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Faithful Chromosome Transmission Requires Spt4p, a Putative Regulator of Chromatin Structure in *Saccharomyces cerevisiae*

MUNIRA A. BASRAI,[1] JEFFREY KINGSBURY,[2] DOUGLAS KOSHLAND,[2]
FORREST SPENCER,[1,3] AND PHILIP HIETER[1]*

Department of Molecular Biology and Genetics[1] and Center for Medical Genetics,[3] The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and Department of Embryology, Carnegie Institute of Washington, Baltimore, Maryland 21210[2]

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A chromosome transmission fidelity (*ctf*) mutant, s138, of *Saccharomyces cerevisiae* was identified by its centromere (CEN) transcriptional readthrough phenotype, suggesting perturbed kinetochore integrity in vivo. The gene complementing the *s138* mutation was found to be identical to the *S. cerevisiae SPT4* gene. The *s138* mutation is a missense mutation in the second of four conserved cysteine residues positioned similarly to those of zinc finger proteins, and we henceforth refer to the mutation as *spt4-138*. Both *spt4-138* and *spt4*Δ strains missegregate a chromosome fragment at the permissive temperature, are temperature sensitive for growth at 37°C, and upon a shift to the nonpermissive temperature show an accumulation of large budded cells, each with a nucleus. Previous studies suggest that Spt4p functions in a complex with Spt5p and Spt6p, and we determined that *spt6-140* also causes missegregation of a chromosome fragment. Double mutants carrying *spt4*Δ*2::HIS3* and kinetochore mutation *ndc10-42* or *ctf13-30* show a synthetic conditional phenotype. Both *spt4-138* and *spt4*Δ strains exhibit synergistic chromosome instability in combination with CEN DNA mutations and show in vitro defects in microtubule binding to minichromosomes. These results indicate that Spt4p plays a role in chromosome segregation. The results of in vivo genetic interactions with mutations in kinetochore proteins and CEN DNA and of in vitro biochemical assays suggest that Spt4p is important for kinetochore function.

The maintenance, replication, and segregation of chromosomal DNA constitute a fundamental aspect of the cell division cycle. The kinetochore (centromere [CEN] DNA and associated proteins) is essential to the high fidelity of chromosome transmission. The kinetochore provides a site of attachment for spindle microtubules and also directs chromosome movement during mitosis and meiosis (for reviews, see references 7 and 32). The kinetochore in *Saccharomyces cerevisiae* is a relatively simple structure (47) compared with the large and complex trilaminar structures seen in multicellular eukaryotes (48, 49). The minimal functional centromere of *S. cerevisiae* is contained within approximately 120 bp of CEN DNA which contains two important conserved DNA sequence elements, termed CDEI (8 bp) and CDEIII (25 bp), flanking a 78- to 86-bp AT-rich CDEII element (17, 23).

Several proteins that bind to CDEI and CDEIII have been identified. The CEP1 (also known as CBF1, CPF1, or CP1) protein binds to CDEI as a homodimer, and a null mutation in *cpf1* causes only a 10-fold decrease in chromosome segregation, indicating that it is important but not essential for kinetochore function (2, 10, 39). A protein complex, CBF3, that contains three major protein species (110, 64, and 58 kDa), was biochemically purified and shown to bind to CEN DNA in a CDEIII sequence-specific manner (36). CBF3 also exhibits a minus-end-directed mechanochemical motor activity in vitro, which is observed as translocation of latex beads covalently attached to CEN DNA along polymerized microtubules (27). *CEP3* encodes the 64-kDa component of CBF3 and contains a putative DNA binding domain (35, 60). The 110-kDa component is encoded by the *NDC10* (also known as *CBF2* or *CTF14*) gene (13, 20, 29); the 58-kDa component is encoded by the *CTF13* gene (13). In addition, recently reported data on genetic interactions suggest that Mif2p, which shows homology to mammalian centromere protein CENP-C, may function as a centromere protein (8, 40).

In addition to the CEN DNA sequence requirements and the *trans*-acting kinetochore proteins, higher-order chromatin structure provides a framework for interactions of histones, CEN DNA, and centromere-specific DNA-binding proteins (7). The kinetochore of *S. cerevisiae* is composed of an approximately 160- to 220-bp nuclease-resistant core that is centered around the CEN DNA sequence flanked by an ordered array of nucleosomes (6, 18). Mutations in CEN DNA and depletion of histones H4 and H2B affect the chromatin structure at the centromere and cause chromosome missegregation (7, 12, 38, 52, 53). Several studies have led to the proposal that the yeast kinetochore may incorporate a modified nucleosome (7). Support for this hypothesis comes from studies of *S. cerevisiae* Cse4p (3, 59). Cse4p shows homology to a family of histone H3-like proteins which includes the mammalian CENP-A protein, which is thought to be a component of a modified nucleosome.

The *ctf* (chromosome transmission fidelity) mutants were originally isolated by the sole criterion of chromosome missegregation (58). In vitro assays suggest that the attachment of CBF3-CEN DNA complexes to microtubules requires additional factors that remain to be identified (31, 56). Hence, in order to identify additional components of the kinetochore, we examined 29 *ctf* mutants in a secondary screen for kinetochore integrity. The secondary screens used previously for a subset of *ctf* mutants led to the identification of two of the essential components of the kinetochore, Ctf13p and Ndc10p (Cbf2p or Ctf14p) (13). One of the assays in the secondary screen detects relaxation of a transcription block formed at the centromere;

* Corresponding author. Mailing address: Dept. of Mol. Biol. & Genetics, 725 N. Wolfe St., Hunterian 617, The Johns Hopkins University School of Medicine, Baltimore, MD 21210-2185. Phone: (410) 955-3482. Fax: (410) 614-2987. Electronic mail address: phil_hieter @qmail.bs.jhu.edu.

TABLE 1. Strains used in this study

S. cerevisiae strain	Genotype	Source or reference
s138 (*spt4-138*)	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 spt4-138*	This study
YPH102	*MAT*α *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1*	58
YPH254	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 his3Δ200 leu2Δ1* CFVIII (*RAD2d.YPH277*) *URA3 SUP11*	F. Spencer and P. Hieter
YPH277	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1* CFVII (*RAD2d.YPH277*) *URA3 SUP11*	58
YPH278	*MAT*α *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	58
YPH279	*MAT***a**/α *ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 TRP1/trp1Δ1 leu2Δ1/leu2Δ1 HIS3/his3Δ200* CFVII (*RAD2d.YPH277*) *URA3 SUP11*	19
YPH656	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ1 his3Δ200 leu2Δ1* [Δ*CEN6*::*LEU2 CEN11*] YAC (*URA3 SUP11 CEN6 TRP1*)	P. Hieter; 54
YPH657	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ1 his3Δ200 leu2Δ1* [Δ*CEN6*::*LEU2 CEN11*] YAC (*URA3 SUP11 CEN6.CDEIII-19*, *20+G TRP1*)	P. Hieter; 54
YPH987	*MAT***a**/α *ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ63/trp1Δ63 leu2Δ1/leu2Δ1 his3Δ200/his3Δ200* CFIII (*CEN3L.YPH983*) *TRP1 SUP11*	P. Hieter
YPH1022	*MAT*α *ura3-52 lys2-801 ade2-101 his3Δ200 trp1Δ63 leu2Δ1* CFIII (*CEN3L.YPH987*) *TRP1 SUP11*	P. Hieter
YPH1218	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ leu2Δ1 his3Δ200* [Δ*CEN6*::*LEU2 CEN11*] YAC (*URA3 SUP11 CEN6.CDEIIΔ31 TRP1*)	This study; 54
YMB53	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 spt4-138* CFVII (*RAD2d.YPH277*) *URA3 SUP11*	This study
YMB54	*MAT***a** *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 spt4-138*	This study
YMB120	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 his3Δ200 leu2Δ1 spt4Δ2*::*HIS3* CFVIII (*RAD2d.YPH277*) *URA3 SUP11*	This study
YMB292	*MAT*α *ura3-52 lys2-801 ade2-101 his3Δ200 trp1Δ63 leu2Δ1 spt6-140* CFIII (*CEN3L.YPH987*) *TRP1 SUP11*	This study
YMB322	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 his3Δ200 spt4Δ2*::*HIS3 ctf13-30*::*TRP1* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	This study
YMB328	*MAT***a**/α *ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ1/trp1Δ1 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 spt4Δ2*::*HIS3*/*spt4Δ2*::*HIS3* CFVIII (*RAD2d.YPH277*) *URA3 SUP11*	This study
YMB369	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 his3Δ200 spt4Δ2*::*HIS3 ndc10-42* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	This study
YMB396	*MAT***a** *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 cbf1Δ*::*TRP1 spt4Δ2*::*HIS3* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	This study
YMB405	*MAT***a** *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 ctf13-30*::*TRP ndc10-42* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	This study
YMB446	*MAT***a**/α *ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ1/TRP1 leu2Δ1/leu2Δ1 spt4-138/spt4-138* CFVII (*RAD2d.YPH277*) *URA3 SUP11*	This study
FY120	*MAT*α *his4-912*δ *lys2-128*δ *ura3-52 leu2Δ1*	37
YVR26	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ1 his3Δ200 leu2Δ1* [Δ*CEN6*::*LEU2 CEN11*] YAC (*URA3 SUP11 CEN6.CDEI-3G TRP1*)	V. Resto and P. Hieter; 54
YJP109	*MAT*α *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 trp1Δ1 ctf13-30*::*TRP* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	J. Puziss and P. Hieter
YVR57	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 cbf1Δ*::*TRP1* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	V. Resto and P. Hieter
ndc10-42	*MAT*α *ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1* CFIII (*CEN3L.YPH278*) *URA3 SUP11*	13

