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Identification and Analysis of a Functional Human Homolog of the *SPT4* Gene of *Saccharomyces cerevisiae*

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Spt4p is a nonhistone protein of *Saccharomyces cerevisiae* that is believed to be required for normal chromatin structure and transcription. In this work we describe the isolation and analysis of a human gene, *SUPT4H*, that encodes a predicted protein 42% identical to Spt4p. When expressed in *S. cerevisiae*, *SUPT4H* complemented all *spt4* mutant phenotypes. In human cells *SUPT4H* encodes a nuclear protein that is expressed in all tissues tested. In addition, hybridization analyses suggest that an *SUPT4H*-related gene is also present in mice. *SUPT4H* was localized to human chromosome 17 by PCR analysis of a human-rodent somatic cell hybrid panel. Thus, like other proteins that are components of or control the structure of chromatin, Spt4p appears to be conserved from *S. cerevisiae* to mammals.

The importance of chromatin structure in the control of transcription has been demonstrated by a combination of genetic and biochemical studies (for a review, see reference 37). In particular, these studies show that nucleosomes can function to repress transcription. However, the mechanisms by which this control is exerted, in terms of establishing, maintaining, and overcoming the repression of transcription by nucleosomes, are not clear. Some clues have come from genetic studies with the yeast *Saccharomyces cerevisiae*. These studies have provided strong candidates for proteins that might regulate the assembly, organization, modification, and disassembly of chromatin structure (52). For example, substantial evidence suggests that the Snf/Swi complex of *S. cerevisiae* serves to overcome nucleosomal repression of transcription by altering nucleosomal structure, thus aiding the binding of transcription factors and the activation of transcription (38).

Another set of *S. cerevisiae* genes, *SPT4*, *SPT5*, and *SPT6*, are candidates to encode functions that help nucleosomes to repress transcription (46, 52). These three genes are members of a large set of genes originally identified as mutations that suppress the transcriptional defects caused by insertions of the retrotransposon Ty or of its long terminal repeat, δ, upstream of the *HIS4* or *LYS2* gene (Spt$^-$ phenotype) (50). On the basis of shared mutant phenotypes, *SPT4*, *SPT5*, and *SPT6* were grouped within a set of genes believed to control transcription by modulating chromatin structure (46, 50). This group also includes *HTA1-HTB1*, one of the two gene pairs encoding histones H2A and H2B (20). Mutations in *spt4*, *spt5*, and *spt6* as well as a deletion of this histone gene pair, (*hta1-htb1*)Δ, cause an Spt$^-$ phenotype (13, 26, 44, 45). In addition, mutations in this group of genes suppress *snf*/*swi* mutations (21, 33, 46). Suppression of *snf*/*swi* mutations by (*hta1-htb1*)Δ occurs at the level of chromatin structure, presumably by reducing the number of nucleosomes via a reduction in the levels of histones H2A and H2B (21). By analogy, *spt4*, *spt5*, and *spt6* mutations may also cause a reduced number of functional nucleosomes.

Although *spt4*, *spt5*, and *spt6* mutants share many phenotypes with (*hta1-htb1*)Δ mutants, the products of these *SPT* genes clearly serve a role distinct from that of histones. Spt4p, Spt5p, and Spt6p have no sequence similarity to histones (26, 44, 45, 48), and they cannot functionally substitute for histones. In addition, they do not appear to regulate histone mRNA or protein levels (51). Furthermore, both biochemical and genetic evidence suggests that Spt4p, Spt5p, and Spt6p function in a complex: mutations in these three genes display synthetic lethality and unlinked noncomplementation, and Spt5p and Spt6p coimmunoprecipitate (46). Thus, Spt4p, Spt5p, and Spt6p are likely to function in a complex, perhaps to modify histones, assemble nucleosomes, or modulate the interactions of nucleosomes with either DNA or other proteins.

A consistent theme in biology is that regulatory processes are highly conserved among eukaryotes. This is true of the general transcription factors and appears to be true of chromatin factors as well. Histones are among the most conserved of all proteins (48), and *SNF*/*SWI* genes have been found in humans (23, for a review, see reference 11), mice (11), and *Drosophila melanogaster* (11, 14). In addition, a Snf/Swi function has been identified in humans (22, 25). Finally, emb-5, a *Caenorhadbitis elegans* protein, is 21% identical to Spt6p, although its function is currently unknown (35). Thus, it is likely that Spt4p, Spt5p, and Spt6p functions exist in other eukaryotes.

The identification of Spt4p, Spt5p, and Spt6p homologs would allow important additional approaches to the study of these functions. First, although *S. cerevisiae* is an ideal organism for genetic studies, its small size makes cytological studies difficult. Therefore, cytological studies of Spt4p, Spt5p, and Spt6p in larger eukaryotes could be useful for determining the time and place of action for these proteins. Second, a number of human genes have been shown to complement mutations in their yeast sequence homologs (1, 24, 30, 31, 54). Thus, for possible Spt4p, Spt5p, and Spt6p homologs, it may be possible to use the methods of yeast genetics to identify mutations that are likely to cause interesting phenotypes when the gene is reintroduced into human cells. Finally, the ability to study these Spt functions from either *S. cerevisiae* or other eukaryotes by using biochemically well-characterized eukaryotic transcription factors should provide additional insights into these functions.

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TABLE 1. Sequences of oligonucleotides used in this study

Oligonucleotide	Sequence
OGH1	5′-CGGAATTCCTGCGGCATCTGCGGGCC-3′
OGH2	5′-CCGCTCGAGTTAGTCTTTAATAGCTGTGTC-3′
OGH3	5′-CGGAATTCATGTCTAGTGAAAGAGCC-3′
OGH4	5′-CCGCTCGAGTTACTCAACTTGACTGCC-3′
OGH24	5′-CAGCAAACAGGCCCGCAGATG-3′
OGH32	5′-CTTTTCTCTTTACTGTCCCACAG-3′
ORM148	5′-GATCTGAATTCGGGGCCGCCGCTGGCGTAGTCGGGCACGTCGTAGGGGTACATGGTA-3′
ORM149	5′-GATCTACCATGTACCCCTACGACGTGCCCGACTACGCCAGCGGCGGCCCCGAATTCA-3′
OPH146	5′-TCACGCCGCTCGAGCTAGGTCTTTATAGCTGTGTC-3′
OPH147	5′-TCACGACGCGTCGACCATGTCTAGTGAAAGAGCC-3′
OPH156	5′-TCAGGACGCGTCGACCCTGCGGCATCTGCGGGCC-3′
OPH183	5′-TGTACTGTGTAATAATTACACCTGGCCACATTCAGTTTGCTGTGCGGTATTTCACACCG-3′
OPH184	5′-AGCAAAAACAAAAGTGCAGAACGTTAAATAAATGAAAGCAAGATTGTACTGAGAGTGCAC-3′

In this work, we describe the identification and analysis of a functionally conserved human homolog of *SPT4*. The human gene (*SUPT4H*), when expressed in *S. cerevisiae*, is able to complement multiple phenotypes caused by a deletion of *SPT4*. In addition, we show that this *SPT4* homolog encodes a nuclear protein that is widely expressed in human tissues. These properties are consistent with the transcriptional role proposed for the yeast Spt4p protein.

