Mutation of *YCS4*, a Budding Yeast Condensin Subunit, Affects Mitotic and Nonmitotic Chromosome Behavior

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The budding yeast *YCS4* gene encodes a conserved regulatory subunit of the condensin complex. We isolated an allele of this gene in a screen for mutants defective in sister chromatid separation or segregation. The phenotype of the *ycs4-1* mutant is similar to topoisomerase II mutants and distinct from the *esp1-1* mutant: the topological resolution of sister chromatids is compromised in *ycs4-1* despite normal removal of cohesins from mitotic chromosomes. Consistent with a role in sister separation, *YCS4* function is required to localize DNA topoisomerase I and II to chromosomes. Unlike its homologs in *Xenopus* and fission yeast, Ycs4p is associated with chromatin throughout the cell cycle; the only change in localization occurs during anaphase when the protein is enriched at the nucleolus. This relocalization may reveal the specific challenge that segregation of the transcriptionally hyperactive, repetitive array of rDNA genes can present during mitosis. Indeed, segregation of the nucleolus is abnormal in *ycs4-1* at the nonpermissive temperature. Interrepeat recombination in the rDNA array is specifically elevated in *ycs4-1* at the permissive temperature, suggesting that the Ycs4p plays a role at the array aside from its segregation. Furthermore, *ycs4-1* is defective in silencing at the mating type loci at the permissive temperature. Taken together, our data suggest that there are mitotic as well as nonmitotic chromosomal abnormalities associated with loss of condensin function in budding yeast.

INTRODUCTION

Cell survival depends on the accurate transmission of a cell's genetic material to its daughters. Coordinating chromosome behavior with the cell cycle machinery ensures that the products of cell division are two viable and genetically identical progeny. Chromosomes replicate to produce two sister chromatids that are held together by topological and protein-mediated linkages. At the onset of mitosis, chromosomes condense into discrete bodies, converting the chromatids into physically strong, rod-shaped structures short enough to segregate away from each other. At anaphase, the protein and topological connections between sisters are resolved and they separate and segregate away from each other to opposite poles of the mitotic spindle. The anaphase

spindle in yeast is 10 μ m in length, implying that the longest chromosome arm (1Mb) must be compacted at least 60-fold relative to the length it would occupy as naked DNA to allow full segregation of chromosome arms.

The cohesin complex is required to hold sisters together (Guacci *et al.*, 1997; Michaelis *et al.*, 1997) (reviewed by Biggins and Murray, 1999; Nasmyth *et al.*, 2000). It consists of two coiled-coil ATPases, Smc1p and Smc3p, and additional regulatory subunits (Guacci *et al.*, 1997; Michaelis *et al.*, 1997; Losada *et al.*, 1998; Toth *et al.*, 1999; Tomonaga *et al.*, 2000); these proteins are loaded onto replicating chromosomes (Uhlmann and Nasmyth, 1998; Toth *et al.*, 1999). In budding yeast, a proteolytic cascade results in sister separation at anaphase. The anaphase-promoting complex mediates destruction of securin (Pds1p) (Cohen-Fix *et al.*, 1996), an inhibitor of a highly specific protease, separase (Esp1p) (Ciosk *et al.*, 1998; Uhlmann *et al.*, 2000). Esp1p cleaves a cohesin subunit, Mcd1p/Scclp, driving the removal of the complex from the chromosomes and sister chromatid separation (Uhlmann *et al.*, 1999). The topological linkage between sisters is also formed during S phase, most likely as a consequence of the collisions between replication forks that

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terminate DNA synthesis (Sundin and Varshavsky, 1980; Sundin and Varshavsky, 1981). At anaphase, DNA topoisomerase (TOP) II enzyme resolves these intertwinings so sisters can fully separate from each other (DiNardo *et al.*, 1984; Holm *et al.*, 1985; Uemura *et al.*, 1987; Shamu and Murray, 1992).

The condensin complex induces mitotic chromosome condensation. Like the cohesins, the condensin complex is composed of two coiled-coil ATPases of the SMC family, Smc2p and Smc4p, and three regulatory subunits, although the latter show no obvious homology between cohesins and condensins (Hirano, 1999). The condensins were isolated biochemically from *Xenopus* egg extracts, are required for mitotic chromosome condensation (Hirano and Mitchison, 1994; Hirano *et al.*, 1997; Cubizolles *et al.*, 1998), and can form loops in DNA molecules in vitro (Kimura and Hirano, 1997; Kimura *et al.*, 1999). The idea that condensins accomplish condensation by the active reconfiguration of chromatin conforms with observations that condensation requires ATP hydrolysis (Kimura and Hirano, 1997) and that members of the SMC family have predicted secondary structures resembling motor proteins that convert chemical energy into movement (Strunnikov *et al.*, 1993; Hirano and Mitchison, 1994).

Experiments in budding and fission yeasts support a role for the condensin complex in chromosome condensation and provide additional insights into their contribution to chromosome segregation (Saka *et al.*, 1994; Strunnikov *et al.*, 1995; Sutani *et al.*, 1999; Freeman *et al.*, 2000; Lavoie *et al.*, 2000; Ouspenski *et al.*, 2000). The fission yeast complex can anneal single-stranded DNA, an activity that may contribute to higher ordered supercoiling consistent with condensation (Sutani and Yanagida, 1997). In *Saccharomyces cerevisiae*, reducing condensin function impairs transmission of the rDNA (Freeman *et al.*, 2000). Recently, the essential role the three non-SMC subunits play has been illustrated in both *Schizosaccharomyces pombe* and *S. cerevisiae* (Sutani *et al.*, 1999; Freeman *et al.*, 2000). One of these subunits, YCS4, is the *S. cerevisiae* homolog of the *Xenopus* XCAPD2 (Kimura *et al.*, 1998) and the *S. pombe* CND1 (Sutani *et al.*, 1999). We isolated a mutant of YCS4 in a screen for defects in chromosome separation or segregation. Our analysis reveals role for the condensins in sister chromatid separation and the recruitment of core chromosomal proteins such as topoisomerases. Interestingly, *ycs4-1* expresses information from the silent mating type loci, whose transcription is normally repressed by the action of a number of chromosomal proteins.

MATERIALS AND METHODS

Microbial Techniques and Yeast Strain Construction

Media and genetic and microbial techniques were essentially as described (Sherman *et al.*, 1974; Rose *et al.*, 1990). All cytological experiments were carried out by arresting cells in 1 μ g/ml α -factor at the permissive temperature (23°C) for 4 h, washing cells twice in prewarmed α -factor-free media, and resuspending them in media at the nonpermissive (37°C) temperature. After 1 h, α -factor was added back to the media to prevent cells from entering the next cell cycle. All experiments were repeated at least twice with similar results. In all experiments, at least 100 cells for each time point were counted. Stock solutions of inhibitors were 60 mg/ml benomyl (DuPont, Boston, MA), 10 mg/ml nocodazole (Sigma, St. Louis, MO), and 10 mg/ml α -factor (Biosynthesis, Lewisville, TX), all in

dimethyl sulfoxide. All stocks were stored at -20°C. For benomyl/nocodazole experiments, cells were released into media with 30 μ g/ml benomyl and 15 μ g/ml nocodazole at 37°C. The strain DH5 α was used for all bacterial manipulations.

