The Condensin Complex Governs Chromosome Condensation and Mitotic Transmission of rDNA

Lita Freeman, Luis Aragon-Alcaide, and Alexander Strunnikov

Unit of Chromosome Structure and Function, National Institutes of Health, National Institute of Child Health and Human Development, Laboratory of Molecular Embryology, Bethesda, Maryland 20892-5430

Abstract. We have characterized five genes encoding condensin components in Saccharomyces cerevisiae. All genes are essential for cell viability and encode proteins that form a complex in vivo. We characterized new mutant alleles of the genes encoding the core subunits of this complex, smc2-8 and smc4-1. Both SM C2 and SM C4 are essential for chromosome transmission in anaphase. Mutations in these genes cause defects in establishing condensation of unique (chromosome VIII arm) and repetitive (rDNA) regions of the genome but do not impair sister chromatid cohesion. In vivo localization of Smc4p fused to green fluorescent protein showed that, unexpectedly, in S. cerevisiae the condensin complex concentrates in the rDNA region at the G2/M phase of the cell cycle. rDNA segregation in mitosis is delayed and/or stalled in smc2 and smc4 mutants, compared with separation of pericentromeric and distal arm regions. Mitotic transmission of chromosome III carrying the rDNA translocation is impaired in smc2 and smc4 mutants. Thus, the condensin complex in S. cerevisiae has a specialized function in mitotic segregation of the rDNA locus. Chromatin immunoprecipitation (ChIP) analysis revealed that condensin is physically associated with rDNA in vivo. Thus, the rDNA array is the first identified set of DNA sequences specifically bound by condensin in vivo. The biological role of higher-order chromosome structure in S. cerevisiae is discussed.

Key words: SM C • condensin • chromosome condensation • chromosome segregation • chromatin

Introduction

The relationship between higher-order structure and function in the eukaryotic chromosome is not well-understood. Chromosomes are functionally complex, supporting DNA replication, transcription, silencing, DNA repair, genetic recombination, and mitotic segregation. The long-range architecture of the chromosomes must accommodate, and in some cases, even determine these disparate chromosomal functions. Identifying molecules involved in long-range chromosome organization and determining their functional roles is crucial to understanding chromosome architecture.

Recent work has identified a family of proteins determining proper chromosome structure in the mitotic cell cycle, the SM C proteins (Koshland and Strunnikov, 1996; Strunnikov, 1998; Hirano, 1999). The predicted structure of SM C proteins includes an NH2-terminal nucleoside triphosphate binding site, two long helical-coil regions separated by a hinge, and a COOH-terminal DA box. These features have become signature motifs for the SM C family. The first SM C protein characterized in Saccharomyces cerevisiae, Smc1p, was identified upon analysis of smc1 mutants in S. cerevisiae (Strunnikov et al., 1993). The S. cerevisiae genome sequencing project revealed the presence of three more SM C family genes, SM C2, SM C3, and SM C4 (Koshland and Strunnikov, 1996). The SM C1 and SM C3 genes are involved in sister chromatid cohesion (Michaelis et al., 1997), whereas the smc2 mutants show defects in long-range structure, i.e., maintenance of condensation (Strunnikov et al., 1995). The Schizosaccharomyces pombe SM C2 and SM C4 orthologues, cut14 and cut3, respectively, and associated factors, are required for mitotic chromosome condensation and segregation (Saka et al., 1994; Futani et al., 1999), perhaps by using an ATP-independent DNA binding and reannealing activity (Futani and Yanagida, 1997). The Bacillus subtilis SM C homodimer, important for proper bacterial chromosome partitioning (Britton et al., 1998; Graumann et al., 1998), reanneals ssDN A in vitro (Hirano and Hirano, 1998).

Studies in higher eukaryotes fully concur with S. cerevisiae and S. pombe data, implicating roles for Smc2 and...
Smc4 proteins in mitotic chromosome structure. The chicken orthologue of Smc2p, Sc11, localizes to mitotic chromosomes (Saitoh et al., 1994). Two Xenopus laevis proteins orthologous to Smc2p and Smc4p, termed XCA P-E and XCA P-C, respectively, copurify with in vitro-assembled mitotic chromosomes and localize to mitotic chromosomes in vivo (Hirano and M Itchison, 1994). XCA P-C and XCA P-E form a 135 condensin complex required for mitotic chromosome condensation in vitro (Hirano et al., 1997), along with XCA P-H, XCA P-D2, and XCA P-G. XCA P-H is the homologue of the Drosofila melanogaster Barren protein, mutations in which cause mitotic defects in embryos with formation of chromosome bridges (Hat et al., 1996). XCA P-D2 is almost identical to peg7, which concentrates on chromosomes during mitosis and which is important for condensation and resolution of mitotic chromosomes (Cubizolles et al., 1998). The 135-condensin complex induces a TP-dependent positive supercoiling of a DNA template as a result of its stoichiometric binding to DNA (Kimura and Hirano, 1997; Kimura et al., 1999). This supercoiling activity depends on phosphorylation, probably by the CDC2 kinase (Kimura et al., 1998).

In addition to the role in mitotic chromosome condensation, some condensin subunits can have additional roles in chromosome structure and function. In Caenorhabditis elegans, a specialized homologue of Smc4p, Dpy-27, is a part of a dosage compensation complex (Chuang et al., 1993, 1994, 1996). A other C. elegans protein M IX-1, an orthologue of Smc2p, is involved in both mitosis and dosage compensation (Lieb et al., 1998).

In S. cerevisiae, chromosome condensation occurs on a fairly minor scale (Guacci et al., 1994). Thus, it was not known whether the condensin complex exists in budding yeast, what the biological role of condensation in S. cerevisiae is, or why condensation of the relatively short S. cerevisiae chromosomes is required. A iso unknown is the mechanism by which smc2 mutations block full separation of chromatids in anaphase (Strunnikov et al., 1995). To disentangle these unresolved issues, we have initiated a systematic analysis of all factors required for chromosome condensation in a genetically and biochemically tractable organism, S. cerevisiae. We investigate the genes encoding condensin subunits, with an emphasis on the core condensin components, Smc2p and Smc4p, with respect to their roles in chromosome transmission in anaphase and in chromosome condensation in vivo.

Materials and Methods

Cloning and DNA Sequencing

Cloning of the Smc2 gene has been reported previously (Strunnikov et al., 1993, 1995). Sequence information for the SM C4, BRN1, YCS4, and YCS5 genes was obtained from the Yeast Genome Project (G Albert et al., 1996; Johnston et al., 1997). The SM C4 gene containing flanking EcoRI and Sphl sites was assembled from two genomic PCR products (5288 basepairs) background. The resulting fragment was cloned into Y Cplac33 (Gietz and Sugino, 1988) giving pA534. The wild-type Smc4 gene was also isolated as a plasmid (pA574) complementing the smc4-1 mutation (library reference in Guacci et al., 1997). DNA was sequenced using the ABI Prism 377 dye-terminator method (PerkinElmer). Sequence analysis was performed using A semblbyLign software (Oxford M de Lecer).