MATERIALS AND METHODS

Oligonucleotides. The sequences of all oligonucleotides used in this work are given in Table 1.

Yeast strains and methods. The four strains used in this study are FY119 (*MAT*α *his4-912*δ *lys2-128*δ *leu2Δ1 ura3-52 trp1Δ63*), FY1114 (*MAT*α *his4-912*δ *lys2-128*δ *leu2Δ1 ura3-52 trp1Δ63 spt4Δ2::HIS3*), s138 [*MAT***a** *trp1Δ1 ura3-52 leu2Δ1 ade2-101 spt4-138 lys2-801* CFVII (*RAD2d.YPH277*) *URA3 SUP11*] (5), and YMB120 [*MAT***a** *trp1Δ1 ura3-52 his3Δ200 leu2Δ1 ade2-101 spt4Δ2::HIS3 lys2-801* CFVII (*RAD2d.YPH277*) *URA3 SUP11*] (5). These strains are isogenic to S288C, and FY119 and FY1114 are also *GAL2*$^+$ (53). FY1114 was constructed for this study by standard yeast methods (39). Media were made as described previously (39). Where indicated, 2% galactose–1% raffinose was used as the carbon source to induce the *GAL1* promoter. For experiments monitoring the loss of the chromosome fragment, medium was supplemented with 6 μg of adenine per ml to enhance the color of the red sectors of the *ade2-101* strains (5). Yeast strains were transformed as described previously (16).

The *spt4Δ2::HIS3* allele, which is a deletion of the sequences between 29 nucleotides upstream of the *SPT4* ATG and 92 nucleotides downstream of the *SPT4* stop codon, was constructed by using a PCR-based method described previously (7). Oligonucleotides OPH183 and OPH184, which are homologous to regions 5′ and 3′ of *SPT4*, respectively, were used to amplify the *HIS3* gene from plasmid pRS303 (42). PCR conditions were as follows: 1 cycle of 94°C for 3 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and a final cycle of 72°C for 10 min. The unpurified PCR product was used to transform strains YPH254 [*MAT***a** *trp1Δ1 ura3-52 his3Δ200 leu2Δ1 ade2-101 lys2-801* CFVII (*RAD2d.YPH277*) *URA3 SUP11*] and FY602 (*MAT***a** *his3Δ200 leu2Δ1 lys2-128*δ *ura3-52 trp1Δ63*) to His$^+$. Correct integration at and deletion of the *SPT4* gene were confirmed by Southern blot analysis. All phenotypes caused by *spt4Δ2::HIS3* are complemented by *SPT4*$^+$.

Spt phenotypes were assayed by the ability of cells to grow in the absence of histidine or lysine. Cells transformed with pJG4-6, pGH78, or pGH127 were grown to saturation in synthetic complete medium (SC)-Trp. These cells were collected by centrifugation, washed twice with sterile water, and resuspended to a density of 10^8 cells per ml in sterile water. Serial fivefold dilutions of cells were transferred to either SC-Trp, SC-Trp-His, or SC-Trp-Lys plates that had 2% galactose and 1% raffinose as a carbon source to induce the *GAL1* promoter. At least two independent transformants were assayed for each strain scored.

Ctf phenotypes were scored by determining the frequency of artificial chromosome fragment loss as described previously (43). Approximately 200 cells were spread on SC-Leu plates with limiting adenine (5). The sectoring phenotype was scored after 5 or 6 days of growth at 25°C. At least two independent transformants were assayed for each strain scored.

Plasmids. The vectors used to express *SUPT4H* and *SPT4* in *S. cerevisiae*, pJG4-6 and pAD5, have been described previously (17, 34). The relevant portions of *SPT4* (codons 1 to 102), and *SUPT4H* (codons 10 to 117) were PCR amplified with primers that added restriction sites for cloning and allowed in-frame fusion to the HA1 epitope tag present in the 5′ end of each vector. PCR conditions were as follows: 1 cycle of 94°C for 5 min; 20 cycles of 94°C for 15 s, 50°C for 15 s, and 72°C for 40 s; and a final cycle of 72°C for 2 min. Plasmid pGH127 (*SUPT4H* in pJG4-6) was created by using oligonucleotides OGH1 and OPH146 to amplify *SUPT4H* from plasmid HIBBB90. Similarly, plasmid pGH78 (*SPT4* in pJG4-6) was created by using primers OGH3 and OGH4 to amplify *SPT4* from plasmid pBM25 (26). These PCR products, which add a 5′ *Eco*RI site and a 3′ *Xho*I site to *SUPT4H* and *SPT4*, were digested with *Eco*RI and *Xho*I, gel purified, and ligated into *Eco*RI-*Xho*I-digested pJG4-6.

Plasmid pMB299 (*SUPT4H* in pAD5) was created by using oligonucleotides OPH156 and OPH146 to amplify *SUPT4H* (codons 10 to 117) from plasmid HBC211. Similarly, plasmid pMB284 (*SPT4* in pAD5) was created by using primers OPH147 and OGH4 to amplify *SPT4* (codons 1 to 102) from plasmid pMB212 (5). The PCR conditions for these two vectors were as described above. These PCR products, which add a 5′ *Sal*I site and a 3′ *Xho*I site to *SUPT4H* and *SPT4*, were digested with *Sal*I and *Xho*I and ligated into *Sal*I-digested pAD5. The DNA sequences of the *SUPT4H* and *SPT4* inserts in these four expression vectors were confirmed by DNA sequence analysis (41) of both strands of the PCR-amplified gene with the Sequenase kit (U.S. Biochemicals) according to the manufacturer's instructions.

To express *SUPT4H* in HeLa cells, a derivative of the plasmid pCDM7 (3) containing the *Xenopus* globin 5′ untranslated region and encoding an N-terminal HA1 epitope tag was created. First, oligonucleotides ORM148 and ORM149 were annealed and ligated into *Bgl*II-digested pCDM6XL (28). This results in a vector with the *Xenopus* globin 5′ untranslated region followed by an initiator methionine codon, sequences encoding the HA1 epitope tag, and a 3′ *Eco*RI site. The *Hin*dIII-*Bam*HI fragment of this vector was isolated and ligated into *Hin*dIII-*Bam*HI-digested pCDM7 to create the plasmid pTAG. To create pGH130, the *Eco*RI-*Xho*I fragment of pGH127 was isolated and ligated into *Eco*RI-*Xho*I-digested pTAG. Like pGH127 and pMB299, pGH130 encodes a protein containing an N-terminal HA1 epitope tag followed by codons 10 to 117 of *SUPT4H*.