the other detects an increase in the mitotic stability of a dicentric test chromosome (13).

This paper describes the characterization of a *ctf* mutant, s138, that tested positive in the CEN transcriptional readthrough assay and is temperature sensitive for growth at 37°C. The gene complementing the *s138* mutation was cloned and shown to be identical to the *S. cerevisiae SPT4* gene (37). The *spt* (suppressor of Ty) mutants were originally isolated in a genetic screen for suppression of transcriptional defects associated with Ty delta insertions in the 5′ regions of the *HIS4* and *LYS2* genes (16, 63, 64). Genetic analyses suggest that Spt4p, Spt5p, and Spt6p interact, and biochemical studies have shown that Spt5p and Spt6p coimmunoprecipitate (61). In the work presented here, genetic and biochemical approaches were taken to further define the role of Spt4p in chromosome segregation and kinetochore function.

MATERIALS AND METHODS

Yeast strains and media. A large reference set of chromosome transmission fidelity mutants, the *ctf* mutants of *S. cerevisiae*, has been described previously (58). Media for yeast growth and sporulation were as described previously (50) except as otherwise indicated. For experiments monitoring the loss of the chromosome fragment, adenine was added to 6 μg/ml to enhance the color of the red pigment in *ade2-101* strains (19). All yeast transformations were done by a previously described method (28). The *S. cerevisiae* strains used in this study are listed in Table 1.

In vivo assay for kinetochore integrity: CEN transcriptional readthrough assay. The CEN transcriptional readthrough assay was done by using a reporter plasmid containing a *GAL10* promoter that initiates transcription of an actin-LacZ fusion gene. A wild-type centromere inserted in the actin intron inactivates the segregational function of the CEN, allowing only 1% of the β-galactosidase activity seen when CEN is absent. The transcriptional block created by the CEN DNA-protein complex is relaxed in putative kinetochore mutants which allow transcriptional readthrough activity from the reporter plasmid (13). Wild-type cells produce white colonies and the kinetochore mutant (*ctf13-30* mutant) produces blue colonies with the reporter plasmids on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)-containing media. The reporter plasmids pKF75 and pKF76 (described previously [13]) contain a β-galactosidase reporter gene in a pRS303 (55)-based vector (selectable marker, *HIS3*). Approximately half of the *ctf* mutants are histidine auxotrophs, and the other half are tryptophan auxotrophs (58). To facilitate the screening of the tryptophan auxotrophs, we constructed plasmids pMB203 and pMB204. The two plasmids are identical except that pMB203 has a wild-type CEN and pMB204 has a mutant CEN (CDEIII G-to-C mutation). Plasmids pMB203 and pMB204 were made by ligating a 6.2-kb *Asp*718-*Nae*I fragment containing the reporter constructs from plasmids pKF75 and pKF76 (13), respectively, to a 3.2-kb *Asp*718-*Nae*I fragment of pRS304 (selectable marker, *TRP*). The plasmids were digested with *Not*I and integrated into chromosome III at the *leu2* locus. The structurally dicentric plasmids were maintained in a functionally monocentric state by keeping the transformed strains on galactose-containing medium, thereby causing transcrip-

tional inactivation of the test centromere. After confirmation of the site of integration by PCR, at least two independent transformants were tested on media containing the chromogenic substrate X-Gal as described previously (13). The color of colonies on X-Gal-containing plates was recorded after incubation at 25°C for 5 to 6 days.

Molecular cloning and characterization of s138. The *s138* gene was cloned by complementation of the temperature-sensitive (*ts*) mutation of *s138* from a library (58a) of 10- to 12-kb fragments of yeast genomic DNA inserted into a pBR322-based *LEU2 CEN4 ARS1* shuttle vector, pSB32 (61a). Two *S. cerevisiae* transformants were obtained at 37°C (a total of 1,000 transformants were plated), and plasmid DNA was isolated from each. One of the plasmids (pMB230) complemented both the *ts* and the *ctf* phenotypes of the *s138* mutant upon retransformation. Appropriate restriction fragments were used for subcloning the insert DNA into the pRS-based vectors (55). Plasmids pMB212 and -232 contain a 2.4-kb *Eco*RI and a 1-kb *Eco*RV fragment, respectively, in pRS314. Plasmids pBM30 (37) and pMB305 contain frameshift mutations created by converting unique restriction sites, *Acc*I and *Bst*EII, to blunt ends and religating. The insert in plasmid pMB232 was sequenced by standard methods (51).

Plasmid pMB217, which contains a 2.4-kb *Eco*RI fragment from pMB212 in the integrating vector pRS304 (selectable marker, *TRP*) (55), was used for genetic linkage studies. The plasmid was linearized at the unique *Nde*I site that lies outside the complementing open reading frame and transformed into a wild-type strain, YPH277. The integration of the plasmid into the genome was confirmed by Southern blot analysis. The transformants were then crossed to YMB53 (*s138* mutant). The resulting diploid strain was sporulated, and tetrads were dissected and analyzed. The *TRP* marker segregated away from the *ts* and *ctf* phenotypes of the *s138* mutation in 10 tetrads analyzed.

The mutant allele of the *s138* gene was cloned and sequenced by a PCR strategy. PCR primers OPH181 (5′-CTG GAA TTC AGT TTG GC-3′) and OPH182 (5′-GAA TAA CTG GAC GGT AG-3′) were used to amplify the *SPT4* gene and flanking DNA from genomic DNAs of the wild-type strain YPH277 and the mutant strain s138 by PCR. The PCR product of 510 bp was cut with *Eco*RI and *Eco*RV, and the resulting 370-bp fragment was cloned into pRS314. Plasmids pMB224A, -224B, and -224C (from wild-type cells) and plasmids pMB225A, -225B, and -225C (from mutant s138) represent three independent *Escherichia coli* transformants. In order to eliminate any possible errors due to PCR, we sequenced the *SPT4* gene in each of the six pMB224 and pMB225 plasmids by standard methods (51).