Strains, Plasmids and Genetic Techniques

Yeast strains used are shown in Table I. In all listed cases of plasmid integration, the confirmation was obtained by Southern blotting, PCR, or Western blotting. The smc4-d1/H53 and smc4-d2/URA 3 deletions were constructed by transforming A5260 (Strunnikov et al., 1995) with fragments from plasmids pA513 or pA533, respectively. Haploid deletion strains were isolated as mitotic progeny of heterozygous strains and were maintained with a minichromosome containing the corresponding wild-type SM C4 gene. Disruption of the SM C2 gene was according to Strunnikov et al., 1995). The ber1-1/URA 3 disruption allele was constructed using primers designed to amplify markers from the prS416 vector (TAGAATACGATGGATGAATATGTTCATGAGCTAGTATAACATAGTAACATAGTCTGGCAGTATGAGCATTACCATTTGATATGCAGGCGTGTTTGATGATGCTGTTAGTTCAACCCCGTTCTTCCGAGCCTGATTTATGGATGTTCGGTGTTTCCCACACCRC). The corresponding PCR product was directly used to transform A540 and A504. The ycs4 and ycs5 deletion alleles were constructed using the same strategy with the primer pairs TACTGGGTATTAATCGATCGTTTGTTCAATATATGCGTAATGCAATGAAACTAACATAGTACCTTTGGCTTCTGTTTAATGGTTGGGCTTTTATTATTCCACCCCGC. The resulting diploids were analyzed by tetrad dissection.

SM C2 mutagenesis by PCR was as described previously (Strunnikov et al., 1995). The SM C2-8 allele was integrated into the chromosome as described previously for smc2-6 (Strunnikov et al., 1995). To generate temperature-sensitive mutants of SM C4, pA534 was transformed into the XL-1 Red strain (Stratagene). Plasmid DNA was isolated and transformed into strain 16aa S335/apS46. Plasmid shuffling resulted in isolation of pA534/1 containing the SM C4-1 temperature-sensitive allele. The smc4-d1 allele was introduced into the chromosome via smc4-d2/URA 3 allele replacement.

The green fluorescent protein (GFP)-based chromosome tagging system for yeast chromosome V was designed by modification of the pA5135 and pA559 vectors (Straight et al., 1996). Plasmid pA5135 expressing LacI-GFP was digested with NsiI, and the PstI-NsiI fragment of pAFS135 and pAFS59 vectors (Straight et al., 1996). Plasmid pAFS135 X-Red strain (Stratagene). Plasmid DNA was isolated and transformed into strain YCS5. The resulting strains containing pA545 and Smc4p was tagged by fusing GFP by PCR to the 3’ end of the gene, creating pL640 (SM C4-GFP). This plasmid was targeted into the chromosomal SM C4 locus by a gel digestion, disrupting the untagged gene. The Smc4p-GFP fusion was fully functional in vivo.

To tag SM C2 with the hemagglutinin (HA) epitope, haploid strain YP499bp transformed with a XbaI-Sall fragment from plasmid pA532, replacing the untagged gene. This fragment carries the SM C2 gene with a PCR-generated 12His-3HA tag inserted into its Spel site and an upstream LEU2 marker. To epitope-tag YCS5, two primers, METCCATAGTAAAGTTAAGTAAGACTTCGTCAGTATGATGCAATGAAACTAACATAGTACCTTTGGCTTCTGTTTAATGGTTGGGCTTTTATTATTCCACCCCGC. The corresponding PCR product was used to amplify a fragment of pA560 (Strunnikov, A., unpublished data) containing the 12His-3HA tag immediately followed by the Spro primer pair: TACTGGGTATTAATCGATCGTTTGTTCAATATATGCGTAATGCAATGAAACTAACATAGTACCTTTGGCTTCTGTTTAATGGTTGGGCTTTTATTATTCCACCCCGC. The corresponding PCR product was used to isolate the SM C2 gene containing flanking EcoRI and Sphl sites in a YCplac33-based chromosome tagging system. For chromosome IV arm probe tandem integration, the confirmation was obtained by Southern blotting, PCR, or Western blotting. The SM C2-1 and SM C2-2::URA 3 deletion alleles were constructed using the same method with the following pairs of primers, respectively: TGGTGCGTTTATTTACCATTTCAACCCCGC. The corresponding PCR product was used to isolate the SM C2 gene containing flanking EcoRI and Sphl sites in a YCplac33-based chromosome tagging system.

Abbreviations used in this paper: ARS, DNA replication origin; ChIP, chromatin immunoprecipitation; FISH, fluorescent in situ hybridization; GFP, green fluorescent protein; HA, hemagglutinin; YAC, yeast artificial chromosome.
Table I. S. cerevisiae Strains

| Strain   | Relevant genotype                                      |
|----------|--------------------------------------------------------|
| AS400    | MATa/MATa LEU2/LEU2 MET15/15 TRP1/1 TRP1 ura3         |
| YPH499bp | MATa ura3 lys2 ade2 trp1 his3 leu2 bar1-D, pep4::HIS3 |
| YPH499p2 | MATa ura3 lys2 ade2 trp1 his3 leu2 bar1-D, pep4::HIS3 |
| YPH499bp5| MATa smc-4-1 ade2 his3 leu2 lys2 ura3                |
| YPH499bp6| MATa smc-4-1 ade2 his3 leu2 lys2 ura3                |
| BY4733bp4| MATa his3 leu2 met15 trp1 ura3 YCS4-12His:3HA(URA3) trp1-D::LEU2 pep4::HIS3 |
| 16aAS353/pAS546| MATa ade2 his3 leu2 lys2 ura3 smc4-1-D::HIS3 URA3 SMP4 |
| lbaAS330| MATa smc-2-8 ade2 leu2 lys2 his3 trp1 ura3           |
| lbaAS344| MATa smc-4-1 ade2 his3 leu2 lys2 ura3                |
| 3-1bAS330/pAS622| MATa ade2 leu2 lys2 his3 trp1 smc-2-8 ura3 bar1-D::SIR2::GFP12(HIS3) SMP4-42::URA3 |
| 6-1bAS344b/pAS622| MATa ade2 his3 leu2 lys2 ura3 smc4-1-D::SIR2::GFP12(HIS3) SMP4-42::URA3 |
| YPH4996V | MATa smc-2-8 ura3-D::HIS3 lacl-GFP::(TRP1 lacO)       |
| lbaAS330bV | MATa smc-4-1 ura3-D::HIS3 lacl-GFP::(TRP1 lacO)       |
| 1aAS342bV | MATa smc-4-1 ura3-D::HIS3 lacl-GFP::(TRP1 lacO)       |
| lbaAS330bIV | MATa smc-4-4 ura3-D::HIS3 lacl-GFP::(TRP1 lacO)       |
| AS335    | MATatx ade2 his3 leu2 lys2 ura3 trp1/1 TRP1 smc4-1-D::HIS3 |
| NOY891/pNOY353 | MATa ade2 ura2 his3 can1 trn1-1-D::HIS3 TRP1 pgal7::rDNA (Oakes et al., 1998) |
| AS401    | MATa/MATa ade2 ura3 his3 can1 trn1-1-D::HIS3 TRP1 pgal7::rDNA |
| L4078    | MATa ade2 leu2 lys2 trp1 ura3 his5 III/(LEU2::ADE2::URA3) (G. Fink) |
| AS389    | MATa/MATa ade2 leu2 lys2 ura3 TRP1/trp1 III/III/(LEU2::ADE2::URA3) |
| AS388    | MATa/MATa ade2 leu2 lys2 ura3 TRP1/trp1 III/III/(LEU2::ADE2::URA3) |
| AS386    | MATa ade2 leu2 lys2 ura3 smc4-1 III/III/(LEU2::ADE2::URA3) |
| 4419     | MATa ade2-1 lys2-1 can1-100 trp1 ura3 his5/5 ADE2 HIS3 (200 kb) |
| 6228     | MATa ade2-1 lys2-1 can1-100 trp1 ura3 his5/5 ADE2 HIS3 (900 kb) |
| 2aAS415  | MATa smc-2-8 ade2 his3 III/III::(LEU2::ADE2::rDNA::URA3) |
| 1aAS416  | MATa smc-4-1 ade2 his3 III/III::(LEU2::ADE2::rDNA::URA3) |
| 5bAS413  | MATa smc-4-1 ade2 his3 III/III::(LEU2::ADE2::rDNA::URA3) |
| 1aAS414  | MATa smc-4-1 ade2 his3 III/III::(LEU2::ADE2::rDNA::URA3) |
| AS260-1  | MATa ade2 his3 leu2-1-D::leu2-1-D::ADE2 trp1 ura3 |
| AS359-1  | MATa ade2 leu2/leu2-D::ADE2 trp1 ura3 smc4-1 II/II/(LEU2::ADE2::URA3) |
| AS362-1  | MATa ade2 his3 leu2-1-D::leu2-1-D::ADE2 trp1 ura3 smc4-1 II/II/(LEU2::ADE2::URA3) |
| WY33     | MATa ade2 his3 leu2 trp1 ura3 net1::9Myc-NET1::LEU2:: pep4-4 (Shou et al., 1999) |

