Pds5p Is an Essential Chromosomal Protein Required for both Sister Chromatid Cohesion and Condensation in *Saccharomyces cerevisiae*

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Abstract. The *PDS5* gene (precocious dissociation of sisters) was identified in a genetic screen designed to identify genes important for chromosome structure. *PDS5* is an essential gene and homologues are found from yeast to humans. Pds5p function is important for viability from S phase through mitosis and localizes to chromosomes during this cell cycle window, which encompasses the times when sister chromatid cohesion exists. Pds5p is required to maintain cohesion at centromere proximal and distal sequences. These properties are identical to those of the four cohesion complex members Mcd1p/Scc1p, Smc1p, Smc3p, and Scc3p/Irr1p (Guacci, V., D. Koshland, and A. Strunnikov. 1997. *Cell*. 91:47–57; Michaelis, C., R. Ciosk, and K. Nasmyth. 1997. *Cell*. 91:35–45; Toth, A., R. Ciosk, F. Uhlmann, M. Galova, A. Schleiffer, and K. Nasmyth. 1999. *Genes Dev.* 13:307–319). Pds5p binds to centromeric and arm sequences bound by Mcd1p. Furthermore, Pds5p localization to chromosomes is dependent on Mcd1p. Thus, Pds5p, like the cohesin complex members, is a component of the molecular glue that mediates sister chromatid cohesion. However, Mcd1p localization to chromosomes is independent of Pds5p, which may reflect differences in their roles in cohesion. Finally, Pds5p is required for condensation as well as cohesion, which confirms the link between these processes revealed through analysis of Mcd1p (Guacci, V., D. Koshland, and A. Strunnikov. 1997. *Cell*. 91:47–57). Therefore, the link between cohesion and condensation is a general property of yeast chromosomes.

Key words: mitosis • chromosomes • cohesion • condensation • yeast

Introduction

After replication, the duplicated chromatids (sister chromatids) are associated along their entire length. During mitosis, the association or cohesion between sister chromatids is maintained while the sisters condense, attach to the mitotic spindle in a bipolar orientation, and congress to the metaphase plate. At the metaphase to anaphase transition, sister chromatid cohesion is dissolved synchronously on all chromosomes and sisters segregate to opposite poles via microtubule-dependent movement. Finally, the segregated sister chromatids decondense.

Eukaryotic chromosomes are large dynamic structures, making it a daunting task to identify the components responsible for cohesion and condensation. Insights into the timing, distribution, and mechanism of sister chromatid cohesion have been provided through studies of mammalian and yeast cells. Classical cytological analyses and fluorescent in situ hybridization (FISH)[1] indicated that in both mammals and yeast, sister chromatids are associated along their lengths from the time of replication until anaphase (Wilson, 1925; Selig et al., 1992; Guacci et al., 1993, 1994). Mammalian and yeast chromosomes also undergo cell cycle–dependent condensation (Wilson, 1925; Lawrence et al., 1988; Trask et al., 1989; Guacci et al., 1994). The similarity of yeast and mammalian chromosomes established budding yeast as a model system to study sister chromatid cohesion and condensation (Guacci et al., 1993, 1994). A method of green fluorescent protein (GFP)–tagging chromosomal loci in vivo further enhanced the yeast system (Straight et al., 1996; Michaelis et al., 1997). The use of FISH- or GFP-tagged loci as cytological tools combined with the powerful genetic and molecular tools available in yeast has led to the isolation of mitotic regulators and chromosomal structural proteins (Guacci et al., 1993, 1994,

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[1]*Abbreviations used in this paper:* ChIP, chromatin immunoprecipitation; FISH, fluorescent in situ hybridization; GFP, green fluorescent protein; HA, hemagglutinin.

1997b; Strunnikov et al., 1995; Funabiki et al., 1996; Yamamoto et al., 1996a,b; Michaelis et al., 1997; Furuya et al., 1998; Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000; Tanaka et al., 2000).

Chromosomal proteins essential for sister chromatid cohesion have been identified in yeast and vertebrates (Guacci et al., 1997b; Michaelis et al., 1997; Furuya et al., 1998; Losada et al., 1998; Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000; Tanaka et al., 2000). In budding yeast, these cohesion factors are Mcd1p (also known as Scc1p), Smc1p, Smc3p, Ctf7p (also known as Eco1p), Scc2p, Scc3p (also known as Irr1p), and Scc4p (Kurlandzka et al., 1995; Guacci et al., 1997b; Michaelis et al., 1997; Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000). These factors can be functionally divided into those that mediate cohesion (i.e., form the molecular "glue") and those that are required to establish cohesion but are not part of the glue.