Plasmid pGH129, containing sequences of the *SUPT4H* cDNA 3′ to the open reading frame (ORF), was made by digesting pHIBBB90 with *Hin*dIII and *Xho*I, blunting with Klenow enzyme, and religating.

Cloning of *SUPT4H*. *SUPT4H* was initially identified by using the amino acid sequence of Spt4p to search a DNA database, the database of expressed sequence tags (dbEST) (9), translated in all six reading frames, with a Blast search (2). Subsequent *SUPT4H* clones were identified by Blast searches (2) either for nucleotide matches to the *SUPT4H* cDNA or for matches to the Supt4hp amino acid sequence following translation of nucleic acid sequences in dbEST in all six possible translational reading frames (i.e., BlastN and TBlastN searches) and by subscription to the XREF database service (described in reference 6). A total of 13 overlapping cDNAs present in various DNA databases were identified in these searches. The accession numbers of these cDNAs are available upon request.

An additional set of *SUPT4H* cDNAs were identified by screening a human muscle cDNA library (a gift from Lou Kunkel) with a probe to the *SUPT4H* ORF generated by PCR amplifying HBC211 with primers OGH1 and OGH2 in the presence of [α-^{32}P]dATP. PCR conditions were as follows: 1 cycle of 94°C for 5 min; 30 cycles of 94°C for 15 s, 57°C for 30 s, and 72°C for 30 s; and a final cycle of 72°C for 3 min. Positive lambda phages were purified, and their *Eco*RI inserts were subcloned into the *Eco*RI site of vector pSP72 for DNA sequence analysis.

Genomic clones of the *SUPT4H* gene were identified by screening a human genomic DNA library, HL1067j (Clontech), with the same probe used to screen the cDNA library. DNAs of positive clones from this lambda phage library were purified, cut with *Sac*I, and subcloned into pSP72. Positive subclones were identified by colony hybridization (10) with the same probe as described above. Sequence analysis of one of these genomic subclones of *SUPT4H* indicates that it contains at least one intron beginning after nucleotide 136 of the *SUPT4H* cDNA.

Sequence analysis of *SUPT4H*. Because only short stretches of sequence data representing a single sequencing gel run are supplied for each clone in dbEST,

we obtained two *SUPT4H* cDNAs which, on the basis of their homology with *SPT4* and their alignment with other *SUPT4H* clones in the database, appeared to encode the full-length or nearly full-length Supt4hp protein: HIBBB90 (accession number T08289) from the American Type Culture Collection and HBC211 (accession number T10957) from Graeme Bell. DNA sequence and restriction endonuclease analyses of these clones showed that they represent full-length or nearly full-length cDNAs of the longer, 1.7-kb *SUPT4H* transcript. The 1.5-kb *SUPT4H* cDNA was sequenced (41) on both strands at least once with the Sequenase kit (U.S. Biochemicals) according to the manufacturer's directions.

Northern (RNA) analysis. The human tissue RNA blot (see Fig. 5A) (human multiple tissue Northern blot [Clontech]), containing approximately 2 μg of poly(A)$^+$ RNA per lane, was a gift from Julie Gastier. RNAs from R772 cells, a human lymphoblastoid cell line, and mouse-derived NIH 3T3 cells were prepared by a standard method (4). The Northern blot in Fig. 5B was prepared as described previously (45). Ten micrograms of total RNA was loaded in each lane. Human brain RNA and mouse heart muscle RNA were gifts from Hart Lidov and Ian Krane, respectively. These blots were probed with the entire *Eco*RI-*Hin*dIII insert of HIBBB90 (see Fig. 5A), the *SUPT4H* ORF probe described above (5′ probe [see Fig. 5B]), and plasmid pGH129 (3′ probe [see Fig. 5B]) according to a published protocol (45). The HIBBB90 and 3′ *SUPT4H* probes were labeled with [α-^{32}P]dATP by random priming by using a kit from Boehringer Mannheim Biochemicals. The RNA molecular size markers used for Fig. 5B were purchased from Boehringer Mannheim Biochemicals.

Transfections and indirect immunofluorescence. HeLa cells were maintained in DME/FCS, which is Dulbecco's modified Eagle's medium (DME) (Media-Tech, Washington, D.C.) supplemented with 2 mM glutamine (JRH Biosciences), 10% fetal calf serum (FCS) (HyClone), and penicillin-streptomycin (JRH Biosciences), at 37°C in a 6% CO_2 atmosphere. HeLa cells were transfected by the $CaPO_4$ procedure (40). Briefly, 12-mm-diameter glass coverslips were placed in 22-mm-diameter wells of 12-well cell culture dishes, and approximately 25,000 cells were plated in 1 ml of DME/FCS. After 18 to 24 h of incubation, the medium was replaced with fresh DME/FCS. One to 2 h later, 100 μl of a $CaPO_4$ precipitate containing 8 μg of DNA, prepared as described previously (40), was added to each well, and chloroquine diphosphate (Sigma) was added to a final concentration of 100 μM. Cells were incubated with the $CaPO_4$ precipitate for 5 h at 37°C and washed three times with 1 ml of phosphate-buffered saline, and then fresh DME/FCS was added. The cells were processed for immunofluorescence 36 to 48 h after transfection as described previously (19). The primary antibody, a monoclonal anti-HA1 antibody (49), was a gift from Frank McKeon. The secondary antibody, CY3 (indocarbocyanine)-labeled goat anti-mouse antibody was from Jackson Immunoresearch Laboratories (West Grove, Pa.). DNA was stained with DAPI (4′,6-diamidino-2-phenylindole dihydrochloride). Transfected cells were visualized with an Axiophot fluorescence microscope with a 40× objective, numerical aperture (NA) 1.25, and photographed with Kodak TMAX-400 black-and-white film.

Mapping *SUPT4H* to a human chromosome. To determine the human chromosomal location of *SUPT4H*, PCR primers that amplify a 175-nucleotide sequence that overlaps the 5′ end of the *SUPT4H* ORF and an intron (see above) were picked. These primers, OGH1, and OGH32, were used to amplify 50-ng aliquots of genomic DNA from the human-rodent hybrid cell lines of National Institute of General Medical Sciences (NIGMS) mapping panel no. 2 (15). PCR conditions were as follows: 1 cycle of 94°C for 5 min; 35 cycles of 94°C for 15 s, 55°C for 30 s, and 72°C for 30 s; and a final cycle of 72°C for 3 min. The PCR products were resolved on a 3% agarose gel and stained with ethidium bromide. To verify that the 175-nucleotide amplification product was derived from the *SUPT4H* gene, this gel was blotted and probed with an oligonucleotide, OGH24, that lies between OGH1 and OGH32. This was carried out as described previously (4) and showed that only the 175-nucleotide amplification product was derived from *SUPT4H.*

Nucleotide sequence accession number. Our DNA sequence of *SUPT4H* has been deposited in GenBank under accession number U43923.