Antigens

To generate chromosome III labeled with 3H until 100% arrest in G1. Cells were washed three times with incubation media and incubated for 2 h at 37°C in the presence of 0.1 mg/ml nocodazole. The quality of arrest at all stages was monitored microscopically and by FACS®.

Cell Cycle Methods

Yeast cultures were maintained according to standard techniques (Rose et al., 1995, 1996). Yeast cell cycle experiments were conducted as described previously (Strunnikov et al., 1995; Guacci et al., 1997) with minor modifications. To double-block with α-factor and nocodazole for GFP-mediated visualization of sister chromatid separation, cells were first grown at 32°C in synthetic drop-out media lacking tryptophan and histidine to 5 × 10⁶ cell/ml. α-Factor was then added to 10-⁴ M and cells were incubated 3 h at 23°C until 100% arrest in G1. Cells were washed three times with incubation media and incubated for 2 h at 37°C in the presence of 0.1 mg/ml nocodazole. The frequency of arrest at all stages was monitored microscopically and by FACS®

Antibodies and Immunoprecipitations

A ntl-Smc2p, Smc4p, Brn1p, and Y cs4p antibodies were generated against the antigens expressed in the BL21 DE 3pLys S strain of Escherichia coli (Novagen). The expression plasmids contained the fragments encoding the last 590, 675, 418, and the first 390 amino acid residues of Smc2p, Smc4p, Brn1p, and Y cs4p, respectively. A Xhol SMC2 fragment from PA534 was ligated into XhoI-digested pSETA, creating pl[F55]. A Sall/XhoI fragment from PA551 containing the 3' end of the gene was ligated into BamHI-digested pSETA, creating pl[F55]. A PCR-generated BamHI fragment of BRN1 containing the 3' end of the gene was ligated into BamHI-digested pSETA, creating pl[F56]. The antigens were purified sequentially by immobilized metal affinity chromatography (ProBond; Invitrogen) and PAGE and then injected into two New Zealand white rabbits (Covance). Production sera were affinity purified on CNBr-Sepharose columns (Amersham Pharmacia Biotech) with the coupled purified recombinant protein and used for Western blotting. A commercially available antibodies were used according to manufacturer recommendations.

Immunoprecipitations were performed with EBA buffer (Liang and Stillman, 1997), except sonication and/or DNAase I (10 μg/ml, 15 min at 15°C) were used to fragment chromatin. All buffers contained Complete™ protease inhibitors (Boehringer). Lysates for immunoprecipitation reactions were prepared by the bead-beating method (Strunnikov et al., 1995). Cell lysates, diluted 1:10 in EBA, were incubated 2 h at 4°C with anti-HA antibodies (2C8A; Boehringer) coupled to CNBr-Sepharose beads. The beads were washed six times with 1 ml EBA in MicroBeadSpin columns (BioRad). Proteins bound to antibodies were eluted in 200 μl 2% SDS at room temperature for 20 min, followed by 5 min incubation at 90°C. Chromatin immunoprecipitation (ChIP) was performed exactly as in M eluh and Koshland (1997), with a cross-linking time of 1 h 15 min. For precipitation of Smc2p-HA-containing chromatin fragments, the polyclonal rabbit anti-HA antibody #2973 was generated by injecting a key...
hole limpet hemacyanin–coupled linear peptide corresponding to one and a half repeats of the HA tag (NH$_2$-GYPYDVDPYAG-COOH) into New Zealand white rabbits (Covance). Immunoprecipitation DNA was diluted at least 1:50 for PCR of rDNA compared with the unique region DNA ChiP.

**Fluorescent In Situ Hybridization and Microscopy**

Fluorescent in situ hybridization (FISH) was performed essentially as described (Guacci et al., 1994) with minor modifications in the cell-swelling protocol. rDNA probes were as in Guacci et al. (1997). Chromosome VIII FISH-painting probes prepared from cosmids C9315, C817, C9666, C9925, C8025, C8082, and C9205 (A TCC) were labeled with digoxigenin separately as described (Guacci et al., 1994). Probes span a 230-kb region of chromosome VIII, including the centromere. All II probes were titrated using a dot-blot filter assay for digoxigenin and then mixed for FISH experiments. The arrest protocol for the establishment of chromosome condensation included α-factor treatment at 23°C for 3 h with release into nocodazole-containing media at 37°C for 2 h. The cells were fixed in 4% formaldehyde for 2 h. The rDNA FISH signal was quantified using the Segmentation function of IP Lab (Scanalytics). The area of the FISH signal was divided by the area of the propidium iodide signal, thus giving the normalized measurement for each nucleus. In the case of multiprobe painting of chromosome VIII, minimal circles were drawn around the FISH signal and around the propidium iodide signal (see Fig. 3 C). The areas of the circles were then treated as segments in the rDNA quantitation.

Fluorescence microscopy was performed using the wide-field Zeiss Axiowert microscope with epifluorescence. The images were collected using a MiroMax cooled charge-coupled device camera (Princeton Instruments) and Z-axis motor assembly (Ludl). Optical sections were converted into a stacked image with IP-Lab software (Scanalytics). For LacI-GFP chromosomal tag visualization, cells were fixed with 3.7% formaldehyde for 5 min, washed extensively with PBS, and mounted for microscopy in a minimal volume of PBS buffer. 10–20 optical sections spanning 5 μM were collected per each field. Distribution of GFP signal was quantified using the recorded three-dimensional images.

**Results**

**Characterization of the Condensin Protein Complex in S. cerevisiae**

The SM C2 gene from S. cerevisiae has been characterized previously and found to be essential for viability and maintenance of chromosome condensation (Strunnikov et al., 1995). We disrupted the previously uncharacterized S. cerevisiae SM C gene SM C4 (Koshland and Strunnikov, 1996) and three open reading frames encoding the putative condensin subunits BRN1 (X CAP-H orthologue), YLR272c (X CAP-D2 orthologue), and YDR325w (X CAP-G orthologue), revealed by the genome sequencing project. All II genes were found to be essential for cell viability (see Materials and Methods). We designated the YLR272c gene Y C4S and the YDR325w gene Y C5S, for yeast condensin subunit four and five, respectively. Detailed sequence analysis of SM C4, Y C5S, and Y C5S (not shown) demonstrates that these proteins have conserved clusters of amino acid residues representing the characteristic signature motifs for each condensin subunit. Fig. 1 A shows classification of S. cerevisiae and some higher eu-
karyotic condensin subunits into corresponding orthologous groups. Smc2p and Smc4p are the most conserved proteins in the complex and Ycs5p is the least evolutionary conserved.