Several lines of evidence indicate that Mcd1p, Smc1p, Smc3p, and Scc3p are part of the molecular glue. First, these four proteins form a soluble complex termed cohesin, and are localized to chromosomes by early S phase (Losada et al., 1998). Second, Mcd1p is required for cohesion and cell viability during or soon after S phase, indicating an essential role at the time cohesion is established (Guacci et al., 1997b; Uhlmann and Nasmyth, 1998). Furthermore, Mcd1p is essential for cohesion in mitosis, demonstrating that it is required to maintain cohesion (Guacci et al., 1997b). Third, a functional site of cohesion was mapped to the yeast *CEN* DNA in vivo (Megee and Koshland, 1999). Subsequently, cohesin complex members Mcd1p and Smc1p were shown to localize to yeast *CEN* DNA, providing a direct link between the cohesin complex and a functional site of cohesion (Blat and Kleckner, 1999; Megee et al., 1999; Tanaka et al., 1999).

Several lines of evidence indicate that Ctf7p, Scc2p, and Scc4p are required for the establishment of cohesion. First, they are required for cohesion and cell viability during or soon after S phase, but are not required after S phase (Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000). Second, Scc2p and Scc4p are required for loading the cohesin complex onto chromosomes, but do not colocalize with the complex (Toth et al., 1999; Ciosk et al., 2000). Third, genetic interactions between Ctf7p and Pol30p (PCNA), a known replication protein, and Ctf18p, a replication factor C homologue, indicate that cohesion and replication are intrinsically linked (Skibbens et al., 1999). Taken together, Scc2p, Scc4p, and Ctf7p function to load the proteins that physically mediate cohesion and link the establishment of cohesion with DNA replication.

Chromosomal proteins essential for chromosome condensation have also been identified in yeast and *Xenopus* (Hirano and Mitchison, 1994; Saitoh et al., 1994; Saka et al., 1994; Strunnikov et al., 1995; Hirano et al., 1997; Sutani and Yanagida, 1997; Lavoie et al., 2000; Ouspenski et al., 2000). In budding yeast, Smc2p and Brn1p are required for chromosome condensation (Strunnikov et al., 1995; Lavoie et al., 2000; Ouspenski et al., 2000). Homologues of Smc2p, Smc4p, and Barren together with two other proteins form a soluble complex termed condensin (Hirano et al., 1997; Sutani et al., 1999). While the mechanism of condensation has not been determined, the condensin complex may function to induce supercoils in DNA to facilitate condensation (Kimura and Hirano, 1997; Kimura et al., 1999). In addition to their roles in cohesion and condensation, members of the Smc protein family have been implicated in dosage compensation, recombination, and transcriptional silencing (Chuang et al., 1994; Jessberger et al., 1996; Donze et al., 1999). One prominent characteristic of Smc protein family members is that they possess large coiled-coil domains that span most of their length (reviewed in Koshland and Strunnikov, 1996). It may be that their broad range of chromosomal functions is a consequence of specific interactions of a variety of proteins with Smc members via their coiled-coil domain.

The cohesin complex proteins are part of the molecular glue that mediates sister chromatid cohesion during mitosis. However, sister chromatid cohesion is a complex and highly regulated process and the cohesin complex is only part of the story (reviewed in Koshland and Guacci, 2000). The establishment of cohesion requires not only the loading of cohesin complex onto chromosomes, but also likely a coupling with DNA replication. The identification of the budding yeast Trf4p as a DNA polymerase required for sister chromatid cohesion supports this connection between cohesion and replication (Wang et al., 2000). Furthermore, the *Drosophila* MEI-S322 protein loads onto centromeric regions of chromosomes in prometaphase and is thought to function in the maintenance of cohesion at the centromere (Kerrebrock et al., 1995; Moore et al., 1998). Thus, there are region-specific cohesion factors that load onto chromosomes well after DNA replication. Finally, Mcd1p is required for condensation as well as cohesion (Guacci et al., 1997b). Therefore, we continued our screen to identify of additional components required for sister chromatid cohesion to help elucidate the mechanisms of sister chromatid cohesion and condensation. Here we have identified a new budding yeast protein, Pds5p (precocious dissociation of sister chromatids). Pds5p has 1,277 amino acids and shares homology with proteins from fungi (*Aspergillus nidulans* {BimD6} and