Yeast strains are listed in Table 1. Yeast strains were constructed by standard genetic techniques. Diploids were isolated on selective media at 23°C and subsequently sporulated at 23°C. The *pGAL- Δ 176-CLB2* fusion that is contained in some strains is not expressed in dextrose media. The *HML* locus was deleted by integrating pJR826 (gift of J. Rine, University of California, Berkeley) and verifying the deletion by polymerase chain reaction (PCR). The marking of the arm of chromosome IV was accomplished by integrating pAFS163 (gift of A. Straight, University of California, San Francisco) at intergenic region 1100000-1102221 of chromosome IV into a strain containing only the *pCUP1-GFP12-LacI12::HIS3* fusion; microscopy verified the integration of the Lac operator repeats. A strain containing the epitope-tagged allele YCS4-3XHA was created by PCR integration. Primers LOC7-3 (5' GTC/ACT/GCA/TTA/TTG/GAG/CAA/GGT/TTC/CAA/GGT/TGT/ATC/CGC/AAA/AGA/AAG/GGA/ACA/AAA/GCT/GG 3') and LOC7-4 (5' TAA/TAA/CAT/ATA/ATA/TAA/AAC/GGA/AGA/AAC/GGG/TAA/ACG/TCA/GTT/CGA/TTA/CTA/TAG/GGC/GAA/TTG/G 3') were used to PCR amplify DNA from plasmid pMPY-3XHA (Schneider *et al.*, 1995; gift of R. Kulberg, University of California, San Francisco), which was integrated into SBY215 to create NBY302. YCS4-13Xmyc was also created by PCR integration. Primers LOC7-10 (5' GAC/GTC/ACT/GCA/TTA/TTG/GAG/CAA/GGT/TTC/AAG/GTT/GTA/TCC/GCA/AAA/GAA/CGG/ATC/CCC/GGG/TTA/ATT/AA3') and LOC7-8 (5' ATA/TAA/TAA/CAT/ATA/ATA/TAA/AAC/GGA/AGA/AAC/GGG/TAA/ACG/TCA/GTT/CGA/GAA/TTC/GAG/CTC/GTT/TAA/AC 3') were used to PCR amplify DNA from pFA6a-13Myc-kanMX6 (Longtine *et al.*, 1998), which was integrated into NBY8 to create NBY333. Strains containing TOP2-3XHA:HIS3 and *pGAL-TOP2-3XHA:LEU2* were a gift of C. Cuomo (University of California, San Francisco). TOP1-3XHA was created by PCR amplifying DNA from pCA6-3HA-His3MX6 (Longtine *et al.*, 1998) by using primers TOP1-1 (5' ATA/AAA/AAA/ATC/TAA/AGG/GAG/GGC/AGA/GCT/CGA/AAC/TTG/AAA/CGC/GTA/AAA/CGG/ATC/CCC/GGG/TTA/ATT/AA 3') and TOP1-2 (5' AAC/TTG/ATG/CGT/GAA/TGT/ATT/TGC/TTC/TCC/CCT/ATG/CTG/CGT/TTC/TTT/GCG/GAA/TTC/GAG/CTC/GTT/TAA/AC 3') and integrating the product into NBY8. TOP1 was deleted by PCR integration by using primers TOP1-2 and TOP1-3 (5' AGA/GAA/AAA/TTT/AAA/TGG/GCC/ATA/GAA/TCG/GTA/GAT/GAA/AAT/TGG/AGG/TTT/CGG/ATC/CCC/GGG/TTA/ATT/AA 3') to PCR amplify DNA from pFA6-kanMX6 (Longtine *et al.*, 1998), which was integrated into NBY8.

Plasmid Construction

DNA encoding only the YCS4 gene (plus 500 base pairs upstream, presumably containing the endogenous promoter) was PCR amplified by using primers LOC7-1 (5' GCG/CGC/GGA/TCC/CGC/GTT/GTT/TTC/TTG/TCG 3') and LOC7-2 (5' GCG/CGC/GGC/CGC/GGG/TAA/ACG/TCA/GTT/CGA 3') that had *Bam*HI and *Not*I sites engineered at the 5' and 3' ends, respectively. The PCR product was digested with *Bam*HI and *Not*I and ligated into the centromeric vector pRS316 (Sikorski and Hieter, 1989) to create pNB27, which complemented the *loc7* ts phenotype. pSB10 was constructed by digesting pAFS78 (gift of A. Straight) with *Bam*HI and ligating the 1-kb *lacI* gene into pGEX-2T digested with *Bam*HI to create a GST-*lacI* fusion protein. pSB14 was constructed by ligating the 1-kb *lacI* *Bam*HI fragment from pAFS78 into the pQE-9 vector to generate 6HIS-*lacI*.

Isolation and Identification of YCS4

The LOC screen was performed on the mutagenized parent strain SBY215 and the details of the screen are published elsewhere (Big-

Table 1. Strains used in this study

All strain are isogenic with the W303 strain background. Plasmids are indicated in brackets.

