Deletion analyses of the Gal4-Spt23p hybrid protein suggest that the first 816 residues of Spt23p have three distinct domains for transcriptional activation, essential function, and suppression activity (Fig. 6). Both the essential function and the suppression activity of Spt23p are involved with transcriptional activation. The transactivation domain of Spt23p has been defined as a 50-amino-acid sequence in its N terminus (codons 26 to 75). This region is acidic (pI = 3.55). Deletion of this acidic sequence eliminates all activities of Spt23p, including transactivation, essential function, and suppression activity. Acidic regions of proteins have been shown to activate transcription in several well-studied transcriptional activators (31, 32, 34). The transactivation domain of Spt23p does not lie

within any of the homologous regions between Spt23p and Mga2p, suggesting that Spt23p and Mga2p activate transcription by different mechanisms. In addition, the transactivation domain of Spt23p is leucine rich (11 of 50 amino acids), which may also contribute to the activity of this region (18). Although DNA-bound Gal4-Spt23p can activate transcription, this result does not necessarily contradict the idea that Spt23p functions through interaction with the chromatin structure. The DNA-bound Gal4-Spt23p may activate transcription by changing the chromatin structure to an active configuration in a manner similar to that of Snf2p and Snf5p. Both DNA-bound Snf2p and Snf5p can activate transcription (37), and it is known that they function by modulating chromatin.

Although we have evidence suggesting that residues 25 to 816 of Spt23p contain three functional domains, the C-terminal region of Spt23p has not been analyzed extensively and therefore may interact with the domains of Spt23p identified in this study or carry out additional functions. It is also formally possible that protein stability of the various fusion proteins may be influencing the results, because we have not reproducibly detected any of the Gal4-Spt23p fusion proteins using antisera against the Gal4p DNA-binding domain or Spt23p (63). However, all the *GAL4-SPT23* fusion genes with the exception of the N-terminal deletion on plasmid pSRZ98 have biological function in vivo, indicating that the fusion protein is being made (Fig. 6). Preliminary results from two-hybrid interaction screens also indicate that Gal4-Spt23p is produced from plasmid pSRZ98 (63).

Our studies of Ty1 transposition in the presence or absence of *2*μm-*SPT23* clearly show that excess Spt23p stimulates Ty1 transposition. The stimulation in Ty1 transposition in a *snf2* mutant containing *2*μm-*SPT23* reflects the increase in the level of Ty1-270*his3-AI* and total Ty1 transcripts (Fig. 5) (63). Although the increase in Ty1 RNA levels probably accounts for the increase in transposition, other factors may also contribute to this process. For example, *2*μm-*SPT23* expression may create better targets for Ty1 transposition via chromatin remodeling, as has been suggested from analyses of *rad6* and *hta1-htb1* mutants (38, 43).

Although we favor a role for *SPT23*/*MGA2* in chromatin accessibility, we do not know how *SPT23*/*MGA2* activates transcription. Our results suggest several possibilities: (i) Spt23p may modulate chromatin structure directly, such as by removing nucleosomes; (ii) Spt23p may negatively regulate the expression or activity of other proteins whose functions are to keep chromatin in a repressive state; and (iii) Spt23p may bind chromosomal DNA and recruit other proteins that can remodel chromatin structure. However, the primary sequence of Spt23p also does not suggest that it is a DNA-binding protein. Therefore, our results do not strongly support the idea that Spt23p binds DNA directly. Spt23p probably works in a complex with another protein(s) that can bind DNA. Taken together, a plausible model for Spt23p transcriptional activation is that Spt23p binds to chromosomal DNA through other proteins and changes the chromatin structure around the bound region to an active configuration for transcription. Identification of other proteins that interact with Spt23p or Mga2p may elucidate how these related proteins activate transcription.

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