Table I. Yeast Strains Used in this Study

Strain	Genotype
VG958-7C	*Mat**a** pds5-3 trp1 leu2 bar1 gal1*
VG982-3A	*Matα trp1 leu2 ura3 bar1 gal1*
VG982-7B	*Matα trp1 leu2 bar1 gal1*
VG982-6A	*Mat**a** trp1 ura3 bar1 gal1*
VG986-5B	*Mat**a** pds5-1 trp1 ura3 bar1 gal1*
VG987-5C	*Mat**a** pds5-2 trp1 ura3 bar1 gal1*
VG988-1C	*Mat**a** pds5-3 trp1 ura3 bar1 gal1*
VG1328-2C	*Matα cdc15-2 trp1 ura3 gal1*
VG2043-8C	*Mat**a** trp1 lys2-801 bar1 gal1*
VG2049-3A	*Mat**a** MCD1-GFP ura3 bar1 gal1*
VG2050-1A	*Mat**a** MCD1-6HA ura3 bar1 gal1*
VG2061-1D	*Matα MCD1-GFP cdc15-2 ura3 leu2 gal1*
VG2063-5C	*Mat**a** PDS5-GFP trp1 ura3 lys2-801 bar1 gal1*
VG2064-2A	*Mat**a** PDS5-6HA trp1 ura3 lys2-801 bar1 gal1*
VG2069-1B	*Mat**a** PDS5-GFP cdc15-2 trp1 ura3 gal1*
VG2072-2D	*Mat**a** mcd1-1 PDS5-6HA trp1 ura3 bar1 gal1*
VG2073-7D	*Mat**a** pds5-1 MCD1-6HA leu2 ura3 bar1 gal1*
VG2074-6C	*Mat**a** pds5-2 MCD1-6HA leu2 bar1 gal1*
VG2073-7D	*Mat**a** pds5-3 MCD1-6HA leu2 ura3 bar1 gal1*
VG1325	*Mat**a** trp1 leu2 ura3 ade2 bar1 gal1*
	Matα trp1 leu2 ura3 ade3 bar1?gal1 can1
VG1359	*Mat**a** pds5::URA3 trp1 leu2 ura3 ade2 bar1 galI*
	Matα trp1 leu2 ura3 ade3 bar1?gal1 can1

Sordaria {Spo76}) and with putative proteins from *Schizosaccharomyces pombe* to humans (Denison et al., 1993; van Heemst et al., 1999). The BimD6 and Spo76 proteins are essential for mitotic and meiotic chromosome segregation, respectively (Denison et al., 1993; van Heemst et al., 1999). Here we demonstrate Pds5p is an essential chromosomal protein required for chromosome segregation and sister chromatid cohesion during mitosis. Thus, Pds5p has functional homology with BimD6 and Spo76. Furthermore, the localization of Pds5p to chromosomes is dependent on the cohesin complex member Mcd1p. Finally, we show that Pds5p is also required for chromosome condensation during mitosis, confirming that sister chromatid cohesion and condensation are linked.

Materials and Methods

Reagents and Media

Reagents and media were described previously (Guacci et al., 1997b; Yamamoto et al., 1996b). Benomyl was a gift from Dupont. Yeast strains are listed in Table I.

Identification of Mutants Exhibiting Mitotic Lethality

A collection of temperature-sensitive mutants (Ts^-) were patched to YEPD plates and grown at 23°C. Cells were replica plated to media containing either α factor (10^{-8} M) or benomyl (70 μg/ml) and incubated at 23°C until cells arrested in either G1 or mid-M phase, respectively. Plates were transferred to 37°C for 3 h to inactivate the mutant gene product while cells were arrested, and then replica plated to YEPD and incubated at 23°C for 48 h to release cells from arrest and allow growth. Mutants showing enhanced lethality after transient arrest in mid-M phase compared with G1 phase were chosen for study.

Cloning the PDS5 Gene

Three mutants with enhanced mitotic lethality and precocious dissociation of sister chromatids fell into a single complementation group based on a failure to grow at 37°C when mated, and so were renamed *pds5-1*, *pds5-2,* and *pds5-3*. Strains were back-crossed and the Ts^- and mitotic lethality phenotypes each segregated 2+:2− and cosegregated. For analysis of *pds5* phenotypes, original isolates were backcrossed five times to wild-type strains to generate haploids VG986-5B (*pds5-1*), VG987-5C (*pds5-2*), and VG988-1C (*pds5-1*). The *PDS5* gene was cloned by transforming haploid VG958-7C (*pds5-3*) with a pRS314 (*CEN ARS TRP1*) vector-based yeast genomic library (provided by P. Hieter, University of British Columbia, Vancouver, BC, Canada). Transformed cells were plated onto Trp^- media, incubated 22 h at 23°C, and then transferred to 37°C for 3 d. Five clones grew at 37°C and contained identical plasmids (8B). A 5.8-kb ClaI fragment from plasmid 8B complemented both the Ts^- and mitotic lethal phenotypes of *pds5* mutants. The ClaI insert ends were sequenced and contained ORF YMR076C from chromosome XIII.

PDS5 Is an Essential Gene

Plasmid pVG177 contains a 5.8-kb ClaI fragment bearing *PDS5* in vector pRS314. The *URA3* gene on a 1.2-kb HindIII fragment (blunt ended using Klenow) was inserted into plasmid pVG177 at the BstEII site (blunt ended using Klenow) to form plasmid, pVG199, which has *URA3* inserted after amino acid 114 of the *PDS5* ORF (*pds5*::*URA3*). Plasmid pVG199 was digested with ClaI and transformed into diploid VG1325. Ura^+ transformants were analyzed by Southern blot to identify clones with *pds5*::*URA3* on one chromosome and wild-type *PDS5* on the homologue, which were renamed as diploid VG1359.