Strain	Genotype
SBY215	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2</i>
SBY376	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-lacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 MCD1:3XHA:URA3:3XHA</i>
NBY8	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ</i>
NBY92	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 top2-4</i>
NBY113	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 mad2Δ::URA3</i>
NBY241	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 hmlΔ::LEU2 ycs4-1</i>
NBY258	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 mad2Δ::URA3 top2-4</i>
NBY259	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ armIV::LacO::URA3 top2-4</i>
NBY275	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 hmlΔ::LEU2 mad2Δ::URA3 ycs4-1</i>
NBY284	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-lacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 MCD1:3XHA:URA3:3XHA hmlΔ::LEU2 ycs4-1</i>
NBY290	<i>MATα ura3-1 leu2,3-112 his3-11::pCUP1-GFP12-lacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ hmlΔ::LEU2 ycs4-1</i>
NBY291	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ armIV::LacO::URA3</i>
NBY292	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ telIV::LacO::LEU2</i>
NBY302	<i>MATa ura3-1 leu2,3-112 his3-11::pCUP1-GFP12-lacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ::pGAL-Δ176-CLB2::LYS2 YCS4:3XHA:URA3:3XHA</i>
NBY316	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ ycs4-1</i>
NBY319	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ hmlΔ::LEU2 ycs4-1</i>
NBY322	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ TOP2-3XHA::HIS3 hmlΔ::LEU2 ycs4-1</i>
NBY323	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ telIV::LacO::LEU2 hmlΔ::LEU2 ycs4-1</i>
NBY327	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ armIV::LacO::URA3 hmlΔ::LEU2 ycs4-1</i>
NBY333	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ YCS4-13Xmyc::KAN</i>
NBY374	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ ADH4::mURA3::tel ppr1Δ::LYS2</i>
NBY377	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ ADH4::mURA3::tel ppr1Δ::LYS2 ycs4-1</i>
NBY455	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ telIV::LacO::LEU2 top2-4</i>
NBY474	<i>MATa ura3-1 leu2-3,112 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ TOP2-3XHA::HIS3</i>
NBY479	<i>MATa ura3-1 leu2-3,112::pGAL-TOP2-3XHA::LEU2 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ hmlΔ::LEU2 ycs4-1</i>
NBY480	<i>MATa ura3-1 leu2-3,112::pGAL-TOP2-3XHA::LEU2 his3-11::pCUP1-GFP12-LacI12::HIS3 trp1-1::LacO::TRP1 ade2-1 can1-100 bar1Δ lys2Δ hmlΔ::LEU2</i>
NBY496	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ TOP1-3XHA::HIS3 hmlΔ::LEU2</i>
NBY498	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ TOP1-3XHA::HIS3 hmlΔ::LEU2 ycs4-1</i>
NBY507	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ YCS4-13Xmyc::KAN {pRDN-URA3}</i>
NBY508	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ YCS4-13Xmyc::KAN rdnΔ {pRDN-URA3}</i>
NBY513	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3</i>
NBY514	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3 top1Δ::KAN top2-4</i>
NBY515	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3 top1Δ::KAN</i>
NBY516	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3 top2-4</i>
NBY518	<i>MATα ura3-1 leu2ΔBstEII::URA3-HOCs::leu2ΔEcoRI his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ top1Δ::KAN top2-4</i>
NBY519	<i>MATα ura3-1 leu2ΔBstEII::URA3-HOCs::leu2ΔEcoRI his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ top1Δ::KAN</i>
NBY520	<i>MATα ura3-1 leu2ΔBstEII::URA3-HOCs::leu2ΔEcoRI his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ top2-4</i>
NBY521	<i>MATα ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3 ycs4-1</i>
NBY522	<i>MATα ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ RDN::URA3</i>
NBY523	<i>MATα ura3-1 leu2ΔBstEII::URA3-HOCs::leu2ΔEcoRI his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ ycs4-1</i>
NBY524	<i>MATα ura3-1 leu2ΔBstEII::URA3-HOCs::leu2ΔEcoRI his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ</i>
NBY585	<i>MATa ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1Δ lys2Δ hmlΔ::LEU2</i>

gins *et al.*, 2001). To confirm that YCS4 was linked to the *loc7* mutation, we performed linkage analysis. NBY302 containing URA3-marked YSC4-HA3 was crossed to NBY290 and the resulting

diploid was sporulated. Of 22 tetrads dissected, the URA3 marker always segregated away from the *loc7* ts phenotype. In addition, a centromeric plasmid (pNB27) containing only the PCR-amplified

YCS4 complemented the *loc7* temperature-sensitive mutation, further confirming that the *YCS4* gene corresponds to *LOC7*.

Generation of *lacI* Antibodies

LacI antibodies were generated against a GST-*lacI* fusion protein, pSB10, expressed and purified from bacteria. The protein was purified as described (Kellogg and Murray, 1995) and 0.5 mg of protein was injected into rabbits at BabCO (Berkeley, CA), followed by 100- μ g boosts. The antibodies were affinity purified by first coupling a 6HIS-*lacI* fusion protein, pSB14, expressed and purified from bacteria, to affi-gel as described (Kellogg and Murray, 1995). Antibodies were purified on the affinity column as described (Harlow and Lane, 1988) and subsequently dialyzed into phosphate-buffered saline.

Immunofluorescence and Microscopy

Microscopy was performed as described (Biggins *et al.*, 1999). CuSO_4 was added to a final concentration of 0.25–0.5 mM to all experiments to induce expression of the green fluorescent protein (GFP)-*LacI* fusion. Immunofluorescence was performed as described (Rose *et al.*, 1990). Monoclonal 9E10 anti-myc (BabCO) and rabbit polyclonal anti-myc (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies were preincubated with an untagged spheroplasted strain two times for 10 min each at 23°C and used at 1:1000 dilution. Anti-Nop1 antibodies were kindly provided by J.P. Aris (University of Florida School of Medicine) and used at 1:5000 dilution. Anti-tubulin antibodies, vol 1/34, (Accurate Chemical & Scientific, Westbury, NY) were used at 1:1000 dilution. 4,6-Diamidino-2-phenylindole (DAPI; Molecular Probes, Eugene, OR) was used at 1 μ g/ml final concentration. Chromosome spreads were performed as described (Michaelis *et al.*, 1997; Loidl *et al.*, 1998). Monoclonal 16B12 anti-hemagglutinin (HA) antibodies (BabCO) were similarly preincubated against an untagged strain and used at 1:1000 dilution for Mcd1-3XHap chromosome spreads and 1:500 for Top2-3XHap and Top1-3XHap spreads. Anti-*LacI* antibodies were used at a dilution of 1:200. Lipsol was obtained from Lip (Shipley, England).

Determining Mitotic Recombination Frequencies

The strains to assay mitotic recombination at the rDNA and the *LEU2* locus were kindly provided by R. Rothstein (Gangloff *et al.*, 1996; Smith and Rothstein, 1999). They were crossed to the appropriate mutant and sporulated to isolate a spore that contained both the mutant allele and the construct to assay recombination. Because we are working with known and hypothesized hyperrecombinant mutants, we maintained the identified spores on –URA media to ensure that the starting colony for the experiment had not already recombined out the marker. Single colonies were inoculated into YPD and allowed to grow until midlog phase. Cultures were then diluted and plated onto YPD solid media. After growth, colonies were counted and the plates replica-plated to –URA solid media. Recombination frequencies were calculated by counting the number of colonies that failed to grow on –URA and dividing that number by the total number of colonies that grew on YPD.

Fluorescence In Situ Hybridization

In situ hybridization was performed as described (Dernburg and Sedat, 1998). The digoxigenin-labeled rDNA probe was a gift of A. Rudner (University of California, San Francisco). Rhodamine-conjugated anti-digoxigenin antibodies (Roche Molecular Biochemicals, Mannheim, Germany) were used at 1:500 dilution. Z-stacks were taken spanning ~ 4 μ m and the optical sections converted into a stacked image with MetaMorph software (Universal Imaging, West Chester, PA).

RESULTS

Sister Chromatid Separation in *ycs4-1*

We generated a temperature-sensitive collection of mutants and visually screened them for defects in mitotic chromosome behavior. Chromosomes were marked with GFP: an array of Lac operator repeats was integrated at the *TRP1* locus (~ 12 kb away from the centromere of chromosome IV) in a strain that expressed a GFP-*Lac* repressor (GFP-*LacI*) fusion (Straight *et al.*, 1996). We isolated nine complementation groups (*loc1*–*9*) that appeared defective in sister chromatid separation or segregation (Biggins *et al.*, 2001). *LOC7* was cloned by complementing the recessive temperature-sensitive phenotype and identified as hypothetical open reading frame YLR272C, the putative XCAPD2 homolog (Kimura *et al.*, 1998). Recent studies have verified that this gene is a regulatory subunit of the condensin complex and it has been named *YCS4* (Freeman *et al.*, 2000).