RESULTS

Identification of a human homolog of *SPT4.* A candidate for a human homolog of *S. cerevisiae SPT4* was identified by a computer database search (2). In this search, a cDNA sequence that is predicted to encode a protein quite similar to Spt4p was identified in the dbEST database (9). By searching for further protein and nucleotide matches to this homolog, a total of 13 overlapping cDNAs of apparently identical sequence were eventually identified (see Materials and Methods for details).

To characterize further the human cDNA, we determined its complete DNA sequence. The 1,499-nucleotide cDNA contains a complete ORF encoding a predicted 117-amino-acid protein (Fig. 1A), followed by a long 3′ noncoding region. The predicted 117-amino-acid human protein is 42% identical to the 102-amino-acid Spt4p protein (Fig. 1B). Although the first methionine is not preceded by an in-frame translation termination codon in the cDNA sequence, sequence analysis of a genomic clone of this region shows that an in-frame UAA codon would occur if the mRNA extends three codons 5′ of our cDNA sequence (data not shown). In addition, Northern hybridization analysis suggests that our cDNA is nearly full length (see below). Thus, the 117-amino-acid sequence shown in Fig. 1 most likely represents the complete human protein. Spt4p contains a single, N-terminal zinc finger motif (26) which appears to be essential for its function since mutations that change any of the cysteines in the motif cause a mutant phenotype (5, 26). Significantly, these cysteine codons are conserved in this human gene (Fig. 1B). Following the nomenclature guidelines for naming human genes (29), we have named the human gene *SUPT4H*. For consistency with *S. cerevisiae* nomenclature, the protein product of *SUPT4H* is designated Supt4hp here.

***SUPT4H* partially complements an *spt4* mutation.** To determine if *SUPT4H* is functionally conserved, we tested the ability of the human gene to complement *spt4* mutations in *S. cerevisiae*. In each test for complementation, we tested plasmids expressing either *SUPT4H*, *SPT4*, or no *SPT4* gene. In all cases, the control plasmid without an *SPT4* gene failed to complement the mutant phenotype. First, we tested for complementation of the Spt$^-$ phenotype, i.e., suppression of insertion mutations in the 5′ regions of the *HIS4* and *LYS2* genes. In an otherwise wild-type strain, the mutations *his4-912*δ and *lys2-128*δ cause His$^-$ and Lys$^-$ phenotypes, respectively (50). In *spt4* null mutants, the insertion mutation phenotypes are suppressed, resulting in a His$^+$ Lys$^+$ phenotype (50). To test if it could complement an *spt4* mutation, the portion of *SUPT4H* homologous to *SPT4*, encoding amino acids 10 to 117 of Supt4hp, was expressed in an *spt4* mutant under the control of the *GAL1* promoter. When this strain was grown on galactose medium to activate transcription from the *GAL1* promoter, *GAL1-SUPT4H* complemented the *spt4* mutation almost as well as the *GAL1-SPT4* control (Fig. 2). As expected, when assayed for their Spt phenotypes on medium containing glucose, which represses the *GAL1* promoter, neither plasmid complemented the *spt4* null mutation (data not shown). In conclusion, *SUPT4H* partially complements the Spt$^-$ phenotype of an *spt4* null mutant.

In addition to the Spt$^-$ phenotype, *SUPT4H* was tested for complementation of two other *spt4*-conferred phenotypes: defects in chromosome transmission fidelity (Ctf$^-$) and temperature sensitivity for growth (Ts$^-$) (5). The Ctf$^-$ phenotype was assayed by a colony color sectoring assay based on loss of a nonessential chromosome fragment containing *SUP11*, which suppresses *ade2-101* (43) (see Materials and Methods). Since phenotypically Ade2$^+$ cells are white and Ade2$^-$ cells are red, loss of the *SUP11*-bearing chromosome fragment can be monitored by the appearance of red sectors in the white colonies. Recently, an *spt4* mutant, the *spt4-138* mutant, was found to give rise to an increased frequency of red-sectored colonies (5). To test the ability of *SUPT4H* to complement this defect, *SUPT4H* and *SPT4* were expressed in *spt4-138* cells under conditions in which loss of the *SUP11*-carrying chromosome could be measured (see Materials and Methods). Because the sectoring assay is difficult to carry out on galactose media, *SUPT4H* and *SPT4* were expressed under the control of the strong constitutive *ADH1* promoter to allow the use of glucose medium. When transformed into *spt4-138* cells, *ADH1-SUPT4H* reduced the frequency of red sectors in these transformants by nearly the same degree as *ADH1-SPT4* (Fig. 3).