To determine whether *PDS5* is an essential gene, wild-type parent VG1325 (*PDS5*/ *PDS5*) and VG1359 (*pds5*::*URA3*/*PDS5*) diploid cells were sporulated and dissected. VG1325 (*PDS5*/ *PDS5*) had a 98% spore viability. In contrast, VG1359 (*pds5*::*URA3*/*PDS5*) had only a 47% spore viability. Of 29 tetrads dissected, 25 were 2:2 (viable:inviable) and 4 were 1:3 tetrads. All viable spores were Ura^- indicating that none contained the *pds5::URA3* allele. Microscopic examination of spores that failed to form colonies revealed 98% had two or more cell bodies, demonstrating that virtually all spores germinated and attempted one or more cell divisions before dying. Finally, strain VG1359 was transformed with plasmid pTH10, a centromere plasmid bearing *PDS5* and *TRP1*, sporulated, and dissected. Spore viability increased to 65%. Of 10 tetrads dissected, 1 was 4:0 (viable:inviable), 4 were 3:1, and 5 were 2:2. Viable Ura^+ spores were obtained and all were also Trp^+, indicating that spores containing the *pds5::URA3* allele grew if they contained plasmid pTH10. These data demonstrate that *PDS5* is an essential gene.

COOH-terminal Tagging of Pds5p and Mcd1p

Strains were constructed to contain either a COOH-terminally tagged Pds5p (Pds5p-6HA or Pds5p-GFP) or Mcd1p (Mcd1p-6HA or Mcd1p-GFP) under control of their endogenous promoters as the sole source of Pds5p or Mcd1p, respectively. Haploids with a tagged Pds5p were made as follows: a BamHI site was generated after the last amino acid of the *PDS5* ORF by PCR and either a 0.26-kb BamHI fragment bearing six tandem hemagglutinin (HA) epitopes or a 0.75-kb BamHI fragment bearing GFP was inserted to form plasmid pVG264 or pVG263, respectively. These plasmids have a pRS406 backbone, *PDS5* promoter, ORF with the tag and 3′UTR. Plasmids pVG264 and pVG263 were linearized with AflII, transformed into haploid VG982-3A, and plated onto Ura^- media to create tandem integrants at the *PDS5* genomic locus. Ura^+ transformants were streaked on FOA-containing plates and Ura^- cells (popouts) were screened by PCR to identify those retaining tagged *PDS5* alleles. These were crossed to haploid VG2043-8C and the resulting diploids sporulated and dissected to generate VG2064-2A (*PDS5-6HA*) and VG2063-5C (*PDS5-GFP)*. Haploid VG2063-5C was crossed to VG1328-2C and the resulting diploid was sporulated and dissected to generate haploid VG2069-1B (*cdc15-2 PDS5-GFP*).

To make haploids containing a tagged Mcd1p, a BamHI site was generated by PCR after the last amino acid of the *MCD1* ORF and either the 6HA or GFP fragment inserted to form plasmid pVG269 or pVG270, respectively. These plasmids have a YIplac211 backbone, the *MCD1* promoter, ORF with the tag, and 3′UTR. Plasmids pVG269 and pVG270 were linearized with AgeI, transformed into haploid VG982-3A. Cells bearing only *MCD1-6HA* or *MCD1-GFP* alleles identified as described for tagged Pds5p. Strains bearing a tagged Pds5p or Mcd1p grew at rates similar to isogenic wild-type strains, indicating that the tag did not compromise protein function.

Yeast Cell Culture Conditions

Yeast cells were grown in YPD at 23°C to mid-log phase then treated as follows.

Arrest at G1, S or Mid-M Phase. α factor (10^{-8} M), hydroxyurea (HU; 100 mM), or nocodazole (15 μg/ml) was added to arrest cells in G1, S, or mid-M phase, respectively, as described (Guacci et al., 1997b).

Synchronous Populations of Cycling Cells Released From S Phase. Cells were treated as described (Guacci et al., 1997b), except that α factor (10^{-8} M) was added to media upon release from S phase.

Synchronous Populations of Mid-M Phase Cells Obtained after Release from G1 Phase. Cells were treated as described (Guacci et al., 1997b).

Fluorescence In Situ Hybridization

FISH was performed as described as described (Guacci et al., 1994, 1997a). Probes used for analysis of cohesion and condensation were described (Guacci et al., 1994, 1997a,b).

Chromosome Spreads

Chromosome spreads were performed as described (Michaelis et al., 1997). HA-tagged proteins were detected using mouse anti–HA antibody 16B12 (Babco) (1:2,500) and Cy3-conjugated goat anti–mouse antibody (Jackson ImmunoResearch Laboratories) (1:3,000).

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) and the PCR analysis was performed as described (Megee et al., 1999).

Fluorescence Microscopy and Flow Cytometry

Indirect immunofluorescence and flow cytometry was described (Yamamoto et al., 1996a). Images were collected using an epi-fluorescence microscope (E800; Nikon) and recorded digitally using a Hamamatso cooled CCD camera with processing software from Phase 3 Imaging, which allowed image superimposition.