We used GFP-marked chromosomes to analyze sister chromatid separation in the *ycs4-1* mutant (Figure 1A). We constructed strains that combined the mutant or wild-type copy of the gene with the Lac operator array integrated near the centromere (at the *TRP1* locus), on the arm, or at the telomere of chromosome IV. Cells were arrested in G1 by treating them with α -factor at the permissive (23°C) temperature and released into media at the nonpermissive temperature (37°C) in the absence of α -factor. Figure 1A shows that in wild-type cells, sister chromatid separation began 80 min after release from G1 and was complete by 120 min. As in wild-type, *ycs4-1* cells began sister separation at 80 min, indicating that the onset of anaphase was normal, but only a fraction of the cells had managed to separate their sisters by 120 min. As the position of the Lac operator array was further from the centromere, the defect became more pronounced: sister separation at the *TRP1* locus occurred in 77% of the cells, whereas only 49% of the cells managed to separate the arms of sister chromatids and 29% the telomeres. The phenotype of *ycs4-1* is reminiscent of that of the *top2-4* mutant, in which the inability to decatenate sister chromatids presents a topological block to sister separation (DiNardo *et al.*, 1984; Holm *et al.*, 1985). In *top2-4*, spindle forces acting at the centromeres pull sisters apart, resulting in chromosome loss and breakage that lead to cell death (Uemura *et al.*, 1987; Holm *et al.*, 1989). We compared the phenotypes of the two mutants and found that although they are qualitatively similar, *top2-4* exhibits more severe sister separation defects than *ycs4-1*, particularly at the arm and telomere of chromosome IV (Figure 1A).

To determine whether the sister chromatid separation in *ycs4-1* was a product of spindle forces, we analyzed chromosome separation in the absence of a spindle. This experiment must be performed in spindle checkpoint mutants because wild-type cells activate the checkpoint to prevent cells from separating their sister chromatids in the absence of microtubules. *mad* and *bub* mutants inactivate this checkpoint (Hoyt *et al.*, 1991; Li and Murray, 1991), allowing activation of the anaphase-promoting complex in the absence of a spindle. Under these conditions, sister chromatids diffuse apart from each other without the aid of microtubules (Straight *et al.*, 1996; Marshall *et al.*, 1997; Straight *et al.*, 1997). If sister separation in *ycs4-1* requires microtubule-dependent forces, a *ycs4-1mad2 Δ* double mutant should not

Wild-type (SBY215), *ycs4-1* (NBY241), *top2-4* (NBY92), *mad2Δ* (NBY113), *ycs4-1mad2Δ* (NBY275), and *top2-4mad2Δ* (NBY258) strains were released from α -factor arrest ($t = 0$) into media with benomyl and nocodazole at the nonpermissive temperature (37°C); all strains contained the Lac operator array at the *TRP1* locus of chromosome IV. After 1 h, α -factor was added back to the media to prevent cells from entering the next cell cycle. Sister chromatid separation was scored over time. In the absence of microtubules, sister chromatid separation is delayed in *ycs4-1*.

separate sisters in nocodazole to the degree that a *mad2Δ* mutant would.

Wild-type, *ycs4-1*, *top2-4*, *mad2Δ*, *ycs4-1mad2Δ*, and *top2-4mad2Δ* strains were arrested in G1 in medium with α -factor at 23°C ; all strains carried the Lac operator array at the *TRP1* locus of chromosome IV. We released them into medium containing nocodazole and benomyl at 37°C . Figure 1B shows that wild-type and the *top2-4* and *ycs4-1* single mutants activated the spindle checkpoint and arrested in metaphase with unseparated sister chromatids. The *mad2Δ* single mutant bypassed the checkpoint, continued cycling in the absence of a spindle and separated sister chromatids in 60% of the cells within 2 h after release from G1. We believe that the sisters were separated in the remainder of the cells, but lie too close to each other to be resolved by the light microscope. Even in the absence of the checkpoint, sister separation was strongly inhibited in *top2-4mad2Δ*. The *ycs4-1* mutant showed an intermediate phenotype. In the absence of microtubules, the *ycs4-1mad2Δ* double mutant separated its sisters but did so more slowly than the *mad2Δ*. Two hours after release from G1, the double mutant separated sisters in only 24% of its cells and required an additional hour and a half to achieve sister separation comparable to that of *mad2Δ* cells at 2 h after release. Therefore, in the absence of spindle forces, the resolution of sister chromatids is compromised in

ycs4-1. The slow sister chromatid separation we observe in *ycs4-1* in the absence of microtubules is dependent upon topoisomerase II activity, as a *ycs4-1top2-4mad2Δ* triple mutant in nocodazole does not separate its sister chromatids (our unpublished results).

Cohesins Are Loaded onto and Removed from Chromosome Spreads in *ycs4-1* as in Wild Type

Sister separation mutants fall into two classes; these are defined by *esp1-1*, which cannot remove cohesins from chromosomes (Ciosk *et al.*, 1998), and *top2-4*, which removes cohesins normally (our unpublished results). We classified *ycs4-1* by monitoring the loading and removal of a cohesin subunit, Mcd1p/Scclp (Guacci *et al.*, 1997; Michaelis *et al.*, 1997) as cells passed through mitosis. Wild-type and *ycs4-1* strains with an epitope-tagged *MCD1/SCC1* gene were arrested in G1 with α -factor at 23°C and released into media at 37°C . Samples were treated with detergent and fixative simultaneously to remove soluble nuclear proteins and retain chromatin-associated proteins, which were then visualized by indirect immunofluorescence. Figure 2 illustrates that the association of Mcd1p/Scclp with chromatin in *ycs4-1* is qualitatively and quantitatively indistinguishable from wild type. The staining pattern and the kinetics of chromatin

Figure 1. YCS4 is required for sister chromatid separation. (A) Sister chromatid separation phenotypes of *ycs4-1* and *top2-4* are similar. Wild-type, *ycs4-1*, and *top2-4* cells released from α -factor arrest ($t = 0$) into media at the nonpermissive temperature (37°C) were scored for sister separation by microscopy at three loci along chromosome IV over time. After 1 h, α -factor was added back to the media to prevent cells from entering the next cell cycle. Strains contained Lac operator repeats integrated at the *TRP1* locus near the centromere (cen-proximal) (wild-type, SBY215, *ycs4-1*, NBY241, *top2-4*, NBY92), on the arm (wild-type, NBY291, *ycs4-1*, NBY327, *top2-4*, NBY259) and at the telomere (wild-type, NBY292, *ycs4-1*, NBY323, *top2-4*, NBY455) of the chromosome. Although sister chromatid separation is normal at all three loci in wild type, the *ycs4-1* mutants, like the *top2-4* mutants, show differential ability to separate loci based on the proximity to the centromere. (B) In *ycs4-1*, sister chromatid separation is compromised in the absence of spindle forces.