To verify whether the putative condensin subunits indeed form a complex in *S. cerevisiae*, we generated epitope-tagged versions of the corresponding genes and raised antibodies against the putative condensin subunits (see Materials and Methods). Immunoprecipitation from extracts containing Ycs5p-HA using anti-HA antibody revealed that the precipitate contains other putative condensin subunits. Fig. 1 B shows that Ycs5p-HA immunoprecipitates contain Smc2p (predicted molecular mass 134 kD), Smc4p (162 kD), Brn1p (83 kD), and Ycs4p (133 kD) in addition to the tagged Ycs5p (126 kD). Only Brn1p showed abnormal PAGE mobility, migrating with an apparent molecular mass of 100 kD. Analysis of extracts from G1, S, and G2/M cells shows little dependence of complex composition on cell cycle stage (Fig. 1 B). Thus, a complex similar to the X. laevis 13S condensin is present in *S. cerevisiae*. We therefore called this five-subunit complex *S. cerevisiae* condensin. A nalysis of whole cell extracts showed that Smc2p-HA is more abundant in the cell than Brn1p-HA, Ycs4p-HA, and Ycs5p-HA throughout the cell cycle (data not shown). This suggests that an incomplete condensin complex similar to the X. laevis 8S complex lacking non-SMC subunits may also exist in *S. cerevisiae*.

We analyzed the stoichiometry of condensin subunits in several experiments. A suspected, complete immunoprecipitation of Smc2p-HA from extract led to complete depletion of Smc4p (Fig. 1 C). When immunoprecipitates of the non-SMC subunits tagged with the HA epitope were analyzed (Fig. 1 D), proportional amounts of Smc2p and Smc4p were detected for each of the tagged subunits, as determined by densitometry. This result suggests that Brn1p, Ycs4p, and Ycs5p are present in a 1:1:1 ratio in the complex. To determine the stoichiometry of the SM C condensin subunits to Brn1p, Ycs4p, and Ycs5p, we performed a small-scale immunoadfinity purification of condensin. One of the non-SMC subunits, Ycs5p, was used to introduce the affinity tag. The protein extract containing Ycs5p-HA was incubated with the anti-HA tag antibodies #2973 (see Materials and Methods) followed by binding to protein A-Sepharose. A fter elution with the corresponding peptide, composition of the eluate was investigated using PAGE and Western blot (Fig. 1 E). Results of this experiment suggest that all five subunits are present in equimolar ratio, without any additional subunits of comparable molecular mass. Whether any substoichiometric subunits are present in *S. cerevisiae* condensin remains to be determined.

**The SMC Components of *S. cerevisiae* Condensin Are Required for Chromosome Segregation and Condensation**

Studies in *X. laevis*, *B. subtilis*, and *S. pombe* predict that Smc2p and Smc4p may be in direct contact with DNA (Kimura and Hirano, 1997; Sutani and Yanagida, 1997; Hirano and Hirano, 1998). We also have shown that at least half of the Smc2p and Smc4p pool is tightly associated with chromatin throughout the cell cycle (see below). Thus, we focused our chromosome segregation and condensation studies on the SM C component of the condensin complex, Smc2p and Smc4p. We used novel conditional alleles of SM C2 and SM C4 for detailed functional studies.

The smc2-8 and smc4-1 mutants undergo growth arrest within one generation time (Fig. 2 A), accompanied by a decrease in cell viability (Fig. 2 B). A t the restrictive temperature, the impairment of chromosome segregation was evident in both the smc2-8 and smc4-1 strains, judging by morphology of their nuclear DNA (Fig. 2, C and D). The terminal phenotype of the mutants was consistent with a defect in mitotic chromosome segregation, specifically a defect in full separation of sister chromatids during anaphase. Surprisingly, however, we did not detect an elevated rate of chromosome III loss at 23°C, 35°C after a 3-h shift to 37°C (data not shown).

To investigate the chromosome segregation defect in smc2-8 and smc4-1 cells, we used the in vivo chromosomal tag approach (Straight et al., 1996). A plasmid expressing LacGFP and an array of LacO sites was introduced into the ura3 locus on chromosome V (see Materials and Methods) in the smc mutants and in the isogenic Smc+ cells. At the restrictive temperature, both smc2-8 and smc4-1 cells showed an increase in the frequency of cells with signals from two sister chromatids located in different cell bodies that are not separated to the maximum possible distance (Fig. 2 E). This finding agrees with the stretched chromosomal DNA morphology frequently seen in these mutants. Thus, the anaphase-like morphology of condensin mutant arrest suggests that the associated block in cell division is determined by improper anaphase progression. Such arrest is analogous to the top2 phenotype and could be attributed to entanglement of sister chromatids (Spell and Holm, 1994). Thus, the cell division arrest in the smc2-8 and smc4-1 mutants could be a result of inability to separate sister chromatids in anaphase completely. A nalysis of the same alleles using chromosomal GFP tags did not reveal, however, any defect in the establishing of sister chromatid cohesion (not shown).

Chromosome condensation, if impaired, can cause chromatids to entangle and prevent their proper separation and anaphase completion. In budding yeast, mitotic chromosome condensation has been characterized mainly through FISH using a procedure involving chromatin spreading (Guacci et al., 1994). We attempted to assess chromosome condensation in intact yeast cells using two approaches. In one case, the distance between two LacO tags introduced into the ura3 and met6 loci was monitored (chromosome V, 225 kb apart). In another strain, the area occupied by the Sir2p-GFP signal (rDNA chromatin) was studied throughout the cell cycle. In both instances, using intact cells and GFP chromosomal tags did not provide sufficient resolution to detect mitotic chromosome condensation (Strunnikov, A., unpublished observation). Thus, FISH remains the only method that allows observation of chromosome condensation in *S. cerevisiae*. Therefore, we used FISH to investigate mitotic changes in chromatin packaging, referred to as condensation.

Previous results suggested that the SM C2 gene has a role in the maintenance of mitotic chromosome condensa-
Viability of Smc1 Characterization of daughter cell body.

Figure 2. Characterization of smc2-8 and smc4-1 mutants. (A) Growth curves of smc mutants (strains 1bA S330 and 1bA S344) at 37°C, compared with the isogenic strain YPH 499 (Smc+). (B) Viability of Smc+ and Smc- strains (YPH 499, 1bA S330, and 1bA S344) at 37°C determined by plating assay. (C) Distribution of cell types within arrested populations of smc mutants (strains listed in A) after a 3-h exposure to restrictive temperature (37°C). (D) Cell morphology (phase-contrast) and nuclear DNA position (4,6-diamidino-2-phenylindole staining) in smc mutant strains after a 3-h exposure to 37°C. Bar, 5 μm. (E) Sister chromatid separation in wild-type cells and smc mutants with a GFP tag at the ura3 locus after 3 h at 37°C. Smc+; YPH 4980V, smc2-8: 1bA S330V; smc4-1: 1aA S342bV. The classes of cells are as follows (only cells with buds were counted): single dot in any cell body; two dots in the same cell body; one dot in each cell body with the distance between two dots < 2/3 of maximal cell axis; one dot in each cell body with the distance between two dots > 2/3 of maximal axial measurement; more than two dots per mother and daughter cell body.