Results

PDS5 Was Isolated in a Screen Designed to Identify Genes Encoding Proteins Important for Sister Chromatid Cohesion

We previously used a cytogenetic screen to isolate mutants defective in sister chromatid cohesion and identified *pds1* and *mcd1* mutants (Guacci et al., 1993, 1997b; Yamamoto et al., 1996a,b). Here we describe the characterization of *pds5* (precocious dissociation of sister chromatids), another mutant identified by this screen. In brief, a collection of mutants temperature sensitive for growth (Ts^-) were screened to identify those exhibiting enhanced lethality after transient arrest in mid-M phase as compared with G1 phase. Mutants that had enhanced mitotic lethality were screened by FISH to identify those exhibiting precocious dissociation of sister chromatids (see below). Three mutants formed one complementation group and were named *pds5-1*, *pds5-2,* and *pds5-3*. All three mutants showed decreased viability at 30°C and no growth at 37°C, indicating that each allele was defective for function at high temperature (Fig. 1 A). Even at 23°C, the *pds5* allele have some growth defects since *pds5* cells form heterogeneously sized colonies and only ~80% of *pds5* cells form colonies on YEPD plates, compared with almost 100% for wild-type cells (data not shown).

To more precisely assess the stage of the cell cycle where Pds5p function is essential, wild-type and *pds5* haploid cells were arrested in either G1, S, or mid-M phases using either α factor, hydroxyurea, or nocodazole, respectively, and then incubated 2 h at 37°C while still arrested (see Materials and Methods). Aliquots of cells were washed free of the arresting agent and plated on YEPD to determine cell viability (Fig. 1 B). In G1 phase, *pds5* cells maintained viability when shifted to 37°C. In S phase, *pds5* cells showed only a 1.5-fold decrease in viability when incubated at 37°C. However, in mid-M phase, *pds5* cells showed a 3-fold decrease in viability at 23°C and a 40-fold decrease to 2% viability when shifted to 37°C. Thus, Pds5p function is not required in G1 phase, but is essential in mid-M phase. The weak effect in S phase *pds5* cells indicates that Pds5p function may be required either during replication or soon after, since Pds5p localizes to chromosomes in early S phase (see below).

PDS5 Is an Essential Gene Required for Proper Chromosome Segregation

The *PDS5* gene was cloned by complementation of the Ts^- and mitotic lethal phenotypes of *pds5* cells (Fig. 1 C). Standard yeast genetic techniques were used to show that *PDS5* is an essential gene (Materials and Methods). The mitotic lethality of *pds5* cells suggested a function for Pds5p in chromosome structure or segregation. To determine the role of Pds5p in cell cycle progression and chromosome segregation, wild-type and *pds5* haploid cells grown to mid-log phase at 23°C were shifted to 37°C, and then cell, spindle, and DNA morphologies as well as DNA content were scored. At 23°C, FACS® (Becton Dickinson)