A

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1     TATTTACTTC CTGCGGGTGC ACAGGCTGTG GTCGTCTATC TCCCTGTTGT TCTTCCCATC GGCGAAGATG GCCCTGGAGA
                                                                   ↓           M   A  L  E  T
81    CGGTGCCGAA GGACCTGCGG CATCTGCGGG CCTGTTTGCT GTGTTCGCTG GTCAAGACTA TAGACCAGTT TGAATATGAT
        V  P  K   D  L  R   H  L  R  A   C  L  L   C  S  L   V  K  T  I   D  Q  F   E  Y  D
161   GGTTGTGACA ATTGTGATGC ATATCTACAA ATGAAGGGTA ACCGAGAGAT GGTATATGAC TGCACTAGCT CTTCCTTTGA
      G  C  D  N   C  D  A   Y  L  Q   M  K  G  N   R  E  M   V  Y  D   C  T  S  S   S  F  D
241   TGGAATCATT GCGATGATGA GTCCAGAGGA CAGCTGGGTC TCCAAGTGGC AGCGAGTCAG TAACTTTAAG CCAGGTGTAT
       G  I  I   A  M  M  S   P  E  D   S  W  V   S  K  W  Q   R  V  S   N  F  K   P  G  V  Y
321   ATGCGGTGTC AGTCACTGGT CGCCTGCCCC AAGGAATCGT GCGGGAGCTG AAAAGTCGAG GAGTGGCCTA CAAATCCAGA
        A  V  S   V  T  G   R  L  P  Q   G  I  V   R  E  L   K  S  R  G   V  A  Y   K  S  R
401   GACACAGCTA TAAAGACCTA GCAAGATGCA AGGCTGCCAG CATCTTTGCT CTCCACCTCC TGCCTCTGCT TATTTCTTGT
      D  T  A  I   K  T  STOP
481   TCTGGAACTA AATGAACAGA ACTTCAAATA CTTCCTACCC TCCAATTCAG ACTCAGCTGA CTGTTGAGAG AGCAGCACAT
561   CATTTTATCA TTTTATCTTC TTTGGACTAC AGGTGGGGTG GGAGGGATTT GGGTTGGTGG ATTAACAGAT GGAATTGAGG
641   AGAGAGTAGG ATGCTGATTT TCCTACCCGT GGCCCAGGTC TGTGCCTTCC CCATGCCAAG GACTCTAGGT CAAATGTCAA
721   TAAATATGAA CCTCGAGAAA GTTCTGAAGG CCATGACACC TGCCTTGCCT CCCTCTTCCA TTCTCTTAGG CACAGTAATA
801   GCTTATTTGC CCTATAAGAA CCTTCCCAGA GCAGCAGAGG CCCTTCTACT CCCTCTTGAC TGTCTCAGCC TCTGGGATTG
881   CAGCCTTTGT AGTGTGCTTC CTTGCTTCCT ATCAGAGGGT GCTGATCCAG AGGCTCAGTA ACCCCATCAA CTTGGTGGCC
961   CTGGTGTCTC ACACTTGTAT CCTTCTGCCC TCGAGACCTG GCACAGCAGT ATCCCTTGAA GAAATCCTGA GGCTTTGTAG
1041  AGTGCTCCTT GACCATGTTT AATAATTCTT CCCTCCCCTG CTTGTCTATT TTCTTCTCTT CACGGCTCTT CCTATACCTT
1121  AGGCCAGTCT CAAGCACTCA CTGGAGACCC TTGGGCCTTG GGCGACCATT GAGTCCTAGT CTCCCTTGTT TGTGCCCCTG
1201  TAGGAGGTAG GTCCTTTTCT CCTCCGGCCT AGTAGGGGAC CTTGGGTAAC ATCCCATTTT TCGGCCAAGG TGAGTTGTTT
1281  TAGGATAAAA AAATTTACCA CAAATTCTCA TTTAAATTTC CACAGAAATC CTGTTCGTAT CCCCATTTTG ATTTCCCTAA
1361  GTTCCTTGTT CTCCCTCTAA AAAGAGAATG ATTGCACCCT GCCTGTTTAC CTCAGGATTG TTGTGATTGT AGAAACGAAG
1441  CTATGTGAAA ATTATATAAG TATTATAAAG GTGAAATACT TTTGCTCTCA AAAAAAAAA
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B

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Supt4hp  1 MALETVPKDLRHLRACLLCSLVKTIDQFEYDGCDNCDAYLQMKGNREMVYDCTSSSFDG 59
                     ||| ||  | |   |  ||| ||       |   |   ||| || |
Spt4p    1          MSSERACMLCGIVQTTNEFNRDGCPNCQGIFEEAGVSTM..ECTSPSFEG 48

Supt4hp 60 IIAMMSPEDSWVSKWQRVSNFKPGVYAVSVTGRLPQGIVRELKSRGVAYKSRDTAIKT 117
              |  |  ||| ||  |     | ||  | ||||   |  |      || ||
Spt4p   49 LVGMCKPTKSWVAKWLSVDHSIAGMYAIKVDGRLPAEVVELL....PHYKPRDGSQVE 102
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FIG. 1. Nucleotide and amino acid sequences of Supt4hp. (A) Nucleotide sequence of *SUPT4H*. The initiator methionine codon, stop codon, and putative internal polyadenylation signal are underlined. The arrow indicates the junction between two exons at nucleotides 136 and 137. (B) Alignment of Spt4p and Supt4hp amino acid sequences. The human and yeast Spt4p protein sequences were aligned by using Blast (2). Two gaps, indicated by periods, have been introduced into Spt4p in this alignment. Vertical bars indicate amino acid identities. Note that Supt4hp has a nine-amino-acid amino terminal extension relative to Spt4p. The four cysteines of the putative zinc fingers of Supt4hp and Spt4p are shown in boldface.

Thus, *SUPT4H* partially complemented the Ctf⁻ phenotype of the *spt4-138* mutant. Similar results were obtained when *SUPT4H* was tested in an *spt4* deletion mutant (data not shown).

To test the ability of *SUPT4H* to complement the Ts⁻ phenotype of *spt4* mutants, an *spt4* deletion mutant was grown at 25, 37, and 38°C (Fig. 4). Cells transformed with *ADH1-SUPT4H*, *ADH1-SPT4*, or vector only grew equivalently at 25°C. At 37°C, cells transformed with *ADH1-SPT4* or *ADH1-SUPT4H*, but not with vector alone, were able to grow. In contrast, at 38°C, only *SPT4* could complement the Ts⁻ growth defect of the *spt4* null mutation. Therefore, as in the cases of the Spt⁻ and Ctf⁻ phenotypes, *SUPT4H* partially complemented the Ts⁻ phenotype of an *spt4* null mutation. Similar results were obtained when *SUPT4H* was expressed in an *spt4-138* strain. Finally, expression of *SUPT4H* or *SPT4* in an *SPT4*⁺ strain did not affect cell growth or cause Spt⁻, Ctf⁻, or Ts⁻ phenotypes (data not shown).

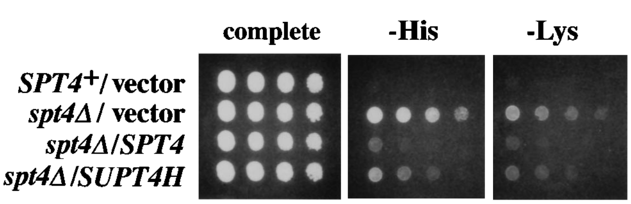

FIG. 2. *SUPT4H* complements the Spt⁻ phenotype of an *spt4* mutant. An *spt4Δ his4-912δ lys2-128δ* strain, FY1114, was transformed with pJG4-6 (vector), pGH78 (*SPT4*), or pGH127 (*SUPT4H*). Additionally, an *SPT4*⁺ *his4-912δ lys2-128δ* strain, FY119, was transformed with pJG4-6 (vector) to serve as a control for the Spt⁺ phenotype. Fivefold serial dilutions of these transformants were transferred to plates containing histidine and lysine, plates lacking histidine (−His) to assay suppression of *his4-912δ*, or plates lacking lysine (−Lys) to assay suppression of *lys2-128δ*. All plates contained galactose to induce the *GAL1* promoter and lacked tryptophan to select for maintenance of each plasmid. The plates were incubated for 3 days at 30°C and photographed. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