No assessment of the contribution of Smc2p, Smc4p, or other condensin subunits in the establishment of the condensed state has been made to date. We used FISH to evaluate the input of Smc2p and Smc4p in the establishment of mitotic chromosome condensation. Smc+ , smc2-8, or smc4-1 cells were arrested with α-factor and released from the G1 arrest into nocadazole-containing media at 37°C. This experimental protocol ensures that each strain progresses synchronously through S phase and then arrests at the same point in G2/M while a given temperature-sensitive protein is inactivated (Guacci et al., 1997). Mitotic chromosome condensation was visualized by FISH using an rDNA probe (Fig. 3, A and B) or a series of chromosome VIII arm probes (chromosome painting) (Fig. 3, C and D). We used a novel approach to quantify changes in mitotic chromosome condensation in S. cerevisiae. Previously, no quantification has been published for rDNA locus condensation, and the published estimates for nonrepetitive region condensation were based on linear measurements, incompatible with the multiprobe approach. In contrast, we applied area measurements of FISH signal corresponding to a chromosomal domain (Fig. 3, A and B) or alized this phenomenon in a series of experiments designed to evaluate the consequences of condensin impairment on different chromosomes and distinct chromosomal domains.

The SMC2 and SMC4 Genes Have a Specific Role in rDNA Chromatin

Despite the fact that the smc2-8 and smc4-1 mutants affect condensation of both unique and repetitive regions of the genome, the different chromosomal domains could have specific requirements for mitotic compaction and thus might be differentially affected by condensation failure and subsequent anaphase block. In this case, some chromosomes may be more sensitive to the loss of condensin function than others. To test whether there is a bias in condensin binding to different chromatin domains, we investigated localization of condensin complex in live intact cells. Previous localization data were obtained using fixed cells overexpressing Smc2p-HA (Strunnikov et al., 1995). Under these conditions, a uniform nuclear signal was observed independently of the cell cycle stage. Using intact live cells and a more sensitive detection system in the absence of protein overexpression allowed us to reveal some novel features of condensin localization in vivo. We followed localization of condensin in intact yeast cells using Smc4p-GFP as a marker. The choice of Smc4p was due to the observation that its intracellular amount does not change in the course of the cell cycle. In an asynchronous cell population expressing Smc4p-GFP from the SMc4 promoter, the GFP signal was seen either as a diffuse nuclear signal or as concentrated in a subnuclear compart-
The shape and position of this compartment is consistent with it being a part of nucleolus (Yang et al., 1989). We confirmed that the area of intensive staining indeed corresponds to nucleolar chromatin by demonstrating that the nucleolar Smc4p-GFP signal is absent in strain NOY891 lacking rDNA repeats (Oakes et al., 1998) (see Materials and Methods). Only a diffuse nuclear GFP signal was observed in NOY891/pLF640 (Fig. 4 C). In the mitotic cells expressing both GFP-tagged Smc4p and Sir2p-GFP, an established rDNA binding protein (Fritze et al., 1997; Gotta et al., 1997), the GFP signal was detected in a subnuclear region of the same shape and position as in the mitotic cells expressing either of the constructs alone (data not shown). Immunofluorescent staining for Net1p-MYC (Shou et al., 1999), a nucleolar protein, largely colocalized with the Smc4p-GFP signal, although the GFP signal was significantly diminished by fixation even as short as 5 min (Fig. 4 D). Observation of a synchronized cell population (Fig. 4 E) established that the subnuclear concentration of Smc4p-GFP is a cell cycle–dependent event. The diffuse nuclear GFP signal observed in interphase cells becomes concentrated in this subnuclear domain before mitosis and persists throughout chromosome separation. This finding indicates that the condensin complex may have a special role in the structure of rDNA chromatin and possibly in mitotic transmission of chromosome XII harboring the ribosomal DNA locus.

Using the Sir2p-GFP marker we followed the rDNA locus position after a 3-h shift to nonpermissive temperature (Fig. 5 A). Surprisingly, we found that in most cells, rDNA did not segregate or even separate into two sister chromatids. Under the same conditions, the pericentromeric regions of chromosome V and the distal arm region of chromosome IV readily separated in more than half of the large-budded cells (Fig. 5, B and C, respectively). Thus, the mutants in the genes for condensin subunits arrest in the middle of anaphase after the separation of chromatids is triggered, but fail to segregate rDNA. Delayed segregation of rDNA is not observed in wild-type yeast cells (Granot and Snyder, 1991). The delay or block in rDNA segregation thus reflects the specific property of condensin mutants. This suggests that condensin may have a more specific role in segregating the rDNA locus, which is structured as an array of tandem 9-kb repeats. To verify whether the tandem repeat structure of the rDNA array plays an important role in its delayed or stalled segregation, we tested the mitotic stability of a plasmid carrying a single rDNA repeat (Nierras et al., 1997). Mitotic stability of the plasmid (81 ± 1% for the Smc1 strain) was only moderately affected by the presence of the smc2-8 (70 ± 1%) or smc4-1 (76 ± 1%) mutations.

Does delayed segregation of rDNA in the smc2-8 and smc4-1 mutants result in the subsequent loss of this chromosome? Unfortunately, any direct assessment of chromosome XII loss in budding yeast is not feasible, as chromosome XII aneuploidy is probably a lethal event (Granot and Snyder, 1991). To circumvent this limitation,
we used strains homozygous for the smc-2 or smc-4-1 mutation but heterozygous for the rDNA locus translocation (Mikus and Petes, 1982). One homologue of chromosome III, a well-marked small chromosome, carried a translocation of the rDNA locus marked with ade2 and leu2 (Fig. 5 D). The mat locus at the opposite arm of chromosome III was used to distinguish gene conversion events from chromosome loss. The mitotic stability of the rDNA-carrying chromosome III was assayed in smc1, smc-2, and smc-4-1 cells using a modification of the half-sector assay (Koshland and Hieter, 1987; Hegemann et al., 1988) (Fig. 5 E). To estimate the loss rate, the cells were grown at the nonpermissive temperature (37°C) for 6 h and then plated and allowed to form colonies at the permissive temperature (30°C). Loss of the ade2 marker with simultaneous loss of leu2 and mat a markers was considered a
chromosome loss event. Loss of ADE2 only was considered a mitotic recombination event. In the Smc+ strain, no loss of the rDNA-carrying chromosome III was detected (frequency below \(3.4 \times 10^{-3}\) per cell division). All events of ADE2 loss shown for the wild-type strain in Fig. 5E were the result of mitotic recombination (2.1 \(\times\) 10\(^{-2}\)). In contrast, in the smc2-8 and smc4-1 mutants, loss rates were high: 4.6 \(\times\) 10\(^{-2}\) and 2.4 \(\times\) 10\(^{-2}\), respectively. Thus, although smc2-8 and smc4-1 only marginally affect transmission of normal chromosome III (Fig. 5F), these mutants do affect transmission of chromosome III when it is carrying rDNA. This provides genetic evidence that the SMC2 and SMC4 genes, and likely the condensin complex as a whole, have a specialized role in the transmission of rDNA in mitosis.

To exclude the possibility that the detected destabilization of chromosome III upon rDNA translocation is due simply to the change in chromosome length, we conducted
an independent test assessing how chromosome length affects its stability in the smc2-8 and smc4-1 mutants. Two homologous telocentric yeast artificial chromosomes (YACs) marked with Ade2 and His3 (van de Vosse et al., 1997), 200 and 900 kb long, were introduced into the mutant and wild-type strains. The length of the YAC arms roughly corresponds to the arm length of chromosome III without and with rDNA translocation, respectively. The loss rate was measured in the half-sectored assay after a 6-h shift to 37°C. As expected, the longer YAC generally had higher mitotic stability than the shorter YAC in all strains tested (Fig. 5 G). This result excludes the possibility that chromosome III carrying the rDNA translocation is destabilized in the smc2-8 and smc4-1 mutants due to its increased size.