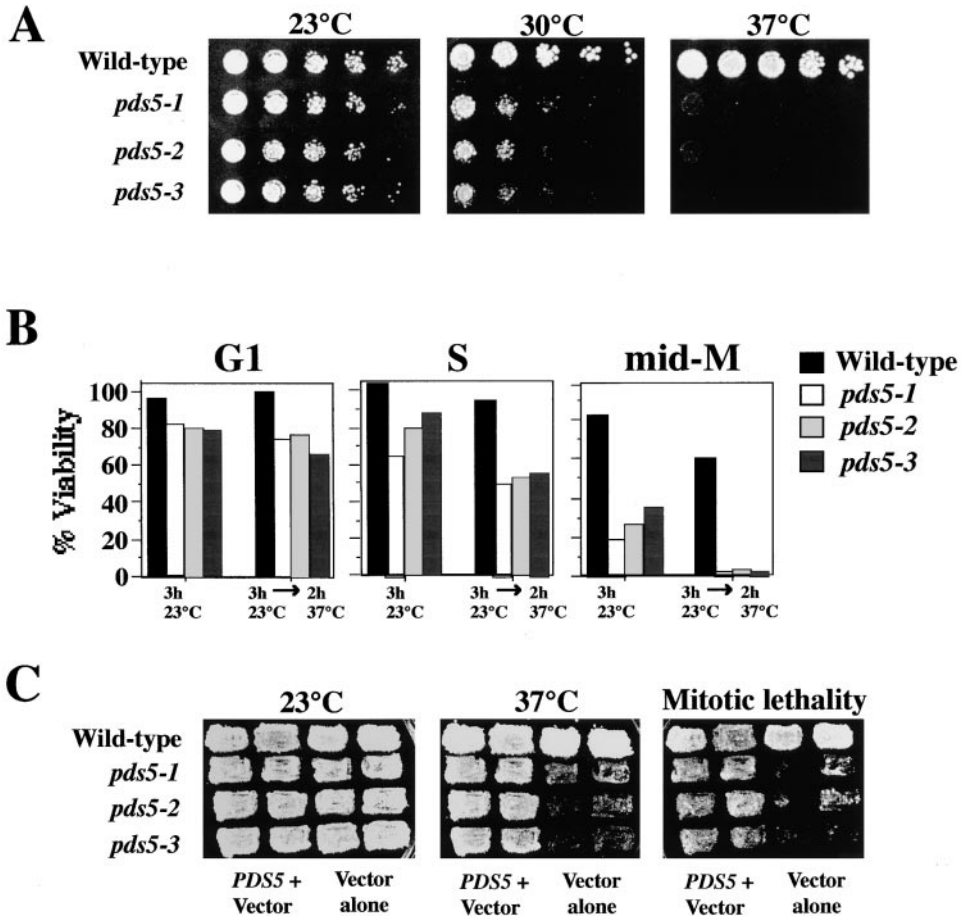

Figure 1. Characterization of *pds5* mutants. (A) Ts^- phenotype of *pds5* cells. Wild-type (VG982-6A), *pds5-1* (VG986-5B), *pds5-2* (VG987-5C), and *pds5-3* (VG988-1C) cells grown at 23°C in YPD liquid were plated in 10-fold serial dilutions on YPD and incubated at 23°, 30°, and 37°C. (B) Cell cycle–dependent lethality of *pds5* cells. Strains in A were arrested at 23°C in either G1, S, or mid-M phase, incubated at 37°C while arrested, and then plated on YPD at 23°C to determine percent viability (Materials and Methods). Data from three independent experiments was used to generate error bars. (C) Cloning of the *PDS5* gene. Strains in A were transformed with either a *CEN* vector alone or one bearing the *PDS5* ORF.