Figure 2. Cohesin binding and displacement is normal in *ycs4-1*. (A) Cohesin subunit Mcd1p/Scclp was localized by indirect immunofluorescence on chromosome spreads in wild-type (SBY376) and *ycs4-1* (NBY284) strains containing 3XHA-epitope tagged Mcd1p/Scclp. Samples were fixed and stained at the indicated times during a synchronous cell cycle at the nonpermissive temperature (37°C). DNA staining (DAPI) is shown in the left panels, anti-LacI antibody staining is shown in the middle panels, and anti-HA antibody staining is shown in the right panels. G1, S phase, and anaphase spreads prepared from wild-type (top) and *ycs4-1* (bottom) cells are shown. Mcd1p is absent from G1 and anaphase spreads but is present on spreads from cells in S phase in both wild-type and *ycs4-1*. Bar, 10 μ m. (B) Quantified results of Mcd1p localization to chromosomes. The percentage of chromosomes with Mcd1p localized to chromosome spreads is represented versus time for wild type (SBY376) and *ycs4-1* (NBY284).

association and dissociation of Mcd1p/Scclp are the same in the two strains. Thus, sister chromatid separation in *ycs4-1* mutants is defective despite the removal of cohesins from chromosomes in anaphase. The similarity to the phenotype of *top2-4* suggests that the condensin complex, which contains Ycs4p, may be required for the rapid resolution of the topological linkage between sister chromatids; alternatively, the condensins may be responsible for the abolition of a previously unsuspected, cohesin-independent, proteinaceous linkage.

YCS4 Regulates Localization of Topoisomerase I and II

Because the lack of YCS4 function results in a *top2-4*-like phenotype, we asked whether YCS4 function was required to localize topoisomerase II. YCS4 and *ycs4-1* strains that contained epitope-tagged TOP2 were arrested in G1 at 23°C and released into fresh media at 37°C. Images of the chromosome spreads are shown in Figure 3A and the data are quantified in Figure 3B. Wild-type nuclei maintained a punctate Top2p association throughout the cell cycle (Figure 3, A and B). However, more than half of the *ycs4-1* nuclei lost their Top2p staining within 30 min of the temperature shift to 37°C (Figure 3, A and B). Immunoblotting of cell lysates verified that the Top2p protein was still present in *ycs4-1* despite the loss of the protein from chromosome spreads (our unpublished results). We also observed a loss of topoisomerase I from *ycs4-1* chromosome spreads at the nonpermissive temperature (Figure 3C). The pattern of topoisomerase I staining on chromosome spreads is similar to that of topoisomerase II staining, punctate and coincident with DNA staining (our unpublished results).

However, the *ycs4-1* phenotype cannot be fully explained by the loss of topoisomerase II from chromosomes. Overex-

pression of Top2p does not suppress the temperature sensitivity or sister separation phenotype of *ycs4-1* despite restoration of Top2p to chromosomes as visualized by chromosome spreads (our unpublished results). A simple interpretation of this failure is that the condensin complex has general effects on mitotic chromosome structure and that Top2 is only one of several proteins whose chromosomal localization and function has been compromised.

Ycs4p Is Nuclear throughout Cell Cycle and Is Enriched at rDNA at Anaphase

To gain a greater understanding of YCS4's role in mitotic chromosome behavior, we localized Ycs4p by indirect immunofluorescence on both whole cells (Figure 4) and chromosome spreads (our unpublished results). In frogs and fission yeast, the condensin complex is only associated with chromatin or in the nucleus during mitosis (Hirano *et al.*, 1997; Sutani *et al.*, 1999). Our experiments reveal that Ycs4p was present in the nucleus (Figure 4, A and B) and associated with chromatin (our unpublished results) throughout the budding yeast cell cycle. This is consistent with the findings of Freeman *et al.* (2000). The only observable shift in localization occurred at anaphase when a general staining of the nucleus was replaced by specific staining of the nucleolus (detected by the nucleolar marker Nop1p; Aris and Blobel, 1988) (Figure 4C); cells arrested in metaphase by overexpression of Mps1p did not exhibit this subnuclear localization (our unpublished results). In cells in which the chromosomal rDNA had been deleted and replaced with a single copy of the repeat on a 2- μ plasmid (*rdn Δ*) (Nierras *et al.*, 1997), there was no anaphase relocalization and Ycs4p was diffusely nuclear throughout the cell cycle (Figure 4D). This effect is not due to the presence of the plasmid-borne

Figure 3. DNA topoisomerases I and II are absent from chromosomes in *ycs4-1*. (A) Top2p was localized by indirect immunofluorescence on chromosome spreads in wild-type (NBY474) and *ycs4-1* (NBY322) strains containing 3XHA-epitope tagged Top2p during a synchronous cell cycle at the nonpermissive temperature (37°C). Wild-type chromosome spreads are on the right and *ycs4-1* spreads are on the left; 0- and 30-min time points are shown. For each, DNA staining (DAPI) is shown on the left and anti-HA antibody staining is shown on the right. Wild-type spreads maintain the punctate Top2p. There is a dramatic loss of Top2p from chromosomes in *ycs4-1* spreads within 30 min of the shift to 37°C. Bar, 10 μ m. (B) Quantified results of Top2p localization. The percentage of chromosomes with Top2p versus time is represented for wild type (NBY474) and *ycs4-1* (NBY322). (C) Quantified results of Top1p localization. Top1p was localized by indirect immunofluorescence on chromosome spreads in wild-type (NBY496) and *ycs4-1* (NBY498) strains containing 3XHA-epitope tagged Top1p during a synchronous cell cycle at the nonpermissive temperature (37°C).

rDNA, because anaphase nucleolar enrichment is restored in a strain that contained the rDNA array on chromosome XII as well as the 2- μ plasmid (our unpublished results). Despite Ycs4p's variation from the behavior of the *Xenopus* and fission yeast condensin complexes, its localization supports a role for Ycs4p in chromosome structure and suggests a specialized role at the rDNA.

ycs4-1 Mutants Exhibit Defects in rDNA Condensation, Function, and Segregation

Because Ycs4p is not localized to the chromatin specifically during mitosis, we asked whether the protein is required for normal mitotic chromosome structure. We monitored mitotic condensation by fluorescent in situ hybridization by using probes against the highly repetitive rDNA array. Condensation defects are assayed at the rDNA primarily because of the ease of interpreting the fluorescence in situ hybridization signal. We arrested wild-type and *ycs4-1* cells in G1 with α -factor and released them into fresh media containing benomyl and nocodazole at 37°C to yield cells arrested in prometaphase. The loops, bars, and horseshoe shapes observed by in situ hybridization to the rDNA during mitosis have been interpreted as condensed rDNA, whereas an amorphous signal at the periphery of nucleus has been interpreted as decondensed rDNA. We saw the latter structure of the rDNA in 69% of the *ycs4-1* cells compared with the intact loops and crescents seen in 95% of wild-type cells in prometaphase (Figure 5, A and B). This observation suggests that YCS4 has a role in maintaining chromosome structure in mitosis and that its functions at the rDNA are not restricted to anaphase. We have not assayed

condensation at single copy sequences but other studies have illustrated the cell cycle dependence of the specific rDNA morphology associated with condensation and its correlation with condensation at single copy loci (Guacci *et al.*, 1994; Freeman *et al.*, 2000; Lavoie *et al.*, 2000).