Despite the fact that condensin plays an important role in rDNA structure, the function of condensin is still essential in the absence of the tandemly repeated rDNA genes. When the condensin subunit genes were disrupted in the strain A S401, which had the only source of rRNA, was a plasmid-borne rDNA repeat (Oakes et al., 1998), the haploid progeny containing disruption alleles of SM C2, SM C4, BRN1, Y C54, or Y C55 could not be recovered. Thus, these genes are still essential for viability in cells without tandem organization of the rDNA genes. Direct integration of the smc2-8 allele in a strain without the rDNA array resulted in a tight temperature-sensitive phenotype (not shown), suggesting the possibility that in the absence of the array, the secondary sites of condensin binding become more affected.

Condensin Binds to rDNA Chromatin Throughout the Cell Cycle

Is condensin physically present at rDNA or are the observed defects in rDNA condensation and segregation indirect? First, we determined whether condensin binding to bulk chromatin changes throughout the cell cycle using a crude chromatin-binding assay (Liang and Stillman, 1997). Extracts of asynchronous cells or cells arrested in G1, S, or M phase expressing Smc2p-HA were fractionated into a chromatin-bound fraction (pellet) and a chromatin-unbound fraction (supernatant). H mo1p, a homologue of known chromatin component HM G1 (Lu et al., 1996), associates with chromatin throughout the cell cycle (Fig. 6 A). Smc2p-HA is partially chromatin-bound and partially extractable, and this distribution does not significantly change throughout the cell cycle. Thus, Smc2p may be used to identify the constitutive sites binding condensin in vivo.

For a detailed analysis of Smc2p binding to chromatin throughout the cell cycle, we turned to ChIP analysis of Smc2p-HA binding to the rDNA locus (Fig. 6 B and C). Cells were cross-linked with 1% formaldehyde, chromatin extracts were prepared, and processed for ChIP as described (Maleh and Koshidlan, 1997). A small aliquot was removed for input sample and the remainder was used for immunoprecipitations with anti-HA antibody. The isogenic strain without a HA tag was used to determine proper template dilution and was included as a negative control in every experiment. After reversing DNA-protein cross-links, immunoprecipitated DNA was purified and analyzed by PCR using a set of primers spanning the rDNA locus (Fig. 6 C). In the asynchronous population, multiple sites of Smc2p-HA binding to rDNA chromatin were detected (Fig. 6 B). To quantify this association, we used synchronized cell populations and compared G1 chromatin to G2/M chromatin (Fig. 6 D), when rDNA is expected to be at its most decondensed and most condensed stages, respectively (Fig. 3 A). Fig. 6 D shows that in α-factor-arrested cells, Smc2p-HA binds to the region upstream of the rDNA replication origin (ARS) (probes 1 and 2) but not to the ARS itself (probe 3). Smc2p binds strongly to the Pol1 promoter (probes 4 and 5) but weakly to the 5′ region of the 375 rDNA transcription unit (probes 6–9), with stronger binding towards the 3′ end of the transcription unit (probes 10–17). Strong binding for α-factor-arrested cells is observed at the replication fork barrier (probe 18) and between the replication fork barrier and the ARS (probes 19–21 and probe 2). The pattern of Smc2p binding to rDNA in nocodazole-arrested cells is very similar to the pattern observed for α-factor-arrested cells, with the notable exception that Smc2p binding to the replication fork barrier (RFB) and 5′ to the ARS (probes 19–21 and probe 2) and 5′ to the RFB (probe 17) is weaker in nocodazole-arrested cells than in α-factor-arrested cells.

ChIP analysis of the non-SMC condensin subunit Ycs5p revealed even more complexity in the binding of condensin to the rDNA repeat. For Ycs5p-HA association with rDNA, some cell cycle dependence was observed. In G1 cells, only five sites are strongly associated with Ycs5p (probes 10, 11, and 13–15), whereas in G2/M cells a strong correlation with the Smc2p-bound sites is observed. Most notably, the Pol1 promoter sites and the 3′ end of the large transcription unit are occupied by Ycs5p in mitosis. Probe 12 again reveals incompatibility of the corresponding region with condensin binding. This result confirms condensin preference for particular binding sites within the rDNA repeat and reveals some heterogeneity between these binding sites. Some of the condensin-binding sites likely have the complete condensin complex bound throughout the cell cycle (probes 13–15), whereas others are associated only with the SMC condensin component in G1 and assemble the full condensin complex only in G2/M. Thus, we presented data pointing to three coexisting modes of condensin recruitment to rDNA. The first mode is constitutive binding of SMC subunits to the specific sites from G1 to mitosis (Smc2p ChIP results), the second is recruitment of additional condensin complexes to rDNA in a mitosis-specific manner (Smc4p-GFP microscopy), and the third, the most speculative, is the mitosis-specific recruitment of the non-SMC subunits to the bound SMC subunits (Ycs5p ChIP data). Repeated structure of rDNA array prevented us from making a quantitative estimate of the relative contributions of these three modes of condensin targeting to rDNA.

Thus, we have identified the first site that is bound by condensin in vivo and uncovered both cell cycle–dependent and cell cycle–independent components in this association. However, condensin binding is not strictly limited to rDNA, as we found several additional sites in the genome, including pericentromeric and peritelomeric regions, that showed association with condensin in the ChIP assay (Freeman, L., unpublished observations), in agree-
ment with FISH data (Fig. 3, C and D). Functional analysis of condensin binding to these sites in strains without the tandem rDNA array is under way.

**Discussion**

**The Condensin Complex of S. cerevisiae**

The S. cerevisiae genome sequence reveals the presence of five genes encoding proteins homologous to the X. laevis condensin subunits (Kimura et al., 1999). We have demonstrated that these five genes encode subunits of the S. cerevisiae condensin complex. All five S. cerevisiae condensin subunits are essential for cell viability. The core SM subunits, Smc2p and Smc4p, were tested and found to be essential for chromosome condensation. We have demonstrated that the condensin complex containing all five subunits is present throughout the cell cycle and has a complex mode of interaction with chromatin. Thus, condensin from S. cerevisiae bears remarkable similarity to the previously characterized 13S condensin from X. laevis and S. pombe condensin (Kimura et al., 1999, Sutani et al., 1999).

Despite these similarities, differences between the con-
densin complexes of different species were found when their cell cycle behavior was examined. S. cerevisiae condensin localization shows a prominent cell cycle-dependent change at the subnuclear level. Smc4p-GFP exhibits a mostly diffuse nuclear staining pattern during most of the cell cycle but shows nucleolar localization at G2/M. Both Smc2p and Smc4p bind chromatin throughout the cell cycle, as assayed by a crude chromatin-binding assay. Further refinement using ChiP analysis revealed that Smc2p binds specific chromatin sites both in G1 and in G2/M phases. Ycs5p binds to many of these sites only in G2/M. Cell cycle-regulated binding of non-SMC condensin subunits to constitutively bound SMC condensin components thus appears to be a unique feature of the S. cerevisiae condensin complex. In contrast, in S. pombe, the entire complex translocates to the nucleus at mitosis. This translocation is due to phosphorylation of a specific site in Cut3, an Smc4p homologue (Sutani et al., 1999). In X. laevis, phosphorylation of the non-SMC components of condensin is responsible for chromosomal targeting of the condensin complex at mitosis and for the positive supercoiling activity of the condensin (Kimura et al., 1998). In this case, phosphorylation occurs on the non-SMC subunits of the X. laevis condensin complex.