A deletion of the *SPT4* open reading frame was generated by a modification of a previously described PCR-based method (4). Oligonucleotides for PCR were synthesized as follows. OPH183 (5′-TGT ACT GTG TAA TAA TTA CAC CTG GCC ACA TTC AGT TTG CTG TGC GGT ATT TCA CAC CG-3′) has 39 bp of homology to the sense strand 39 nucleotides upstream of the *SPT4* ATG followed by 20 bp of sequence from the plus strand of pRS vectors (55) adjacent to the vector selectable marker. Oligonucleotide OPH184 (5′-AGC AAA AAC AAA AGT GCA GAA CGT TAA ATA AAT GAA AGC AAG ATT GTA CTG AGA GTG CAC-3′) has 40 bp of homology to the antisense strand 92 nucleotides downstream of the *SPT4* stop codon followed by 20 bp of sequence from the minus strand of pRS vectors adjacent to the vector selectable marker. The oligonucleotides OPH183 and -184 can be used to incorporate any one of the markers from the pRS vectors (*URA3*, *HIS3*, *LEU2*, or *TRP1*) by PCR. The two oligonucleotides OPH183 and -184 were used to amplify a *HIS3* marker from pRS303 by PCR. All PCRs were done on a Perkin-Elmer 9600 instrument with the following cycling parameters: 1 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and then 10 min at 72°C. The PCR product was transformed into the haploid yeast strain YPH254 and the diploid strain YPH987 (Table 1). Homologous recombination replaced the *SPT4* open reading frame with the selectable marker. Four transformants from a total of 15 haploid transformants tested had a deletion of *SPT4* as verified by Southern blot analysis. The *spt4Δ2::HIS3* strain (YMB120 [Table 1]) was tested for *ts* and *ctf* phenotypes.

A two-step gene replacement strategy was used to integrate the cloned *spt6-140* (11) allele in plasmid pAB56 (kindly provided by Fred Winston) into a wild-type strain (YPH1022) containing a chromosome fragment (CF). Strain YPH1022 was transformed with plasmid pAB56 linearized with *Sac*I. Ura^+ transformants were colony purified and streaked on 5-fluoroorotic acid medium for a pop-out of the vector with stable replacement of the wild-type *SPT6* gene with the *spt6-140* allele. Since *spt6-140* mutants are *ts* for growth at 37°C, we tested 20 5-fluoroorotic acid-resistant transformants for the *ts* phenotype. Four transformants were *ts* for growth at 37°C, and plasmid pMS33 (61), which contains *HA-SPT6*, was used to test for complementation of the *ts* phenotype. The *spt6-140*-containing strains (e.g., YMB292 [Table 1]) were tested for their abilities to maintain the nonessential CF by plating cells on synthetic complete (SC) medium with limiting adenine at 25, 30, and 32°C.

Epitope-tagged constructs. Plasmid pMB237 was constructed by cloning *HA-SPT4* on a *Hin*dIII fragment from plasmid pBM65 (37) (kindly provided by Fred Winston) into the *Hin*dIII site of pRS315 (55). *GST SPT4* (pGH84) was kindly provided by Grant Hartzog. The epitope-tagged *SPT4* constructs were found to complement the *ts* and *ctf* phenotypes of YMB120 (*spt4Δ2::HIS3*). *HACTF13* is as previously described (13), and *HACBF1* (pPM81) was generously provided by Pam Meluh.

Flow cytometry. Logarithmically growing cells were pelleted, resuspended in 0.2 M Tris (pH 7.5)–70% ethanol, and fixed overnight at 4°C. The cells were pelleted, resuspended in 0.2 M Tris (pH 7.5) containing 1 mg of RNase A per ml, and incubated at 37°C for 3 to 4 h. Cells were washed, resuspended in 1 ml of 0.2 M Tris (pH 7.5) containing 3 μg of propidium iodide (Sigma) per ml, and incubated at least overnight at 4°C. Samples were sonicated for 2 to 3 s, and flow cytometry was performed on a Coulterepics 752 apparatus with a gain of 10 and high voltage of 935 V. Propidium iodide-stained cells were excited with 400 mW of 488-nm light, and integrated red (wavelength, >590 nm) fluorescence was recorded. Nuclear morphology was determined by DAPI (4′,6-diamidino-2-phenylindole) staining as described previously (13).

Gel mobility shift assays. Gel mobility shift assays with whole-cell yeast extracts and radiolabeled CEN DNA probes were performed by previously described procedures (13, 56). We assayed activities of extracts from cells grown at the permissive temperature (25°C) or nonpermissive temperature (37°C) for binding to CEN DNA at 25 or 38°C. Increases in CEN DNA-protein complex formation were linear with increasing concentrations of whole-cell extract in the range of 10 to 60 μg of total protein per 30-μl reaction mixture. The 96-bp sequence of CDEI+II was isolated as a *Bam*HI fragment from BPH567, which contains the CDEI+II fragment from pRN505 (43) in pRS314. The CDEIII probe was made by purifying a *Hin*dIII-*Eco*RI fragment containing 88 bp of CDEIII from pSF262a (kindly provided by Peter Sorger). The 3′-terminal transferase activity of *Taq* polymerase was used to label the CDEI+II probe with [α-^{32}P]dGTP and the CDEIII probe with [α-^{32}P]dATP as described previously (44). Binding reaction mixtures were electrophoresed on 4% polyacrylamide gels, and relative band intensities were determined on a PhosphorImager.

Minichromosome-microtubule binding assays and loss of minichromosome pDK370. The microtubule-minichromosome binding assays were performed at 23°C essentially as described previously (30), with minor modifications (31). The mitotic stability of pDK370 was calculated by measuring the fraction of the cells that retained the plasmid after growth in selective medium and then growth for seven to nine generations in nonselective medium at 23°C (30).

RESULTS

s138 missegregates a chromosome fragment and tests positive in a secondary screen for kinetochore integrity, the CEN transcriptional readthrough assay. When applied to a subset of *ctf* mutants, the CEN transcriptional readthrough assay led to the identification of two essential components of the *S. cerevisiae* kinetochore, Ctf13p and Ndc10p (Ctf14p) (13). Because of the success with these assays, we decided to screen the remaining *ctf* mutants for kinetochore integrity. The reporter plasmid pMB203 was integrated into the genomes of the *ctf* mutants (see Materials and Methods), and transformants were plated on medium containing X-Gal as described previously (13). As expected, wild-type cells produced white colonies, and the known kinetochore mutant (the *ctf13-30* mutant) allowed transcriptional readthrough and produced blue colonies. Upon screening of 29 additional *ctf* mutants (one-third of the total mutant collection [57]), five mutants, s138, s141, s145, s149, and s151, were identified as positive (blue colonies on X-Gal plates) in the CEN transcriptional assay, suggesting that kinetochore integrity is partially altered in these *ctf* mutants. The s138 mutant, which missegregates a chromosome fragment, also shows a *ts* phenotype for growth at 37°C (data not shown). Genetic analysis showed that the *ts* and *ctf* phenotypes of *spt4-138* are recessive when tested in a heterozygous diploid (*spt4-138*/*SPT4* strain). Sporulation of this diploid indicated that the *ts* and *ctf* phenotypes cosegregated 2:2 in the 20 tetrads analyzed, suggesting that the two phenotypes were due to a single mutation. The known kinetochore mutations *ctf13-30* and *ndc10-42* also confer temperature sensitivity for growth at 37°C, and the mutant strains missegregate a chromosome fragment and test positive in the CEN transcriptional readthrough assay (13). The phenotypic similarities of the *s138* mutant and the known kinetochore mutants prompted us to study it further.