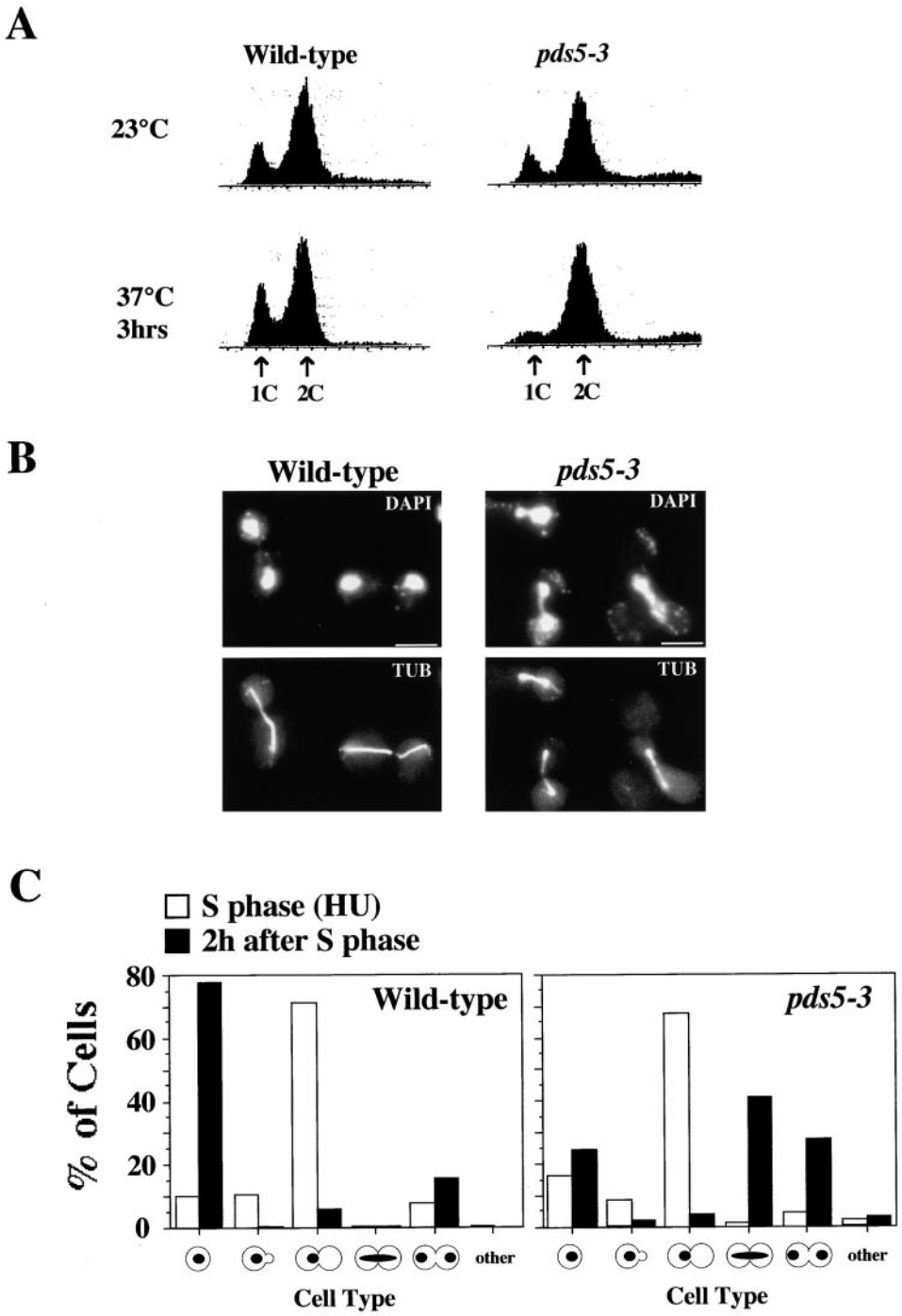

Figure 2. Cell cycle progression in wild-type and *pds5* mutant cells. (A) DNA content of cells. Wild-type (VG982-6A) and *pds5-3* (VG988-1C) cells were grown asynchronously at 23°C or shifted to 37°C for 3 h, and then subjected to FACS® analysis to measure DNA content (Materials and Methods). (B) Micrographs showing the mitotic spindle in cells 1 h after release from S phase at 37°C. Strains in A were synchronously released at 37°C from S phase arrest, and, after 1 h, cells were processed for immunofluorescence. Chromosomal DNA (gray) and Tubulin (white). (C) Quantitation of cell and DNA morphologies after release from S phase at 37°C. Strains in B were scored for cell and DNA morphologies in S phase arrested cells at 37°C [S phase (HU)] and in cells 2 h after release at 37°C (2 h after S phase). Data from 400 cells from two independent experiments were scored to generate error bars.

profiles of *pds5* cells were similar to wild-type cells (Fig. 2 A). However, after 3 or 6 h at 37°C, the *pds5* cultures were enriched for cells with 2C DNA content and showed a twofold increase in the frequency of large budded cells with short or partially elongated spindles (Fig. 2 A and data not shown). These phenotypes indicate that *pds5* cells accumulate in G2 and M phases of the cell cycle when grown at 37°C. This result is consistent with a defect in chromosome structure delaying cell cycle progression through mitosis.

Pds5 mutant cells were further characterized using synchronous populations of cycling cells obtained after release from S phase arrest. Asynchronously growing cultures of wild-type and *pds5* cells were grown to mid-log phase at 23°C, arrested in S phase by addition of hydroxyurea, and then transferred to 37°C for 1 h while in the presence of hydroxyurea to inactivate the mutant Pds5p in S phase (Materials and Methods). Finally, cells were released from S phase by transferring to YEPD media containing α factor at 37°C. This regimen allows cells to progress through mitosis at nonpermissive temperature for *pds5,* and then arrests cells in G1 phase to prevent progression through a new cell cycle (Materials and Methods). In S phase arrested cells at either 23° or 37°C, wild-type and *pds5* cells had a 1C DNA content, large bud, undivided DNA mass, and short spindle (Fig. 2 C, and data