The mechanism of regulation of S. cerevisiae condensin activity and targeting throughout the cell cycle still remains to be elucidated. We found no evidence of nuclear import being involved in condensin regulation. We also demonstrated that expression levels of SM-C proteins in the condensin complex do not vary throughout the cell cycle, whereas transcription (Spellman et al., 1998) and protein levels of BRN1, YCS4, and YCS5 are under only mild cell cycle control (data not shown). It is therefore unlikely that expression level is the regulatory mechanism responsible for the cell cycle control of condensin activity in S. cerevisiae. Regulation of S. cerevisiae condensin activity by posttranslational modifications is an open possibility, although neither of the phosphorylation sites identified in X. laevis or S. pombe condensin are conserved in S. cerevisiae condensin.

In conjunction with the observations discussed above, it is clear that onset of mitosis induces a fundamental and complex change in the nature of chromatin-bound condensin complex. This study is focused on the biological role of chromatin condensation in budding yeast and on characterization of the functionally critical chromatin sites affected by condensin activity.

**Condensin and Mitotic Chromosome Transmission**

Previous observation of bulk nuclear DNA in the smc2-6 mutant has established that chromosome transmission in mitosis is disrupted in this mutant. Here we demonstrate that both the novel temperature-sensitive allele smc2-8 as well as the smc4-1 mutation in the newly characterized SM-C4 gene impair proper partitioning of chromosomes in mitosis (Fig. 2 E). Both of these mutants also disrupt establishment of mitotic condensation of rDNA and chromosome VIII (Fig. 3), suggesting that this defect may be responsible for the resulting chromosome segregation block. This reinforces previous conclusions that one biological role of condensin in vivo is to establish the higher order structure of mitotic chromosomes necessary for successful sister chromatid separation at anaphase (Koshland and Strunnikov, 1996).

Since the initial characterization of the smc2 mutant, one question has, however, remained unresolved: why obvious impairment of mitosis in a condensin component mutant does not translate into a genetically tractable event of chromosome loss and/or nondisjunction. We find that mutations in condensin components can indeed be detected genetically as chromosome instability, but the effect depends on the composition of the chromosome. The presence or absence of rDNA on a chromosome affects its loss rate in condensin mutants.

S. cerevisiae chromosomes lacking rDNA appear to show only a transient block in mitotic segregation in condensin mutants. A typical S. cerevisiae chromosome contains mostly unique nonrepetitive DNA, with the exception of the telomeric regions. For such chromosomes, exemplified by chromosomes III, V, and IV analyzed here, a high frequency of separated, properly oriented chromatids that did not complete anaphase B (Fig. 2 E and Fig. 5 B) is observed in condensin subunit mutants. There is also evidence that chromosomal arms frequently exhibit delayed segregation (Fig. 5 C). Yet increased loss of chromosome III is not observed in these mutants. It is not presently known whether this anaphase lock is due to an unknown feedback control arresting the cell cycle at anaphase or to the structural interlocks between the chromatid arms holding them together. The genetic interaction of condensin subunit mutants with topoisomerase mutants (Saka et al., 1994; Bhat et al., 1996) and the supercoiling activity of condensin in vitro (Kimura et al., 1999) support the latter theory. We can hypothesize that inability to detect high rates of chromosome III loss in our study was due to the eventual resolution of these tangles by topoisomerase II activity, allowing for recovery after the temperature shift and resulting in relatively high viability, despite the clear condensation defects throughout the genome (Fig. 3).

For the rDNA-containing chromosome XIII, however, the condensin mutations lead to a phenotype consistent with a virtually complete block of mitotic segregation. In this case, we found an almost complete absence of sister chromatid separation (Fig. 5 A). Unsegregated rDNA resided in one of the cells in the dividing pair, potentially leading to lethality of one half of the newly generated cells. Indeed, both smc2 and smc4 mutants display 30–50% viability after the 4-h temperature shift (Fig. 2 B). If nondisjunction had affected other chromosomes, this value should have been significantly lower. These observations, coupled with the impairment of rDNA condensation in condensin mutants, suggest that condensin defects may affect rDNA segregation in a distinct manner.

This hypothesis is supported by our finding that genetic instability of a chimeric chromosome III containing a rDNA translocation increases dramatically in the smc2 and smc4 mutants compared with the wild-type cells. More corroborating evidence for this theory is the discovery that condensin localization in vivo is strongly biased towards rDNA. We found that the rDNA array has a high density of Smc2p and Ycs5p binding sites and that Smc4p-GFP
concentrates in the nucleolar region at the G2/M transition. We can speculate that both the high density of binding and the mitotic-specific relocation reflect the important role of condensin in the formation of higher order structure of the rDNA region. Impaired condensation of the whole rDNA chromosomal region may explain the severity of the defects observed in chromosomes carrying rDNA in the condensin subunit mutants.

Specificity of Condensin Binding to Chromatin

The data presented in this study argue that in a cell containing a chromosomal rDNA array, an essential function of condensin is to properly condense and thus facilitate segregation of this chromatin domain. ChIP analysis of condensin binding to rDNA chromatin at 300-bp resolution reported here suggests that condensin binding is strongly biased towards specific chromatin sites. This is the first evidence of such bias in vivo. In vitro, however, condensin preferably binds to structured DNA (Kimura and Hirano, 1997). Thus, we are now in a position to uncover what the rules are for condensin binding to chromatin in live cells. Many factors may contribute to this specificity, such as structure of the underlying DNA, kinetics of chromatin assembly on this DNA, or occupancy of the condensin-specific sites by nonhistone proteins. For example, the rDNA ARS may not be compatible with strong condensin binding due to occupation by the origin recognition complex. At the same time, some condensin binding sites found within the rDNA locus correlate well with known Sir2p and Net1p binding sites in rDNA (Gotta et al., 1997; Straight et al., 1999). A region of condensin binding in the nontranscribed spacer (probes 18–21 in Fig. 6) also correlates well with the major rDNA sir-responsive region SRR1 observed by Fritze et al. (1997), whereas the second described sir-responsive region SRR2 does not appear to correlate with sites of condensin binding. Thus, it appears theoretically possible to identify chromatin proteins, including histones, and corresponding mutants that will interfere with condensin binding or will be indifferent. Mutations in Sir2 and NET1, for example, do not affect condensin targeting (our unpublished observation).

The high density of short inverted repeats in the rRNA genes may itself attract condensin binding, since it has been shown that condensin binding in vitro prefers structured or cruciform DNA (K. Iwama and H. Hirano, 1997). This hypothesis correlates well with our experimental result that artificial chromosomes, containing multiple mammalian satellite sequences, were less stable in vivo than in the artificial chromosomes, containing multiple mamma-

Condensin Function and the Cell Cycle

When rDNA segregation and centromere-driven segregation are uncoupled, as in strains with the plasmid-born rDNA, condensin function still remains essential. The provocative findings that the condensin complex is partially assembled on rDNA chromatin throughout the cell cycle opens the possibility that the condensin complex in S. cerevisiae may have another role in rDNA chromatin that is not directly associated with mitotic chromosome segregation. Such function may be related to the unique heterochromatin-like properties of the rDNA locus (Bryk et al., 1997, Fritze et al., 1997). Alternatively, binding to the condensation site throughout the cell cycle may provide an effective way to complete condensation in the small window of time allowed by the short yeast cell cycle.