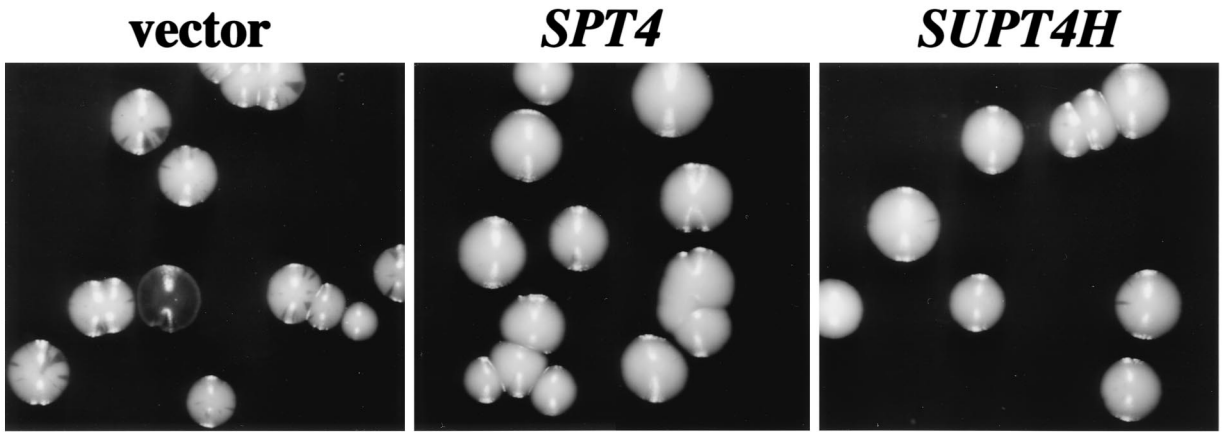

FIG. 3. The Ctf⁻ phenotype of an *spt4* mutant is complemented by *SUPT4H*. An *ade2-101 spt4-138* strain (s138) carrying a nonessential *SUP11* chromosome fragment was transformed with pAD5 (vector), pMB284 (*SPT4*), or pMB299 (*SUPT4H*). Approximately 200 cells of each transformant were spread on SC plates lacking leucine and with limiting adenine and grown for 5 to 6 days at 25°C. Red sectors, which appear as black sectors or lines in this photograph, indicate clones of cells that have lost the nonessential *SUP11* chromosome fragment during growth of the colony (43). The single completely dark colony in the vector panel arose from a cell that had lost the *SUP11* chromosome fragment prior to being plated. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

Analysis of *SUPT4H* expression. To study the expression of the *SUPT4H* mRNA in human cells, a radioactively labeled *SUPT4H* cDNA probe was hybridized to a blot containing RNAs prepared from a wide variety of human tissues (Fig. 5A). This analysis revealed two *SUPT4H* transcripts, approximately 1.7 and 0.9 kb in length, in all tissues examined. Although the 0.9-kb message was present at very low levels in brain RNA (Fig. 5A, lane 2), analysis of a separate sample of human brain RNA indicates that the short message is expressed in human brain and that the signal shown here is not due to spillover from an adjacent lane (18).

The size of the larger *SUPT4H* transcript indicates that, assuming a normal-length poly(A) tail (250 to 300 nucleotides [8]), the 1.5-kb cDNA must be close to full length. To study the origin of the shorter *SUPT4H* transcript, we probed a human muscle cDNA library to identify additional clones. This screen yielded seven clones, one approximately 1.5 kb and the others less than 300 bp in length. The longer clone had the same 5′ and 3′ sequences as the original *SUPT4H* cDNA. Two of the short clones were sequenced and were shown to have DNA sequences corresponding to the 3′ region of the *SUPT4H* ORF and to have 3′ termini that end just downstream from a consensus polyadenylation element (Fig. 1A, nucleotides 719 to 724) that is 3′ to the *SUPT4H* ORF. To test the idea that the shorter and longer *SUPT4H* messages are alternate transcripts of the same gene, related by alternative polyadenylation, we probed a Northern blot with probes derived from sequences 5′ and 3′ of this putative polyadenylation signal. Consistent with our hypothesis, the 5′ probe hybridized to both the long and short *SUPT4H* messages, whereas the 3′ probe hybridized only to the longer message (Fig. 5B).

Since Spt4p is conserved between *S. cerevisiae* and humans, it seemed likely that it would also be conserved in mice. To test this possibility, we also hybridized radioactively labeled *SUPT4H* probes to a Northern blot containing RNA from the mouse tissue culture cell line NIH 3T3. From this analysis, we identified an 0.9-kb message with the 5′ *SUPT4H* probe and no signal with the 3′ probe (Fig. 5B). Analysis of RNA derived from mouse heart muscle gave identical results (18). These data are consistent with Southern hybridization analysis showing that sequences closely related to *SUPT4H* exist in the mouse (described below).

Supt4hp is a nuclear protein in human cells. Previous immunolocalization studies with *S. cerevisiae* were unable to detect Spt4p, even when the protein was overexpressed (27). To determine the localization of an Spt4p protein, then, we attempted to determine the cellular location of Supt4hp in tissue culture cells. To do this, *SUPT4H* was HA1 epitope tagged and expressed under the control of the constitutive cytomegalovirus immediate-early promoter. This construct was transiently transfected into HeLa cells, and the resulting transfectants were examined for HA1-Supt4hp expression and localization

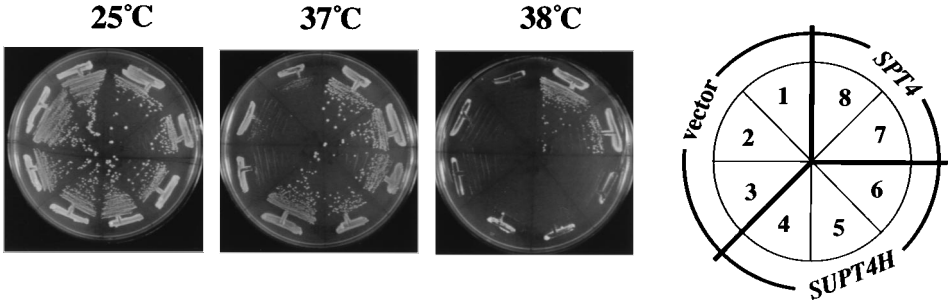

FIG. 4. The Ts⁻ phenotype of an *spt4* mutant is complemented by *SUPT4H*. The *spt4*Δ strain YMB120 was transformed with pAD5 (vector), pMB299 (*SUPT4H*), or pMB284 (*SPT4*). Transformants were restreaked on plates lacking leucine to select for the plasmid, allowed to grow 3 days at 25, 37, or 38°C, and photographed. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