We asked whether YCS4 plays a role in the stability of the rDNA locus. Topoisomerase I and II have been implicated in maintaining the stability of the rDNA array by suppressing mitotic recombination at the locus (Christman *et al.*, 1988; Kim and Wang, 1989). Because *ycs4-1* impairs topoisomerase I and II's association with chromosomes, we measured recombination within the rDNA array by measuring the loss of a *URA3* marker inserted into the rDNA locus (Gangloff *et al.*, 1996). Control strains, in which the *URA3* marker was integrated between a pair of direct repeats of the *LEU2* locus, were used to determine whether effects were specific for the rDNA locus (Smith and Rothstein, 1999). Table 2 illustrates the frequency of loss of the *URA3* marker in *top1 Δ* , *top2-4*, *top1 Δ top2-4* double, and *ycs4-1* mutants at both the rDNA and the *LEU2* locus. The single and double topoisomerase mutants showed higher rates of mitotic recombination at the rDNA locus (38-fold higher for *top1 Δ* and 83-fold higher for *top1 Δ top2-4*) than wild-type with substantial but smaller increases in recombination at the *LEU2* locus. *ycs4-1* cells grown at the permissive temperature had a much more specific defect: a 63-fold elevation in recombination at the rDNA locus with only a twofold increase in recombination at *LEU2*.

A requirement for the budding yeast condensin complex has been implicated in rDNA segregation during mitosis (Freeman *et al.*, 2000). We examined the segregation of the rDNA locus in synchronized cells passing through anaphase. In wild type, 90% of the cells have segregated the

Figure 4. Ycs4p is nuclear protein throughout the cell cycle and becomes enriched at the nucleolus at anaphase. Indirect immunofluorescence was performed on Ycs4p-13Xmyc in cells with the chromosomal rDNA array (NBY333) and without, *rdnΔ* (NBY508). DNA staining (DAPI) is shown in the panels in the first set of vertical panels, anti-myc antibody staining that recognized Ycs4p-13Xmyc in the second set, anti-Nop1p staining in the third set, and a merge of the Ycs4p and Nop1p staining in the final set of panels. Bar, 10 μ m.

Figure 5. YCS4 is required for mitotic condensation, stability, and segregation of rDNA. (A) The rDNA is decondensed in *ycs4-1* mutants. Fluorescent in situ hybridization with probes against the rDNA was performed on wild type (NBY8) and *ycs4-1* (NBY319) arrested in metaphase at the nonpermissive temperature (37°C). Compared with the loops present in wild-type rDNA, the rDNA in *ycs4-1* cells appears collapsed into an amorphous mass at the periphery of the DNA staining. Bar, 10 μ m. (B) Results of in situ hybridization were quantified. In 69% of *ycs4-1* cells arrested in metaphase, the rDNA appears decondensed, as compared with 4.5% of wild-type cells. (C) Segregation of the nucleolus is defective in *ycs4-1*. Indirect immunofluorescence for the nucleolar marker Nop1p was performed on wild-type (NBY8) and *ycs4-1* (NBY319) cells in anaphase (120 min after release from G1). The percentage of cells with segregated or unsegregated nucleoli was scored and graphed. Only 10% of wild-type cells in anaphase have not segregated their nucleolus; 45% of *ycs4-1* cells with elongated spindles still contain the nucleolus in the mother bud (as identified by the persistence of the α -factor induced shmoo). (D) Loss of nucleolar structure may inhibit its segregation. A wild-type (NBY8) cell is shown on the left and a *ycs4-1* mutant (NBY319) cell on the right. DNA staining (DAPI) is shown in the top pair of panels, anti-tubulin antibody staining in the middle, and anti-Nop1p antibody staining on the bottom. The wild-type cell has segregated its crescent-shaped nucleolus into each cell body. The nucleolus in the *ycs4-1* cell has lost its structure and is found in the mother cell body; the amorphous Nop1p-containing mass is typical of the 45% of cells with an unsegregated nucleolus. Bar, 10 μ m.

nucleolar marker Nop1p to both mother and bud, and only 10% of cells contained Nop1p only in the mother (identified by the pheromone-induced shmoo morphology) (Figure 5C). In *ycs4-1* cells undergoing anaphase, 45% of the cells exhibited Nop1p only in the mother (Figure 5C). Furthermore, these cells had a perturbed nucleolar structure: the nucleolus is not bar- or crescent-shaped as in wild type, but diffuse and amorphous, consistent with the in situ hybridization results (Figure 5D). The remaining 55% of the cells had segregated their nucleoli and exhibited normal Nop1p staining.

ycs4-1 Is Defective in Silencing of Silent Mating Type Locus

Initial attempts to arrest *ycs4-1* MATa cells in media containing α -factor at the permissive temperature failed. When

these cells were plated on YPD plates containing α -factor, they did not respond to the pheromone and continued to grow (Figure 6A). *ycs4-1*'s α -factor resistance was overcome when the silent mating locus *HML* α was deleted (Figure 6A), suggesting that the mutant was defective in silencing at the mating type loci. Silencing defects were not observed at the telomere at the permissive temperature (Figure 6B), and silencing at the rDNA could not be assayed because the integration of the reporter construct (Smith and Boeke, 1997) at the rDNA is synthetically lethal with the *ycs4-1* mutation (our unpublished results).

DISCUSSION

We have shown that YCS4, a regulatory subunit of the condensin complex, is required for accurate sister chromatid

Table 2. Mitotic frequency of marker loss

Recombination frequencies were determined as described in MATERIALS AND METHODS. Values are reported as the means and standard deviations and were determined on at least three independent trials for each genotype.