Molecular cloning of the gene complementing the *s138* mutation. The *s138* gene was cloned by complementation of the *ts* phenotype (see Materials and Methods). A 1-kb *Eco*RV fragment in plasmid pMB232 complemented the *ctf* and *ts* phenotypes of mutant strain s138. Contour-clamped homogeneous electric field gel analysis and Olson blots (lambda clone grid

filters) placed the *s138* gene on chromosome VII proximal to the *RAD2* locus (data not shown). Integrative transformation of the cloned DNA and tetrad analysis indicated that the cloned DNA is linked to the *s138* mutation (see Materials and Methods). DNA sequence analysis revealed overlap with a 3-kb sequence, deposited in GenBank by Malone et al. (37), containing the *S. cerevisiae SPT4* gene. Plasmid pBM30 (37), which contains a frameshift in the *Acc*I site within the *SPT4* open reading frame, did not complement the *s138* mutation. The *SPT4* gene encodes a 102-amino-acid protein that contains an N-terminal zinc finger motif (37). Direct sequence analysis proved that the *s138* mutant phenotype is due to a mutation in the *SPT4* gene (see below). Henceforth we refer to the *s138* mutation as *spt4-138*.

***SPT4* is essential for haploid growth at 37°C.** Previous work has shown that a partial deletion of the *SPT4* gene has no effect upon haploid growth (37). We used a PCR-mediated technique to create the complete deletion allele *spt4*Δ*2*::*HIS3* (see Materials and Methods). Transformation of the PCR product into haploid strain YPH254 and diploid strain YPH987 gave roughly the same number of transformants. Four of the 15 independent haploid transformants tested were found to have the precise deletion of the *SPT4* gene as verified by Southern blots. Therefore, *SPT4* is not essential for haploid growth at 25°C. However, the *spt4*Δ*2*::*HIS3* mutation was found to cause temperature sensitivity for growth at 37°C. The deletion mutant also exhibited a *ctf* phenotype and grew more slowly, with doubling times 1.5 to 2 times that of the wild-type strain at 25°C (data not shown).

The *spt4-138* mutant has a mutation in the cysteine of the zinc finger of Spt4p. Since the *spt4-138* and *spt4*Δ*2*::*HIS3* (YMB120) strains were *ts* for growth at 37°C and had a *ctf* phenotype, the *spt4-138* mutation was likely to be a loss-of-function mutation. Hence, we determined the nature of the *SPT4* mutation in the *spt4-138* mutant (see Materials and Methods). Spt4p has a C-X_2-C-X_{13}-C-X_2-C pattern at amino acid positions 7, 10, 24, and 27 (37), similar to that found in some zinc-binding proteins (5). Previous studies showed that the cysteines at position 7, 24, and 27 are essential for *SPT4* function (37). Sequence comparison showed that the *spt4-138* mutation is a missense mutation that changes the second cysteine at position 10 in the zinc finger domain to tyrosine. The plasmid pMB225 (containing *spt4-138*) failed to complement the *ts* or the *ctf* phenotype of the *spt4*Δ mutant, indicating that the cysteine at position 10 is essential for *SPT4* activity.

The *spt6-140* mutant missegregates a chromosome fragment. Synthetic lethality and unlinked noncomplementation studies suggest that Spt4p functions in a complex with Spt5p and Spt6p (61). Since *spt4-138* has a chromosome missegregation phenotype, we examined if the *ts* allele *spt6-140* has a *ctf* phenotype at a semipermissive temperature. A two-step gene replacement method was used to integrate a *ts spt6-140* allele into a wild-type strain, YPH1022, containing a CF. The resulting strain, YMB292 (*spt6-140*), was *ts* for growth at 37°C, and the stability of the CF was monitored by a sectoring assay at 25, 30, and 32°C. The colony color sectoring assay is based on loss of a nonessential CF containing *SUP11*, which suppresses an *ade2-101* mutation (58). The loss of the *SUP11*-containing CF can be monitored by the appearance of red sectors in the white colonies. Strain YMB292 (*spt6-140*) showed a rare sectoring phenotype at 25°C; however, at 32°C a clear sectoring phenotype was observed (Fig. 1). The wild-type control strain YPH1022 showed no sectoring, while the *spt4-138* strain showed the expected sectoring phenotype (Fig. 1). The *ctf* and *ts* phenotypes of YMB292 (*spt6-140*) were complemented by pMS33, a plasmid containing *HA-SPT6* (data not shown). These results indicate that *spt6-140* has a *ctf* phenotype.

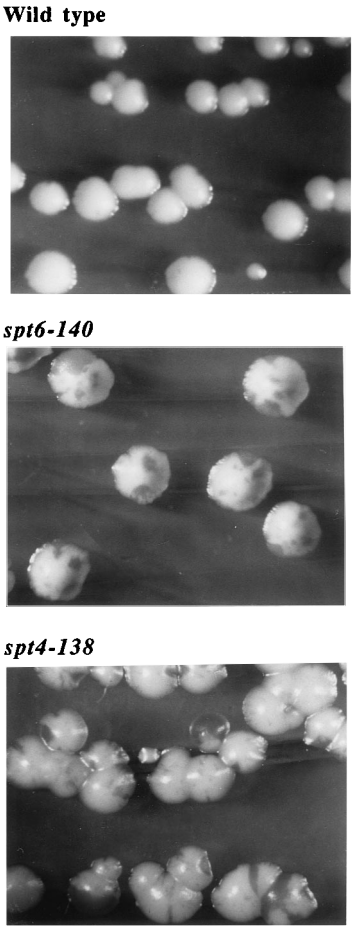

FIG. 1. *spt6-140* causes missegregation of a CF. Wild-type (YPH1022) and *spt6-140* mutant (YMB292) cells were plated on SC plates with limiting adenine and photographed after incubation for 5 to 6 days at 32°C (see Materials and Methods). The phenotype of an *spt4-138* strain assayed similarly at 25°C is shown for comparison. The dark sectors in the white colonies arise as a result of loss of the *SUP11*-containing nonessential CF.

***spt4-138* is able to suppress the *lys2* delta insertions.** *spt4* mutants were originally isolated in a genetic screen for suppression of transcriptional defects associated with Ty delta insertions in the 5′ end of the *HIS4* or *LYS2* gene (64). In a wild-type strain, the mutations *his4-912*δ and *lys2-128*δ cause His^- and Lys^- phenotypes, respectively (63, 64). In *spt4* mutants, the insertion mutation phenotypes are suppressed, resulting in a His^+ Lys^+ phenotype (37). We determined whether the *spt4-138* mutant allele had an *spt* phenotype. This was done by crossing strain YMB53 (*spt4-138*) to FY120 (*SPT4*$^+$) (37). The diploid strain (*spt4-138*/*SPT4 lys2-128*δ/*lys2-801*) was sporulated, and tetrads were analyzed. The *ts* phenotype segregated 2:2 in the 10 tetrads analyzed and 10 out of the 20 *ts* spores were Lys^+, suggesting that *spt4-138* can suppress the *LYS2* delta insertion. We conclude that *spt4-138* has an *spt* phenotype.