The failure to segregate rDNA in mitosis described here for condensin subunit mutants is reminiscent of the mutant cdc14 phenotype (Granot and Snyder, 1991). In this mutant the nucleolus fails to segregate, unlike in another late mitotic mutant, cdc15. Interestingly, the cdc14 mutant was found to be defective in rDNA condensation (Guacci et al., 1994), whereas the cdc15 mutant was not. Cdc14p is an abundant nucleolar protein playing a key role in mitotic regulation (Visintin et al., 1998, 1999; Shou et al., 1999). A’condensin is highly enriched in the nucleolus, it is intriguing to speculate that condensin targeting to rDNA chromatin and its function there could be linked to the anaphase entry and exit from mitosis, processes dependent on nucleolar proteins.

We thank G. Fink, A. Straight, A. Murray, E. Jones, S. Brill, S. Liebman, Y. Chernoff, S. Roeder, M. VanDeRber, J. Den Dunnen, M. Nomura, O. Cohen-Fix, and V. Larionov for research materials; D. Koshland, I. Ouspenski, O. Cabello, V. Zakian, and M. Christman for advice and communicating results before publication; A. Wolfe for the comments on the manuscript; and R. Jenkins for technical help.

L. Freeman and L. Aragon-Alcaide were recipients of the National Institute of Child Health and Human Development Intramural Research Training Awards.

Submitted: 18 May 1999
Revised: 22 March 2000
Acepted: 29 March 2000

References

Bhat, M.A., A.V. Philp, D.M. Glover, and H.J. Bellen. 1996. Chromatid segregation at anaphase requires the barren product, a novel chromosome-associated protein that interacts with Topoisomerase II. C. Cell. 87:1103–1114.

Britton, R.A., D.C. Lin, and A.D. Groisman. 1998. Characterization of a prokaryotic SMC protein involved in chromosome partitioning. Genes Dev. 12:1254–1259.

Bryk, M., M. Banerjee, M. Murphy, K.E. Knudsen, D.J. Garfinkel, and M.J. Curcio. 1997. Transcriptional silencing of Yt1 elements in the RDN1 locus of yeast. Genes Dev. 11:255–269.

Chuang, P., D. Albertyson, and B. Meyer. 1994. D. PY: -27: a chromosome condensation protein homolog that regulates C. elegans dosage compensation through association with the X chromosome. Cell. 79:459–474.

Chuang, P.T., J.D. Lieb, and B.J. Meyer. 1996. Sex-specific assembly of a dosage compensation complex on the nematode X chromosome. Science. 274:1736–1739.

Cubizolles, F., V. Legagneux, R. Le Guicelle, I. Chartrain, R. Uzkov, C. Ford, and K. Le Guellec. 1998. pex7, a new xeropus protein required for mitotic chromosome condensation in egg extracts. J. Cell Biol. 143:1437–1446.

Fritze, C.E., K. Verschueren, R. Strich, and R. Easton Esposito. 1997. Direct evidence for Sir2 modulation of chromatin structure in yeast rDNA. EMBO (Eur. Mol. Biol. Organ.) J. 16:6495–6509.

Galibert, F., D. Alexandraki, À. Bau, E. Boles, N. Chalwatzis, J.C. Chuat, F. Coster, C. Cziepluch, M. De Haan, H. Domdey, et al. 1996. Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X. EMBO (Eur. Mol. Biol. Organ.) J. 15:2031–2049.
Gietz, R.D., and A. Sugino. 1988. New yeast - Escherichia coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair re- 
striction sites. Gene. 76:527-534.

Gotta, M., S. Straath-Bösinger, H. Renaud, T. Laroche, B.K. Kennedy, M. Grunstein, and S.M. Gasser. 1997. Localization of Sir2p: the nucleolus as a compartment for silent information regulators. EMBO (Eur. Mol. Biol. Or- 
gan.) J. 16:3243–3255.

Ganot, V., D. Koshland, and A. Strunnikov. 1997. A direct link between sister chromatin cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47–57.

Hirano, T., R. Kobayashi, and T. Mitchison. 1994. A heterodimeric coiled-coil protein re- 
quired for mitotic chromosome condensation and segregation. J. Bacteriol. 180:5749–5755.

Hirano, T., R. Kobayashi, and M. Hirano. 1997. Condensins, chromosome condensation and sister chromatid pairing in budding yeast. J. Cell Biol. 135:517–530.

Hirano, T., K. Kimura, M. Hirano, and T. Hirano. 1998. Phosphorylation of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. 18:2523–2528.

Hirano, T., and T. Hirano. 1999. SMC-mediated chromosome mechanics: a conserved scheme that the nucleolus lies opposite the spindle pole body. Curr. Biol. 9:3273–3279.

Hirano, T. 1999. SMC-mediated chromosome mechanics: a conserved scheme for a bacterial SC homodimer. EMBO (Eur. Mol. Biol. Or- 
gan.) J. 17:7139–7148.

Hirano, T., M. Iino, and T. Hirano. 1998. A TP-dependent aggregation of single- 
stranded DNA by a bacterial SC homodimer. EMBO (Eur. Mol. Biol. Or- 
gan.) J. 17:7159–7171.

Hirano, T., M., and T. Hirano. 1999. SM C-mediated chromosome mechanics: a conserved scheme from bacteria to vertebrates? Genes Dev. 13:11–19.

Hirano, T., and T. Mitchison. 1994. A heterodimeric coiled-coil protein re- 
quired for mitotic chromosome condensation and segregation. J. Bacteriol. 176:449–458.

Hirano, T., R. Kobayashi, and M. Hirano. 1997. Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a Xenopus homolog of the Drosophila Barren protein. Cell. 99:511–521.

Koshland, D., and A. Strunnikov. 1996. Mitotic chromosome condensation. Trends Cell Biol. 6:145–149.

Koshland, D., and A. Strunnikov. 1997. A direct link between sister chromatin cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47–57.

Kosman, H., J., H. Shero, G. Cottarel, P. Philippens, and P. Hieter. 1988. Mutational analysis of the centromere DNA from chromosome VI of Saccha- romyces cerevisiae. Mol. Cell. Biol. 8:2523–2528.

Kimura, K., M. Hirano, R. Kobayashi, and T. Hirano. 1998. Phosphorylation of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. 18:2523–2528.

Koshland, D., and A. Strunnikov. 1996. Mitotic chromosome condensation. Trends Cell Biol. 6:145–149.

Koshland, D., and A. Strunnikov. 1997. A direct link between sister chromatin cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47–57.

Kosman, H., J., H. Shero, G. Cottarel, P. Philippens, and P. Hieter. 1988. Mutational analysis of the centromere DNA from chromosome VI of Saccha- romyces cerevisiae. Mol. Cell. Biol. 8:2523–2528.

Koshland, D., and A. Strunnikov. 1996. Mitotic chromosome condensation. Trends Cell Biol. 6:145–149.

Koshland, D., and A. Strunnikov. 1997. A direct link between sister chromatin cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47–57.

Kosman, H., J., H. Shero, G. Cottarel, P. Philippens, and P. Hieter. 1988. Mutational analysis of the centromere DNA from chromosome VI of Saccha- romyces cerevisiae. Mol. Cell. Biol. 8:2523–2528.

Koshland, D., and A. Strunnikov. 1996. Mitotic chromosome condensation. Trends Cell Biol. 6:145–149.

Koshland, D., and A. Strunnikov. 1997. A direct link between sister chromatin cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47–57.

Kosman, H., J., H. Shero, G. Cottarel, P. Philippens, and P. Hieter. 1988. Mutational analysis of the centromere DNA from chromosome VI of Saccha- romyces cerevisiae. Mol. Cell. Biol. 8:2523–2528.

Koshland, D., and A. Strunnikov. 1996. Mitotic chromosome condensation. Trends Cell Biol. 6:145–149.

Koshland, D., and A. Strunnikov. 1997. A direct link between sister chromatin cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 91:47–57.