Genotype	<i>RDN::URA3</i>		<i>leu2::URA3::leu2</i>	
	Recombination frequency $\times 10^{-3}$	Fold increase	Recombination frequency $\times 10^{-3}$	Fold increase
wildtype	0.4 ± 0.3	1	0.3 ± 0.3	1
<i>top1</i> Δ	15 ± 3	38	5 ± 3	17
<i>top2-4</i>	1 ± 1	3	0.8 ± 0.2	3
<i>top1</i> Δ <i>top2-4</i>	33 ± 5	83	8 ± 4	27
<i>ycs4-1</i>	25 ± 4	63	0.5 ± 0.5	2

Figure 6. *ycs4-1* is defective in silencing at the silent mating type loci but not at the telomeres. (A) Deletion of the *HML α* locus suppresses *ycs4-1*'s growth on media containing α -factor. Serial dilutions of wild-type (NBY8), wild-type with the *HML α* silent mating type locus deleted (*hml Δ*) (NBY585), *ycs4-1* (NBY316), *ycs4-1* with the *HML α* silent mating type locus deleted (*hml Δ*) (NBY319) on YPD with and without 1 μ g/ml α -factor (all strains are *bar1 Δ*). Deletion of the *HML α* locus suppresses *ycs4-1*'s growth on media containing α -factor. (B) Silencing at the telomeres is unaffected by the *ycs4-1* mutation at the permissive temperature. Serial dilutions of wild type (NBY374) and *ycs4-1* (NBY377), both containing a *URA3* reporter construct at the telomere to assay silencing.

separation; the mutant phenotype resembles that of *top2-4*, suggesting that *ycs4-1* mutants have a topological block to sister separation. Consistent with the sister separation phenotype, Top2p and Top1p are absent from chromosome spreads prepared from *ycs4-1* cells at the nonpermissive temperature. Ycs4p is intimately associated with the array of rDNA genes on chromosome XII: the protein localizes to the nucleolus in anaphase cells, nucleolar structure and segregation are abnormal in *ycs4-1*, and interrepeat recombination in the rDNA array is specifically elevated in *ycs4-1*. The mutant exhibits defects in silencing at the silent mating type loci at the permissive temperature, suggesting that yeast condensins function at all stages of the cell cycle and influence processes other than mitotic chromosome condensation.

Condensin Function Is Required to Separate Sister Chromatids

The phenotype of *ycs4-1* resembles that of topoisomerase II mutants; sister chromatid separation becomes more defective as the distance from the centromere increases. In *top2-4*, the separation observed near the centromere requires microtubule-dependent forces and the inability to fully resolve the catenated sister chromatids leads to lethal events such as nondisjunction and chromosome breakage (Holm *et al.*, 1989). In *ycs4-1*, the sister chromatids have difficulty separating but this block can eventually be resolved, even in the absence of spindle forces. This observation may explain why chromosome loss phenotypes are difficult to detect in condensin mutants, especially given the small size of reporter constructs used in such assays (Hieter *et al.*, 1985; Spencer *et al.*, 1990). We suggest that condensins establish and maintain mitotic chromosome structure, which in turn facilitates the resolution of topological linkage between sister chromatids. In the absence of full condensin function, the decatenation, separation and proper segregation of sister chromatids are impaired, despite the normal timing of cohesin removal at anaphase.

Depending on the state of the substrate DNA, topoisomerase II can either catenate or decatenate DNA circular DNA molecules. Increasing DNA condensation favors decatenation, because two compact DNA molecules are less likely to collide with each other and become catenated than two extended DNA molecules (Holmes and Cozzarelli 2000). Thus, condensins could promote sister separation by affecting the amount or directionality of topoisomerase II activity. Studies on the bacterial SMC homolog *MukB* support the latter possibility (Sawitzke and Austin, 2000). Sawitzke and Austin (2000) found that the chromosome partitioning defects of the *mukB*, *mukE*, and *mukF* mutants in *Escherichia coli* were suppressed by mutations in the bacterial topoisomerase I gene *topA*. Reducing topoisomerase I activity allows DNA gyrase activity to increase the negative supercoiling of the nucleoid; in the absence of Muk function, this increased negative supercoiling provided a level of chromosome organization that allowed proper segregation of the nucleoid. In eukaryotes, it is possible that the action of the condensin complex contributes to the decatenation of sister chromatids by introducing the higher level organization typical of mitotic condensation (reviewed by Holmes and Cozzarelli, 2000).

Catenation of eukaryotic chromosomes is believed to arise as replication forks collide at the completion of DNA replication (Sundin and Varshavsky, 1980; Sundin and Varshavsky, 1981) and topoisomerase II activity is required during anaphase to allow sister chromatid separation (Holm *et al.*, 1985; Uemura *et al.*, 1987; Holm *et al.*, 1989; Shamu and Murray, 1992). What changes to favor decatenation at anaphase? We can exclude two obvious possibilities, microtubule-dependent forces and increased topoisomerase II activity. Sisters can separate in the absence of microtubules (Straight *et al.*, 1996; Straight *et al.*, 1997), and topoisomerase activity falls as *Xenopus* extracts enter anaphase (Shamu and Murray, 1992).

We suggest that the extent of chromosome condensation reflects a dynamic balance between the activities of cohesins

and condensins. We speculate that the complete removal of cohesins at anaphase allows condensins to induce further DNA compaction that makes anaphase chromosomes more condensed than metaphase ones. In this scenario, cohesins and condensins have opposing effects on chromosome condensation. This idea explains the relationship between cohesin behavior, topoisomerase activity, and chromosome condensation as vertebrate cells enter mitosis. Unlike budding yeast, most cohesin leaves vertebrate chromosomes as the cells enter mitosis, corresponding to an increase in chromosome condensation, which requires topoisomerase II activity. The removal of cohesin would allow condensin to increase chromosome compaction, thus driving topoisomerase II to remove topological linkages that would interfere with full chromosome condensation. Opposing roles of condensin and cohesin are not easily reconciled with the condensation defects observed in budding yeast cohesin mutants. We cannot exclude the possibility that there may be some collaboration between cohesin and condensin function in preparing condensed mitotic chromosomes for segregation in vertebrate cells.

Condensins Are Required to Localize Topoisomerase I and II

We found that the condensin complex is required to localize topoisomerase I and II to chromosomes. This observation differs from that of Hirano *et al.* (1997) who showed that immunodepletion of the condensin complex from *Xenopus* frog egg extracts did not affect the association of topoisomerase II with chromosomes. There are a number of differences between the experiments. First, the frog egg extract was made from cells in metaphase of meiosis II and yeast cells were studied in mitosis. Second, chromosomes in the egg extracts had not gone through replication. Third, there are large stockpiles of numerous essential proteins in the extract. A high concentration of topoisomerase II may allow condensin-independent binding to chromosomes. Indeed, we may be recapitulating such a scenario when we overexpress Top2p; under these conditions, Top2p binds to chromosomes despite defects in YCS4.

Studies on the Barren mutant in *Drosophila* suggested an interaction between the condensin complex and topoisomerase II. Barren is the fly counterpart of *Xenopus* XCAP-H, budding yeast *BRN1*, and fission yeast *CND2*. The fly protein colocalized, biochemically associated with, and enhanced the enzymatic activity of topoisomerase II (Bhat *et al.*, 1996). Attempts to recapitulate these findings in yeast and *Xenopus* have been unsuccessful (Hirano *et al.*, 1997; Lavoie *et al.*, 2000). Our investigations reveal that a relationship between the complex and topoisomerase II does exist; condensin function is required to localize the protein to chromosomes. However, we do not observe a biochemical interaction between Ycs4p and Top2p (our unpublished results), suggesting that yeast condensins stimulate topoisomerase binding indirectly.