***spt4*/*spt4* homozygous diploids show a high rate of CF loss.** The *spt4-138* and *spt4*Δ*2*::*HIS3* strains both show a CF loss phenotype suggestive of a chromosome missegregation phenotype. Chromosome missegregation can result from two types of events, chromosome loss (1:0 segregation) and chromosome nondisjunction (2:0 segregation) (22). We examined the relative

TABLE 2. Analysis of CF loss (1:0) and nondisjunction (2:0) events in *spt4*/*spt4* homozygous diploids[a]

Strain	Genotype	CF missegregation rate		No. of colonies[b]
		% 1:0 events[c]	% 2:0 events[d]	
YPH279	*SPT4*/*SPT4*	0.03	0.03	29,046
YMB446	*spt4-138*/*spt4-138*	3.20	0.32	937
YMB328	*spt4*Δ/*spt4*Δ	0.42	<0.05	2,611

[a] The CF missegregation rate (loss [1:0] and nondisjunction [2:0] events) for the nonessential CF were measured as described previously (19). The rates for wild-type strain YPH279 are from previously reported data (19). The rates for the *spt4* mutants were determined in duplicate for two isogenic homozygotes.
[b] Total number of pink colonies (colonies with one CF).
[c] Number of half-red, half-pink colonies divided by the total number of colonies with one CF.
[d] Number of half-red, half-white colonies divided by the total number of colonies with one CF.

contributions of each of these events for the missegregation phenotype observed in the *spt4* mutants by using the *SUP11* colony color assay. A 1:0 missegregation event for the chromosome fragment in the first division results in a half-red, half-pink colony, while a 2:0 missegregation event results in a half-red, half-white colony (22). These rates were measured for *spt4-138*/*spt4-138* (YMB446), *spt4*Δ*2*::*HIS3*/*spt4*Δ*2*::*HIS3* (YMB328), and *SPT4*/*SPT4* (YPH279) homozygous diploid strains at 25°C. The *spt4-138* homozygous diploid (YMB446) showed about a 100-fold-higher chromosome loss phenotype (1:0) and about a 10-fold-higher chromosome nondisjunction phenotype (2:0) compared with wild-type cells (Table 2). The *spt4*Δ homozygote (YMB328) showed a 14-fold-higher chromosome loss in comparison with the wild-type strain (YPH279). These results show that both *spt4-138* and *spt4*Δ strains have a chromosome missegregation phenotype, with the predominant phenotype being chromosome loss (1:0) events. An increase in chromosome loss (1:0) events is not inconsistent with a role in kinetochore function, since the known *ctf13-30* kinetochore mutant exhibits a comparable increase in both 1:0 and 2:0 events (13).

***spt4* mutants exhibit a three-budded phenotype upon shift to the nonpermissive temperature.** The *spt4-138* and *spt4*Δ*2*::*HIS3* strains are *ts* for growth at 37°C. In order to examine the cell cycle profiles, we performed flow cytometry of cells in the logarithmic phase of growth at 25°C and then after a shift to 38°C for 4 and 6 h. Both the *spt4-138* and the *spt4*Δ cells showed a nearly wild-type profile at 25°C (Fig. 2). After a shift to 38°C for 4 h, the *spt4* mutants showed an accumulation of cells with a 2C DNA content, with the peak trailing to the right. Upon further incubation for an additional 2 h (6 h total), the 2C peak was reduced in size with a concomittant increase in the 1C DNA peak and a peak with >2C DNA content.

The nuclear morphology of the cells was examined by DAPI staining, and the results are summarized in Fig. 3. Microscopic examination of cells at 25°C showed that *spt4* mutants accumulate with a large budded morphology with either a divided nucleus or a nucleus at or near the neck. Upon a shift to the nonpermissive temperature of 38°C for 4 h, the *spt4* mutants showed an increase in both the large-bud, divided-nucleus phenotype and a three-budded cell phenotype. Further incubation at 38°C (6 h) showed that the three-budded cell morphology was the predominant phenotype for the *spt4* mutants (42% for the *spt4-138* strain and 36% for the *spt4*Δ strain), and depending on the size of the third bud, it is either with or without a nucleus. All samples were sonicated (see Materials and Methods) before analysis; hence, the three-budded phenotype is not due to cell clumps. These results suggest that the *spt4* mutants do not show a classical *cdc* (cell division cycle) arrest phenotype. However, in the *spt4* mutants there appears to be a failure or delay in cytokinesis.

***spt4* mutants show synergistic chromosome instability in combination with CEN DNA mutations.** As described above, the *spt4-138* mutant missegregates a chromosome fragment with a wild-type centromere (Fig. 1). If Spt4p plays a role in kinetochore structure or function, we might expect a combination of an *spt4* mutation with a *cis*-acting CEN DNA mutation to give a synthetic acentric phenotype as manifested by a synergistic effect of the two mutations. Using this rationale, Strunnikov et al. (60) designed a screen which led to the identification of an essential component, CEP3, of the CBF3 complex of the yeast kinetochore. These studies prompted us to examine the stabilities of yeast artificial chromosomes (YACs) containing either a wild-type CEN or a mutant CEN in *spt4* mutants. The YAC-containing strains, YPH 656 (wild-type CEN), YVR26 (CDEI-3G), YPH1218 (CDEIIΔ31), and YPH657 (CDEIII-19, 20+G) (Table 1), were mated to *spt4-138* and *spt4*Δ*2*::*HIS3* strains. Heterozygous diploids were sporulated, and the spores containing the YAC and the *spt4* mutation were used for further analysis. We compared the sectoring phenotype of a YAC in a wild-type strain with that of the same YAC in the *spt4* mutant (Fig. 4). A YAC containing a wild-type CEN is stable in wild-type (*SPT4*) cells and is lost at a low frequency in the *spt4-138* and *spt4*Δ*2*::*HIS3* mutants (Fig. 4). Although a mutant CDEI YAC (CDEI-3G) was lost at a low frequency in the wild-type cells, it was lost at a higher frequency in the *spt4-138* and *spt4*Δ*2*::*HIS3* strains (Fig. 4). Similarly, a mutant CDEII YAC (CDEIIΔ31), which is slightly unstable in wild-type cells (Fig. 4), was further destabilized in the *spt4-138* and *spt4*Δ*2*::*HIS3* strains. Furthermore, a mutant CDEIII YAC (CDEIII-19, 20+G), which was slightly unstable in wild-type cells, was more unstable in the *spt4-138* strain and moderately unstable in the *spt4*Δ*2*::*HIS3* strain (Fig. 4). These results show that the *spt4* mutations interfere with centromere function in vivo and show a synergistic instability of YACs containing either CDEI, CDEII, or CDEIII mutations.

***SPT4* genetically interacts with genes that encode components of the kinetochore.** To further examine the role of Spt4p in kinetochore function, we examined possible genetic interactions of double mutants containing *spt4*Δ*2*::*HIS3* with muta-

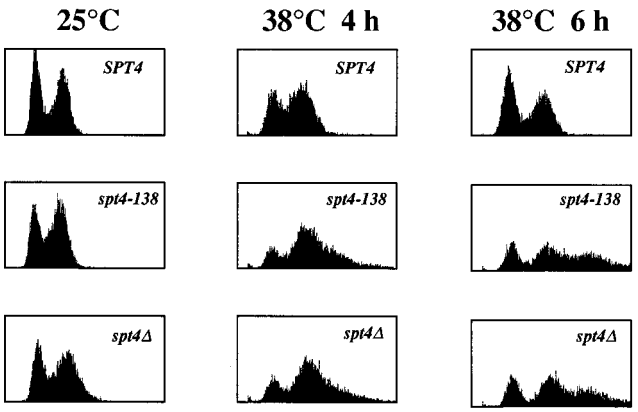

FIG. 2. Cell cycle distribution of *spt4* mutants. Flow cytometry analysis of logarithmically growing *SPT4*, *spt4-138*, and *spt4*Δ*2*::*HIS3* cells at 25°C and after a shift to the nonpermissive temperature of 38°C for 4 or 6 h was done as described in Materials and Methods. The number of cells is depicted on the vertical axis, and the fluorescence intensity of emitted light (proportional to the DNA content) is shown on the horizontal axis. Cells were sonicated for 3 to 4 s before analysis.