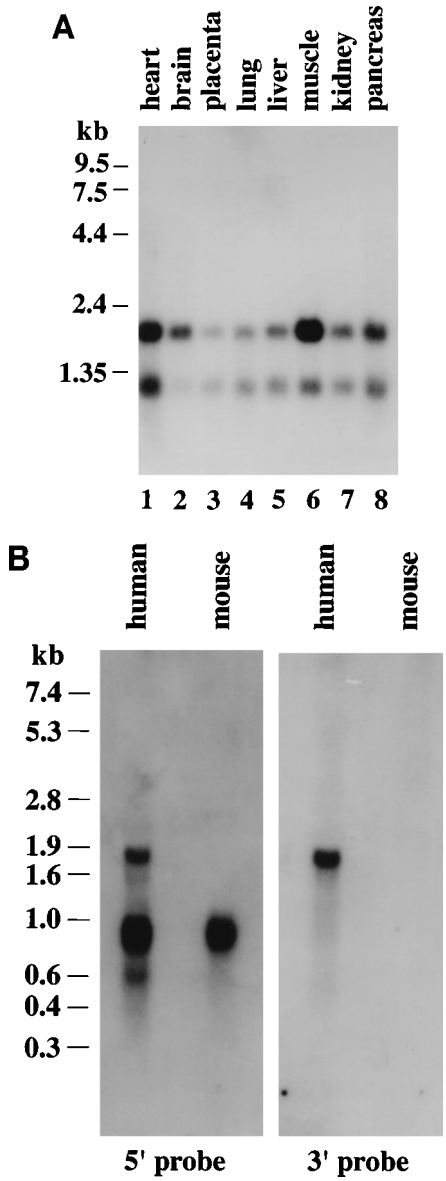

FIG. 5. Northern blot analysis of *SUPT4H*. (A) The *SUPT4H* cDNA was labeled with ^{32}P and hybridized to a blot of RNAs from the indicated human tissues. The positions to which RNA markers migrated are indicated on the left. (B) RNA from human- or mouse-derived tissue culture cells was separated on an agarose gel and hybridized to a probe containing sequences 3′ of the consensus polyadenylation signal (3′ probe). The filter was then stripped of probe and rehybridized with a probe 5′ to the polyadenylation signal (5′ probe) that corresponds to the *SUPT4H* ORF. The faint bands observed below the 1.7- and 0.9-kb bands with the 5′ probe were not seen reproducibly (see, for example, Fig. 5A) and are therefore likely due to degradation of the human RNA sample used in this experiment. The positions to which RNA size markers migrated are given on the left. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

by indirect immunofluorescence microscopy with an anti-HA1 antibody. In this analysis, HA1-Supt4hp was localized predominately to the nucleus and excluded from nucleoli (Fig. 6). In cells expressing higher levels of Supt4hp, the protein was also detectable at low levels in the cytoplasm. No unusual cellular phenotypes were observed in cells expressing Supt4hp. In control experiments, no signal was observed in cells transfected with vector lacking the *SUPT4H* gene (18). Thus, we conclude that this ectopically expressed Supt4hp protein is localized to the nucleus.

***SUPT4H* maps to human chromosome 17.** To map the chromosomal location of *SUPT4H*, PCR primers that amplify a 175-nucleotide sequence of *SUPT4H* were used to screen a human-rodent hybrid chromosome mapping panel. Although these primers gave rise to a variety of bands in hamster, human, and mouse genomic DNAs, the 175-nucleotide band was amplified only from human genomic DNA, the cloned *SUPT4H* gene, and DNA from the cell hybrid that contains human chromosome 17 (Fig. 7). No amplification products were observed in the absence of template DNA. The other bands observed in this experiment are likely due to the presence of a *SUPT4H* homolog in the mouse genome (Fig. 5B) and, presumably, the hamster genome as well. Additionally, pseudogenes of *SUPT4H* (see below) may present additional targets for amplification. Thus, the *SUPT4H* gene is located on human chromosome 17. Other data, described in Discussion, support this map assignment.

DISCUSSION

In this study, we have identified a functional human homolog of the *S. cerevisiae SPT4* gene. This gene, *SUPT4H*, encodes a predicted 117-amino-acid protein that is 42% identical to the 102-amino-acid Spt4p protein (Fig. 1B). The conservation between Supt4hp and Spt4p includes all four cysteines of a putative zinc finger, which have been shown to be essential for Spt4p function. When expressed in *S. cerevisiae*, *SUPT4H* partially complemented every phenotype of an *spt4* null mutation tested: suppression of insertion mutations (Fig. 2), defects in chromosome transmission fidelity (Fig. 3), and temperature sensitivity for growth (Fig. 4). Immunoblots of strains expressing Spt4p or Supt4hp showed that Supt4hp is expressed at lower levels than Spt4p (18). Thus, the partial complementation of *spt4* mutant phenotypes by *SUPT4H* is likely due to its lower expression levels. Therefore, the function of Spt4p is likely to have been conserved throughout eukaryotes.

Northern blot analysis showed that *SUPT4H* encodes two transcripts (Fig. 5A and B). These two transcripts appear to be related by alternative polyadenylation 3′ of the *SUPT4H* open reading frame (Fig. 5B and data not shown). The biological significance of the two *SUPT4H* transcripts is not clear. They may have different stabilities or be translated with different efficiencies. However, since only a single *SPT4* transcript was detected in mouse RNA (Fig. 5B), the presence of two *SUPT4H* transcripts in humans may have no functional consequence.

We also found that *SUPT4H* is widely expressed. First, *SUPT4H* transcripts were found in RNAs from eight of eight tissues examined by Northern hybridization (Fig. 5A). In addition to these tissues, *SUPT4H* cDNAs were found in libraries made from human ovary, olfactory epithelium, leukocyte, fetal liver and spleen, and fetal brain RNAs (see Materials and Methods). While this work was in progress, two other groups also found that *SUPT4H* is widely expressed (12, 47). Such ubiquitous expression is a property consistent with a protein involved in general rather than cell-type-specific transcription-regulatory processes.