Do condensins recruit other chromosomal proteins other than topoisomerases? The normal binding and displacement of Mcd1p/Scclp indicates that at least one protein binds normally in the absence of condensins. However, condensins may recruit additional chromatin-associated proteins required for mitotic chromosome behavior, some of which may collaborate with condensins to condense chro-

mosomes and drive sister chromosome separation and segregation.

Ycs4p Is Localized to Chromatin throughout Cell Cycle

The behavior of the budding yeast condensin complex differs from that of the complexes characterized in *Xenopus* egg extracts and fission yeast. In frogs, phosphorylation of a subset of the regulatory subunits by the mitotic Cdc2/Cyclin B complex controls the association of the complex with chromatin at mitosis (Hirano *et al.*, 1997) and activation of its supercoiling activity (Kimura and Hirano, 1997; Kimura *et al.*, 1998). The fission yeast complex is regulated by compartmentalization; nuclear import, and thus access to the chromatin, is limited to mitosis. Import depends on the phosphorylation of Cut3p, the SMC4 homolog, by the Cdc2/CyclinB complex (Sutani *et al.*, 1999). The *S. cerevisiae* complex, specifically Smc2p and 4p, associate with chromatin throughout the cell cycle; strikingly, the only change in localization occurs at prometaphase when Smc4p and Ycs5p, another condensin regulatory subunit, concentrate at the rDNA (Freeman *et al.*, 2000). We observe a similar dramatic shift in localization with Ycs4p. However, our analysis of the protein's localization indicates that its exclusive binding at the rDNA occurs only during anaphase; cells arrested in metaphase exhibit the nuclear and general chromatin localization observed in every other stage of the cell cycle. Could this shift in localization be a modification of the mitosis-specific chromatin association observed in fission yeast and *Xenopus*? Or does the nucleolar association we observe in anaphase indicate a budding yeast-specific requirement for condensin function in the decatenation, separation, and proper segregation of the chromosomal rDNA array?

Condensins Play a Special Role at Chromosomal rDNA Array

Freedman *et al.* recently illustrated a special role for the condensin complex at the rDNA array (Freeman *et al.* 2000). They provided evidence that strongly suggests that the complex is required for the mitotic transmission of rDNA. Herein, we show that the condensin complex affects the structure and stability of the chromosomal array as well as its segregation during mitosis. We observed three defects specific to the rDNA array. First, mitotic recombination at the rDNA array is increased 63-fold over wild type in the *ycs4-1* mutant at the permissive temperature. Second, integration of a reporter construct designed to assay transcriptional silencing at the rDNA is synthetically lethal with the *ycs4-1* mutation (our unpublished results). Third, the anaphase structure and segregation of the nucleolus is abnormal in *ycs4-1* cells. When we used Nop1p to visualize segregation of the rDNA array in *ycs4-1*, we saw two phenotypes. In 55% of cells, the nucleolus had segregated normally and had a normal condensed, crescent-shaped structure, whereas 45% of cells contained a single amorphous mass that stained with Nop1p antibodies and remained in the mother. We do not know whether defects in nucleolar structure lead to defects in nucleolar segregation or vice versa. The defects in nucleolar segregation in condensin mutants (Freeman *et al.*, 2000) suggest that nucleolar enrichment of condensin subunits during anaphase could be

an attempt of the cell to facilitate the separation and segregation of this heterochromatin-like locus (Bryk *et al.*, 1997; Fritze *et al.*, 1997; Smith and Boeke, 1997).

The rDNA differs from the remainder of the genome in two ways: it is present as a large array of tandem repeats, and a fraction of the repeats is transcribed at very high rates. Transcription produces topological effects that may interfere with proper chromosome segregation. Plant and animal cells deal with this problem by shutting down transcription during mitosis, but in budding yeast, transcription continues during mitosis, which can occupy a large fraction of the cell cycle. We speculate that the presence of condensin at the nucleolus relieves the topological constraints produced by transcription, thus facilitating separation and segregation of the rDNA.

Condensin also appears to be required for the stability of artificial chromosomes containing repetitive satellite DNA (Freeman *et al.*, 2000), which are probably not transcribed, suggesting that repetitive DNA presents additional challenges to chromosome segregation that require condensin function. Annealing of single-stranded regions from one repeat to another repeat within the same array will form structures that stimulate recombination, leading to repeat loss, repeat gain, and breaks within the array. Such single-stranded DNA could appear during DNA replication or as a result of topological stress induced by transcription. The observed strand annealing activity of condensins (Sutani and Yanagida, 1997) may help to prevent the formation of single-stranded intermediates that could trigger such dangerous reactions. This role in DNA metabolism may explain the observed localization of condensin subunits to specific regions of chromatin during interphase in human cells (Schmiesing *et al.*, 1998) and fruit flies (Lupo *et al.*, 2001). Recruiting condensins to repeated DNA sequences during interphase could be the basis of heterochromatin formation.

YCS4 Is Required for Silencing at Silent Mating Loci

We observed defects in silencing at the mating type loci in *ycs4-1* at the permissive temperature: *ycs4-1* cells arrest in response to α -factor only when the *HML α* locus is deleted, suggesting that defects in condensin function interfere with silencing. At the permissive temperature these defects are mild; the loss of silencing at *HML α* is not severe enough to prevent mating (Whiteway and Szostak, 1985) and we could not detect derepression of a reporter gene integrated at the telomere, although this assay may lack the sensitivity of the assay at *HML α* . Furthermore, the silencing defects we observe may be less severe because we must assay for them at the permissive temperature; the loss of silencing may be more dramatic if we could assay it with the complete lack of *YCS4* function.

Recently, topoisomerase II and Barren have been implicated in regulating epigenetic gene expression in fruit flies (Lupo *et al.*, 2001). A *YCS4* homolog, DPY-28, is required for dosage compensation in *Caenorhabditis elegans* (Meyer, 2000), making it tempting to infer a direct requirement for members of the condensin complex in silencing in budding yeast, perhaps with other partners. Indeed, this may explain its association with chromatin throughout the cell cycle. However, the silencing defect may be one more indirect consequence of the requirement for condensin function to maintain chromosome architecture throughout the cell cycle in

budding yeast; like topoisomerase I and II, proteins required for silencing that may be lost from chromosomes as a result of perturbed chromosome structure. Two observations argue against this hypothesis: 1) indirect immunofluorescence on chromosome spreads against Sir2p reveal no gross loss of this chromosome-associated silencing factor from chromatin (our unpublished results); and 2) mutants containing temperature-sensitive alleles of *SMC2* do not exhibit the α factor resistance phenotype, whereas the *smc4-1* mutant does (our unpublished results). In addition to the resolution of sister chromatids, our investigations have revealed a role for the condensins in regulating the behavior of budding yeast chromosomes throughout the cell cycle.

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