Temp	Genotype	Nuclear morphology			
					
25°C	WT	71	16	12.6	0.4
	spt4-138	47	25	25	3
	spt4Δ2::HIS3	60	22	16	2
38°C 4 h	WT	74	15	11	0
	spt4-138	22	15	44	19
	spt4Δ2::HIS3	33	17	40	10
38°C 6h	WT	69	24	7	0
	spt4-138	26	12	20	42
	spt4Δ2::HIS3	28	12	25	35

FIG. 3. *spt4* mutants show a three-budded phenotype. The nuclear distributions of *SPT4*, *spt4-138*, and *spt4Δ2::HIS3* cells grown at 25°C and shifted to the nonpermissive temperature of 38°C for 4 or 6 h were determined by DAPI staining of fixed cells. The nucleus is depicted as a black circle. At least 200 cells were counted for each sample in two independent experiments (variance, ±3%), and the numbers represent the percentages of cells that show the depicted morphologies.

tions in genes that encode kinetochore proteins. The *spt4Δ2::HIS3* strain was mated to the kinetochore mutants carrying *ctf13-30*, *ndc10-42*, or *cbf1Δ::TRP1* and to a nonkinetochore mutant carrying *chl12Δ::LEU2*, and heterozygous diploids were sporulated for tetrad analysis. As a control we crossed the *ctf13-30* and *ndc10-42* kinetochore mutants. Figure 5 shows the growth phenotypes of the haploid parents and the double mutants at 25 and 30°C. We analyzed 32 tetrads for the *spt4Δ2::HIS3 ctf13-30* cross and obtained 16 double mutants that formed small colonies after 5 to 6 days at 25°C. Half of the expected *spt4Δ2::HIS3 ctf13-30* double mutants (16 of 32) failed to form colonies at 25°C. Examination by light microscopy

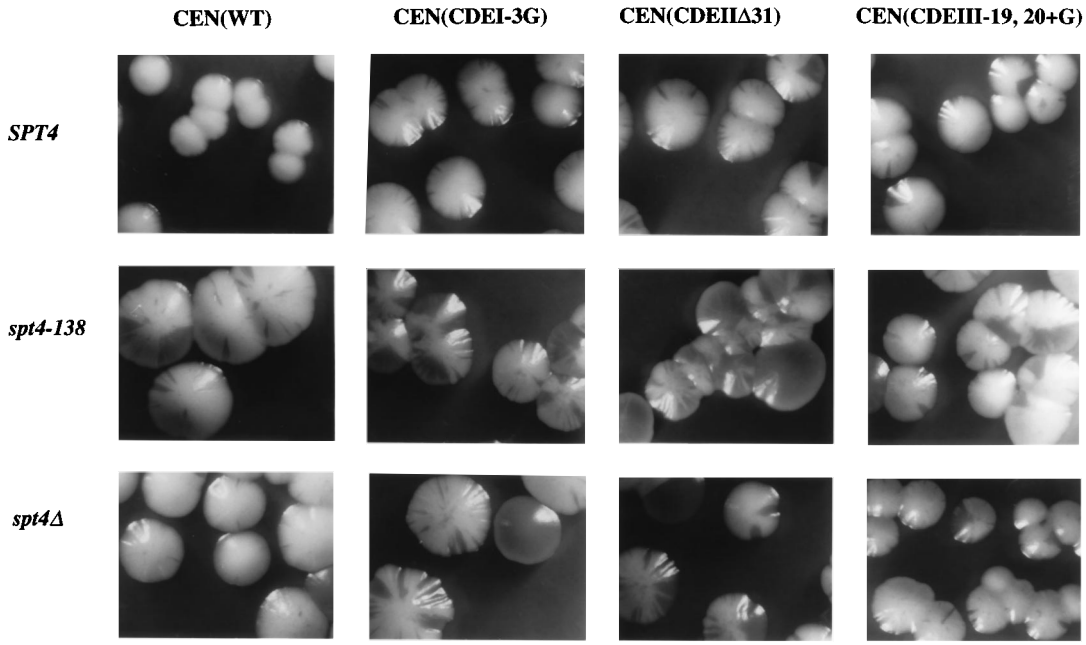

FIG. 4. *spt4* mutants show synergistic chromosome instability in combination with CEN DNA mutations. The stabilities of YACs containing either a wild-type CEN or a mutant CEN were determined by plating cells on SC with limiting adenine. Photographs were taken after incubation for 5 to 6 days at 25°C. The sectoring phenotypes of *SPT4*, *spt4-138*, and *spt4Δ2::HIS3* (*spt4Δ*) cells containing either a wild-type (WT) CEN (YAC from YPH656) or mutant CEN CDEI-3G (YAC from YVR26), CDEIIΔ31 (YAC from YPH1218), or CDEIII-19, 20+G (YAC from YPH 657) are shown. The dark sectors in the white colonies are due to loss of the *SUP11*-containing YAC. Two independent strains were tested for each YAC.

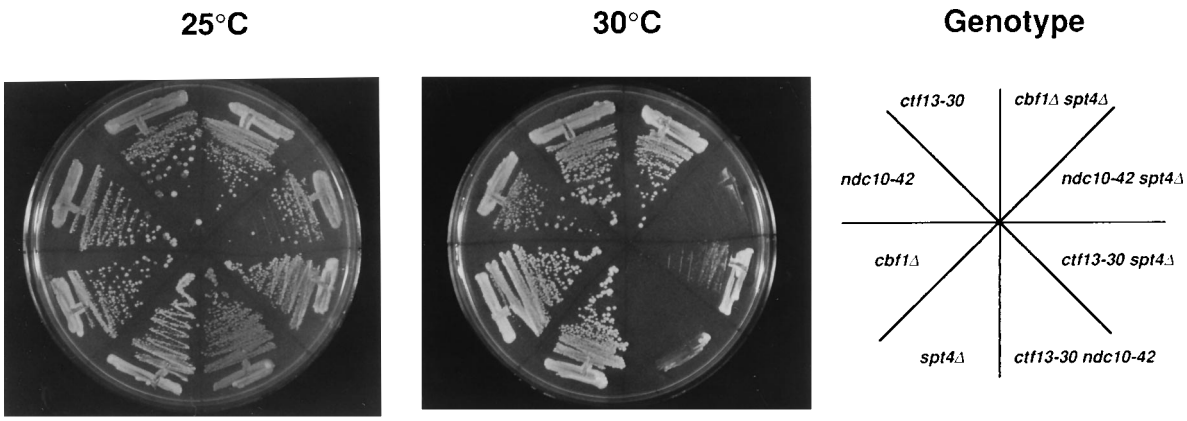

FIG. 5. *SPT4* genetically interacts with genes that encode components of the kinetochore. Growth of the haploid parents (*ctf13-30*, *ndc10-42*, *cbf1*Δ::*TRP1* [*cbf1*Δ], and *spt4*Δ*2*::*HIS3* [*spt4*Δ] strains) and the double mutants (*ctf13-30 ndc10-42*, *ctf13-30 spt4*Δ*2*::*HIS3*, *ndc10-42 spt4*Δ*2*::*HIS3*, and *cbf1*Δ::*TRP1 spt4*Δ*2*::*HIS3* strains) (Table 1) was determined by streaking the cells on SC medium. At least three independent mutants were tested for each genotype. Plates were photographed after 3 to 4 days of incubation at 25 or 30°C.

showed that the spores had germinated and divided two or three times. The *spt4*Δ*2*::*HIS3 ctf13-30* mutants that did form colonies at 25°C failed to do so at 30°C or developed very small colonies ($<10^6$ cells) after 3 days. We analyzed 20 tetrads for the *spt4*Δ*2*::*HIS3 ndc10-42* cross and obtained 18 *spt4*Δ*2*::*HIS3 ndc10-42* double mutants. All of the double mutants grew at 25°C and failed to grow at 30°C. We obtained 45 *spt4*Δ*2*::*HIS3 cbf1*Δ::*TRP1* double mutants from 46 tetrads. Although the double mutants grew at both 25 and 30°C, they formed smaller colonies than their haploid parents at 25°C (data not shown). *ctf13-30 ndc10-42* double mutants grew at 25°C and failed to grow at 30°C, similar to the case for the *spt4*Δ*2*::*HIS3 ndc10-42* mutants. Haploid parents of each cross grew at 25 and 30°C. In order to determine if the synthetic growth phenotype was a true genetic interaction between *spt4*Δ*2*::*HIS3* and the known kinetochore mutations, we examined the growth of *spt4*Δ*2*::*HIS3 chl12*Δ::*LEU2* spores (the *chl12* strain is a nonkinetochore mutant exhibiting very high rate of chromosome missegregation [33]). We analyzed seven tetrads and obtained four *spt4*Δ*2*::*HIS3 chl12*Δ::*LEU2* spores. All four *spt4*Δ*2*::*HIS3 chl12*Δ::*LEU2* spores showed no growth defects at both 25 and 30°C (data not shown). These results show that *SPT4* genetically interacts with kinetochore genes.