One of the major attractions of studying Spt4p in human cells was the promise of being able to carry out higher-resolution cytological studies than are possible with *S. cerevisiae*. Attempts to demonstrate the location of Spt4p in *S. cerevisiae* have been unsuccessful (27), although we have presumed it to be a nuclear protein, given its proposed role in controlling chromatin structure and its genetic interactions with *SPT5* and

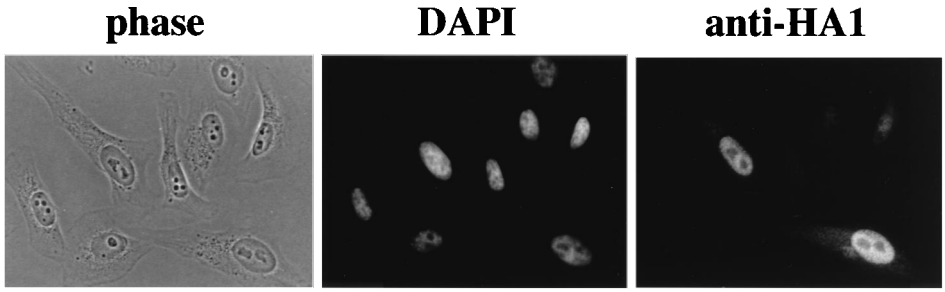

FIG. 6. Supt4hp is a nuclear protein in human cells. HeLa cells were transiently transfected with HA1 epitope-tagged *SUPT4H* and subsequently assayed for HA1-Supt4hp expression by indirect immunofluorescence. Phase-contrast, DAPI, and anti-HA1 indirect immunofluorescence images of the same field are shown. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

SPT6. In Fig. 6, we demonstrate that Supt4hp is nuclear when transiently expressed in human tissue culture cells. Neither Supt4hp nor Spt4p has an obvious nuclear localization signal; thus, Spt4p may be targeted to and retained in the nucleus via interactions with another protein(s). Because it is a small protein, 11 kDa in *S. cerevisiae* (26) and predicted to be 13 kDa in humans, Spt4p should, in the absence of other protein-protein interactions, be able to freely diffuse into and out of the nucleus (36). Thus, the weak cytoplasmic staining observed in transfectants expressing high levels of Supt4hp may be due to the titration of some factor that normally directs Supt4hp to or retains Supt4hp in the nucleus.

The immunofluorescence data in Fig. 6 indicate that Supt4hp is not localized to any particular subnuclear structure. This result suggests that even if Supt4hp affects centromere function directly (5), it also plays a more general role throughout the nucleus. Alternatively, the Ctf^- phenotype observed in *S. cerevisiae spt4* mutants may reflect an indirect effect of either altered chromatin structure or altered transcription on centromere function. Consistent with this model, altered histone gene dosage, in addition to causing Spt^- defects (13), is known to cause defects in mitotic chromosome transmission (32). From our data, we cannot exclude the possibilities that overexpression of Supt4hp in HeLa cells has perturbed its localization or that Supt4hp localization changes during the cell cycle. Future studies, using anti-Supt4hp antibodies, should allow us to examine the cellular localization of endogenous Supt4hp throughout the course of the cell cycle.

During the course of these studies, we screened a human genomic DNA library for the *SUPT4H* gene. We isolated three classes of clones. The first class contained *SUPT4H*. Sequence analysis indicates that *SUPT4H* contains at least one intron (see Materials and Methods). The other two classes of clones isolated appear to encode processed pseudogenes of *SUPT4H*, as they contain frameshift, missense, and nonsense mutations in the coding regions and the intron observed in *SUPT4H* is not present (18). In addition, none of the cDNAs that we identified in database searches or library screens arose from either of these putative pseudogenes.

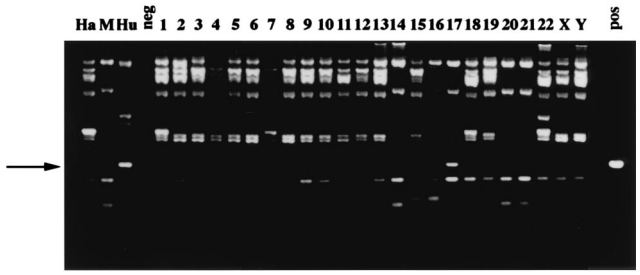

FIG. 7. *SUPT4H* maps to human chromosome 17. Primers designed to amplify a 175-nucleotide fragment of *SUPT4H* were used to amplify DNA from a human-rodent monochromosomal mapping panel. Lanes containing amplification products derived from hamster (Ha), mouse (M), and human (Hu) genomic DNAs, a cloned genomic fragment of *SUPT4H* (pos), and a no-template negative control (neg) are shown. Lanes containing amplification products derived from genomic DNAs of particular hybrids are indicated by the number of the human chromosome present in that hybrid. The amplified *SUPT4H* DNA fragment is indicated by the arrow. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

By screening a monochromosomal mapping panel with primers specific to the expressed *SUPT4H* gene, we mapped *SUPT4H* to human chromosome 17 (Fig. 7). *SUPT4H* was also mapped to human chromosome 17 by this methodology in a recent independent study (12). In addition, using PCR- and hybridization-based approaches, three groups have also identified *SUPT4H*-related sequences on human chromosomes 2, 12, 17, and 20 (6, 12, 47). Consistent with our identification of processed pseudogenes of *SUPT4H*, Chiang et al. found that the loci they identified on chromosomes 2, 12, and 20 apparently lack introns and are therefore likely to represent processed pseudogenes of *SUPT4H* (12).

By Northern blot analysis (Fig. 5B), we found that *SUPT4H* has an apparent mouse homolog. Consistent with these data, the XREF database identified and mapped regions of the mouse genome that contain sequences related to *SUPT4H*. Those data indicate that there are three *SPT4*-related sequences in the mouse genome that are located on chromosomes 6, 10, and 11 (6). Significantly, the mouse chromosome 11 locus maps to a region that is syntenic to human chromosome 17 (6). In total, these data are consistent with the idea that both the human and mouse genomes contain one expressed *SPT4* gene, located on human chromosome 17 and mouse chromosome 11, and at least two *SPT4* pseudogenes located elsewhere.

This study serves to illustrate some of the strengths of using multiple organisms to study evolutionarily conserved proteins. First, by taking advantage of the rapid progress of the human genome effort, we were able to identify *SUPT4H* merely by searching a database. Second, analysis of *SUPT4H* expression revealed two important properties, i.e., expression in all human tissues tested and nuclear localization, consistent with the idea that Spt4p plays an important role in fundamental processes of transcription. Finally, *SUPT4H* maps to human chromosome 17, and its mouse homolog maps to mouse chromosome 11. These assignments will allow consideration of *SPT4* as a candidate gene in studies mapping disease genes to these chromosomes in humans or mice.

We expect that this approach can be extended to *SPT5* and *SPT6*, the other two *SPT* genes that functionally interact with

SPT4. Eventually, such studies should lead to assays to study and compare these functions in different organisms. Already, human expressed sequence tags with good matches to *SPT5* and *SPT6* exist in databases (18). Additionally, in *C. elegans*, *emb-5* is a potential homolog of *SPT6* (35), and there is an expressed sequence tag with homology to Spt5p (18). The genes encoding at least some members of the Snf/Swi complex, which may interact with Spt4p, Spt5p, and Spt6p, have homologs in humans, mice, and *D. melanogaster* (11, 14, 23). Also, a protein complex that contains Snf/Swi family members and exhibits activities similar to the yeast Snf/Swi complex has been purified from human cells (22, 25). Thus, it appears that the Spt and Snf/Swi proteins and their functions are conserved from yeasts to humans. Further cross-species studies of these proteins should yield new insights into their mechanisms of action.

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