Spt4p is not a structural component of the CEN DNA-protein complex. In order to correlate the phenotypes of *cis*- and *trans*-acting mutations observed in vivo, we tested for specific biochemical defects in vitro. Extracts from *spt4-138* or *spt4*Δ*2*::*HIS3* cells grown at 25 or 37°C did not show any defects in gel mobility shift when assayed for binding to either a CDEI+II or CDEIII probe at 25°C (data not shown). Extracts made from *spt4*Δ*2*::*HIS3* cells grown at 25°C showed a modest decrease (to about 35 to 50% of the wild-type level) in binding to a CDEI+II or CDEIII probe when assayed at 38°C (data not shown).

The relative mobilities of the CEN DNA-protein complex were nearly the same for extracts from *spt4*Δ and *SPT4* cells (data not shown). In order to confirm the absence of Spt4p in the CEN DNA complex, we performed gel mobility shift experiments with tagged *SPT4* in the presence of a monoclonal antibody to the tag. Whole-cell extracts from *spt4*Δ*2*::*HIS3* strains expressing either HA-tagged *SPT4* or GST-tagged *SPT4* failed to show a supershift of the CBF1- or CBF3-specific gel mobility shifts in the presence of anti-HA or anti-GST monoclonal antibodies (data not shown). The positive control reactions of *cbf1*Δ::*TRP1* with *HACBF1* (for the CDEI+II probe) and *ctf13-30* with *HACTF13* (for the CDEIII probe) did show the expected supershifts with HA antibody. These results suggest that Spt4p is not a structural component of the CEN DNA-protein complex.

***spt4* chromatin extracts exhibit reduced microtubule binding to minichromosomes.** The gel mobility shift assays suggest that Spt4p is probably not a structural component of the CEN DNA-protein complex. Previous work suggests that kinetochore function can be perturbed by a failure either to form the core CEN DNA-protein complex or to assemble components that interact with the microtubules to ensure faithful chromosome segregation (31). To test the latter hypothesis, we used an assay developed previously to measure the ability of minichromosomes formed in vivo to bind to microtubules in vitro (30, 31). In this assay, cells containing minichromosomes are lysed, and the minichromosomes are isolated as chromatin in a clarified extract. Taxol-stabilized bovine microtubules are added to the extracts, and the binding of minichromosomes to microtubules is measured by the percentage of minichromosomes that cosediment with the microtubules. The sensitivity of this assay is improved by preparing lysates from cells staged in mitosis with nocadazole. Isogenic wild-type and *spt4* mutant strains were transformed with minichromosome pDK370 (*CEN3 ARS1 URA3*). Mitotic cultures of these strains were prepared with nocadazole (30, 31), and then minichromosomes in clarified extracts of these cultures were assayed for binding to the taxol-stabilized microtubules.

When minichromosomes were isolated from mitotic wild-type cells, approximately 40 to 50% of them quantitatively precipitated with taxol-stabilized bovine microtubules at saturating microtubule concentrations (Fig. 6). Approximately 5% of acentric minichromosomes will cosediment with microtubules under these conditions. These values are very similar to those reported previously for minichromosomes isolated from other nocadazole-arrested wild-type strains (30, 31). In contrast, only 15% of minichromosomes from *spt4-138* mitotic cells bound microtubules at saturating microtubule concentrations. A similar 2- to 2.5-fold difference in minichromosome binding to microtubules was observed in an independent experiment using wild-type and *spt4*Δ::*HIS3* strains (Fig. 6). Thus, for different alleles of *spt4*, the decrease in minichromosome binding to microtubules is small but reproducible. The intermediate value of minichromosomes bound to microtubules in

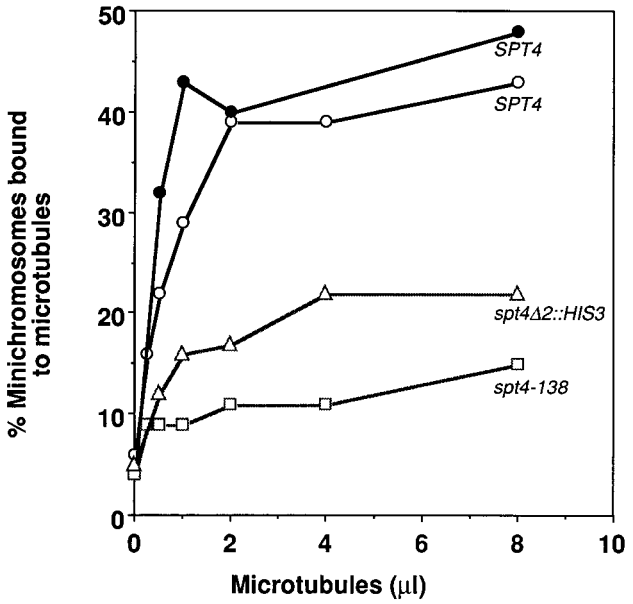

FIG. 6. *spt4* chromatin extracts exhibit reduced microtubule binding to minichromosomes. Cleared lysates were prepared from nocadazole-treated *SPT4*, *spt4-138*, and *spt4Δ2::HIS3* cells containing minichromosomes and grown at 23°C. Different amounts of bovine microtubules were added to the lysates and incubated for 15 min at 23°C. Microtubules were pelleted, and the percentage of minichromosomes that cosedimented with microtubules was determined (see Materials and Methods).

spt4 mutants (15 to 20%) compared with the values for centric (40 to 50%) and acentric (5%) minichromosomes in wild-type cells strongly suggest that in *spt4* mutants the centromere function is partially impaired. Further support for the relevance of this in vitro defect came from analysis of the minichromosome loss rate. Previous studies have shown a very good correlation between the in vivo minichromosome loss rate and the amount of centromere-dependent binding of minichromosomes to microtubules (30, 31). In particular, minichromosomes with specific CEN DNA mutations have intermediate values for both minichromosome loss rate and minichromosomes binding to microtubules, between those for wild-type and acentric minichromosomes. Therefore, we asked whether the minichromosome loss rate in *spt4* cells was intermediate between those for wild-type and acentric minichromosomes as indicated by the partial reduction in microtubule binding of minichromosomes in the two *spt4* strains. Indeed, we observed that the rate of loss of minichromosomes (per cell division) was 0.08 in *spt4* mutants compared with 0.01 in wild-type cells. Acentric minichromosomes have a loss rate of 0.34. The minichromosome loss rate in *spt4* mutants is intermediate between those observed for centric and acentric minichromosomes in wild-type cells, as expected given the partial centromere defect observed in the in vitro assay.

DISCUSSION

Our results indicate that Spt4p is an important determinant of chromosome transmission fidelity and strongly suggest that Spt4p plays an important role in kinetochore function. The *spt4-138* mutation was identified within the set of *ctf* mutants (isolated initially by the sole criterion of chromosome missegregation) (58) by screening for a secondary phenotype (CEN transcriptional readthrough) that was designed to monitor kinetochore integrity in vivo (13). The *spt4-138* point mutant, as well as the *spt4* deletion mutant, also exhibited additional phenotypes characteristic of previously studied known kinetochore mutants (13). The *spt4-138* and *spt4Δ* strains missegregate a chromosome fragment, are *ts* for growth at 37°C, show synthetic conditional phenotypes with the *ctf13-30* and *ndc10-42* kinetochore mutants, and exhibit synergistic chromosome instability in combination with mutant centromere DNA. Furthermore, minichromosomes purified from *spt4* mutants exhibit a defect in binding to microtubules in vitro, and protein extracts of an *spt4Δ* strain exhibit a modest *ts* defect for CEN DNA-protein complex formation in vitro.

Even though the *spt4-138* and *spt4Δ2::HIS3* mutants show similar phenotypes, we observed differences between the two mutants. For several of the assays it appears that the presence of the mutant protein in the *spt4-138* mutant results in a dominant interfering phenotype compared with its absence in the *spt4Δ* strain. First, the *spt4-138/spt4-138* homozygous diploids showed about a 100-fold-higher rate of chromosome missegregation than wild-type cells, whereas the *spt4Δ2::HIS3* homozygote showed only a 14-fold increase in the chromosome missegregation phenotype. Second, the in vitro microtubule binding activity is about twofold lower in the *spt4-138* mutant than in the *spt4Δ2::HIS3* strain. Finally, unlike the *spt4-138* mutant, the *spt4Δ2::HIS3* strain does not show a CEN transcriptional readthrough phenotype (data not shown). The *spt4-138* mutant phenotypes could be due to the mutation in the zinc finger of Spt4p which results in a defective interaction of Spt4p with either DNA or other proteins producing phenotypes more severe than a complete absence of the interaction.

The *spt* (suppressor of Ty) mutants, which fall into 20 complementation groups, were identified as extragenic suppressors of transcriptional defects caused by Ty or Ty delta element insertions in the 5′ regions of the *HIS4* and *LYS2* genes (16, 64). On the basis of the ability to suppress particular Ty insertions, the *SPT* genes can be placed in different groups. One of these groups includes the histone genes *HTA1* (*SPT11*) and *HTB1* (*SPT12*) as well as *spt4*, *spt5*, and *spt6* (21, 61, 63). In addition to suppressing Ty delta insertions, mutations in the histone group of *SPT* genes also suppress the defect in *SUC2* transcription caused by *snf2*, *snf5*, and *snf6* (*SNF* genes are required for transcriptional activation of the *SUC2* gene [26, 42, 61]). Genetic and biochemical experiments suggest that Spt4p, Spt5p, and Spt6p function in a complex (61). Our results show that both *spt4-138* and *spt6-140* mutants missegregate a chromosome fragment, suggesting that the two proteins Spt4p and Spt6p (possibly along with Spt5p) may also function in a complex to mediate their role in chromosome segregation.

Another group of *SPT* genes includes *SPT15*, which encodes the TATA-binding protein TBP, and the *SPT3* gene (63). The *spt3* mutant was also independently isolated in a screen designed for mutants defective in kinetochore structure (46). Studies with *SPT3* suggest that it has a role in the function of TBP (Spt15p) rather than a role in chromatin structure (14). In contrast, Spt6p, and by inference Spt4p and Spt5p, exert their effects on transcription via changes in chromatin structure (7a).

What is the relationship between the kinetochore-related phenotypes and the phenotypes previously described for *spt4* mutants, and what does this tell us about the role of Spt4p in chromosome transmission? The suppression of *snf/swi* mutations by (*hta1-htb1*)Δ and *spt6* has been shown to be due to a change in chromatin structure (7a, 26). Therefore, one possible explanation for the chromosome missegregation and other kinetochore-like phenotypes of *spt4-138* and *spt4Δ2::HIS3* could be that they are a result of altered chromatin structure at the centromere in the *spt4* mutants. The altered chromatin could affect the assembly or stability of the kinetochore complex that

assembles on the CEN DNA or of protein complexes required to achieve microtubule attachment. The synthetic conditional growth phenotypes of *spt4*Δ with known kinetochore mutations could reflect genetic interactions of *SPT4* with other components of the kinetochore. The effects of alteration in the nature and composition of the chromatin in an *spt4* mutant could be further accentuated by a mutation in the CEN DNA, thereby resulting in a synergistic instability of YACs containing mutant CENs. It is also possible that Spt4p alone or in combination with Spt5p and Spt6p is a structural or regulatory component of a specialized nucleosome at the yeast kinetochore. Several studies suggest that the yeast kinetochore may incorporate a modified nucleosome (7). It is known that CEN DNA mutations and histone H4 and H2B depletion affect chromosome segregation and alter the chromatin structure at the *S. cerevisiae* centromere (6, 12, 38, 52, 53). It has been proposed that the *S. cerevisiae* Cse4p protein (59), which shows homology to a family of histone H3-like proteins that includes the mammalian CENP-A protein, may be a component of a specialized nucleosome at the *S. cerevisiae* centromere (3, 59).

On the basis of the previously described phenotypes of *spt4*, *spt5*, and *spt6* (26, 61, 63) and the kinetochore phenotypes described in this paper, it is tempting to speculate that the Spt4p, Spt5p, and Spt6p proteins, along with other proteins, may mediate the establishment of a transcriptionally repressive state at the *S. cerevisiae* centromere. Such transcriptionally repressed regions, referred to as heterochromatin, remain highly condensed throughout the cell cycle (25). Hence, the chromosome missegregation phenotypes of *spt4* mutants could be due to a loss of heterochromatic character within the centromeric chromatin itself. Although no evidence for heterochromatin at the *S. cerevisiae* centromere currently exists, it is known that high levels of transcription towards the centromere perturb centromere function (24). Heterochromatin, however, is found at centromeres and telomeres of *Drosophila melanogaster* (62), mice (9), and *Schizosaccharomyces pombe* (1, 45) and within the mammalian inactive X chromosome (41). Whether heterochromatin itself contributes to centromere function is unknown. Recent studies with *S. pombe* suggest that heterochromatin at the centromere is important for centromere function. These studies showed that the *S. pombe swi6* gene product, a chromodomain protein, which is important for maintaining transcriptional repression at silent mating loci, is also associated with centromeres and telomeres (15). Studies with *S. pombe* have also shown that regions adjacent to the centromere are silenced and that mutations derepressing silent centromeric domains disrupt chromosome segregation, suggesting that heterochromatic structure is an absolute requirement for a fully functional centromere (1). Although centromere region-specific silencing has not been documented for *S. cerevisiae*, it would be worthwhile to examine whether *spt4*, *spt5*, or *spt6* mutants have an effect on the expression of genes immediately adjacent to centromeres.

It has been proposed that the functional kinetochore contains two domains: (i) a stable core of CEN DNA and CEN DNA-binding proteins and (ii) a microtubule binding domain (31). Microtubule-based motors have been implicated in the mechanisms of kinetochore-microtubule interaction in mammalian cells (27). In vitro assays suggest that the CBF3 complex is essential but not sufficient for microtubule binding activity of a yeast centromere-binding protein complex (56). With *spt4* mutants we observed a reduction in binding of minichromosomes to microtubules. This assay tests the ability of a complex preformed on the minichromosomes in vivo to mediate attachment to microtubules in vitro. Thus, the perturbed state is stably maintained during minichromosome purification, arguing against a direct effect of ongoing transcription in perturbing function. It is possible that Spt4p directly affects microtubule binding via changes in chromatin structure at the kinetochore.

The accompanying paper (21) reports the characterization of the human *SPT4* gene. So far only two proteins that are implicated in kinetochore function, namely, Mif2p (a putative centromere protein) and Cse4p (a putative modified histone at the yeast kinetochore), have been reported to show an evolutionary conservation from *S. cerevisiae* to humans (8, 34, 40, 59). Connelly and Hieter (11a) have recently characterized Skp1p, a 21-kDa component of CBF3 that is highly conserved throughout eukaryotic evolution. These studies suggest that detailed analysis of chromosome segregation in the experimentally tractable yeast *S. cerevisiae* will help us understand how chromosome segregation operates in higher eukaryotes. It is hoped that future experiments will lead to the identification of structural and regulatory components of the kinetochore and of cell cycle checkpoint components involved in monitoring the proper completion of chromosome segregation, which may eventually lead to the definition of the basic activities necessary for eukaryotic mitosis.

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