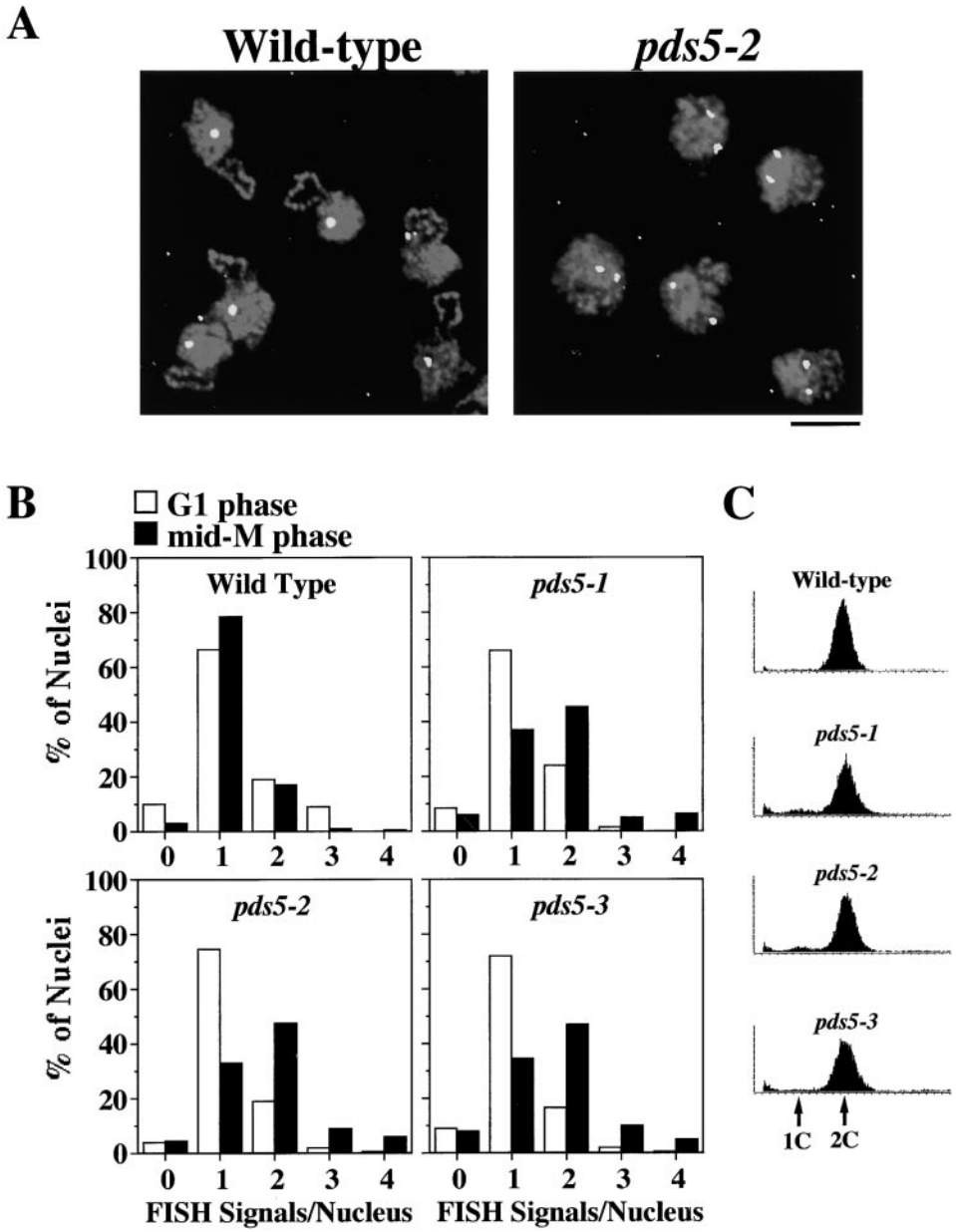

Figure 3. Sister chromatid cohesion wild-type and *pds5* cells. (A) Micrographs of mid-M phase cells subjected to FISH using a chromosome XVI *CEN*-distal probe distal (295 kb from *CEN16*). Wild-type (VG982-6A) and *pds5-2* (VG987-5D) cells were arrested in G1 phase [G1 (αF) 23°C], shifted to 37°C, and then released from G1 and arrested in mid-M phase {M (nocodazole) 37°C}. Mid-M cells were processed for FISH. Chromosomal DNA (gray) and hybridized probe (white). Bar, 5 μm. (B) Quantitation of FISH of G1 and mid-M phase cells. Wild-type (VG982-6A), *pds5-1* (VG986-5B), *pds5-2* (VG987-5C), and *pds5-3* (VG988-1C) were arrested and subjected to FISH as described in A. The number of FISH signals in each DNA mass was determined and plotted as a percentage of total nuclei. 200 nuclei from G1 and mid-M cells were scored to generate data. (C) DNA content of mid-M phase cells. Aliquots of cells from strains in B were fixed and processed for flow cytometry to analyze for DNA content in mid-M phase–arrested cells at 37°C.

not shown). 1 h after release from S phase at 37°C, both wild-type and *pds5* cells had completed DNA replication as indicated by a 2C DNA content (data not shown). Most wild-type cells had also completed chromosome segregation since they had an elongated spindle and segregated DNA masses (Fig. 2 B). In contrast, most *pds5* cells had a stretched nuclear DNA mass and partially elongated spindle, indicative of a chromosome segregation defect (Fig. 2 B). 2 h after release, 75% of wild-type cells were unbudded, which demonstrated that they had exited mitosis and arrested in G1 phase (Fig. 2 C). In contrast, only 25% of *pds5-3* cells were unbudded while the majority remained in mitosis as large budded cells in either anaphase with a stretched DNA mass or in telophase with separated DNA masses (Fig. 2 C). After 4 h, the number of unbudded *pds5-3* cells increased to 63%. Similar results were obtained with *pds5-1* and *pds5-2* cells (data not shown). These data suggest that *pds5* inactivation causes a mitotic defect that disrupts chromosome segregation and delays, but does not prevent, exit from mitosis.

Pds5p Is Required for Sister Chromatid Cohesion

We next assayed whether *pds5* cells are defective in sister chromatid cohesion. Wild-type, *pds5-1*, *pds5-2,* and *pds5-3* haploid strains were arrested in G1 phase at 23°C incubation in YEPD liquid containing α factor, and then transferred to 37°C for 1 h to inactivate the mutant Pds5p in G1 phase (Materials and Methods). Cultures were then released from G1 phase arrest at 37°C by transferring cells to YEPD liquid-containing nocodazole to arrest cells in mid-M phase at 37°C (Materials and Methods). In this protocol, cells were at the nonpermissive temperature for the *pds5* mutation through S, G2, and mid-M phases, which encompasses the time when sister chromatid cohesion is established and maintained. Aliquots of G1 and mid-M phase cells were processed for FISH using a chromosome XVI centromere-distal probe to assay cohesion at a site 295 kb from the centromere (Materials and Methods). Consistent with our previous studies, most wild-type cells in mid-M phase had one FISH signal per nuclear DNA mass, demonstrating that sister chromatids were paired

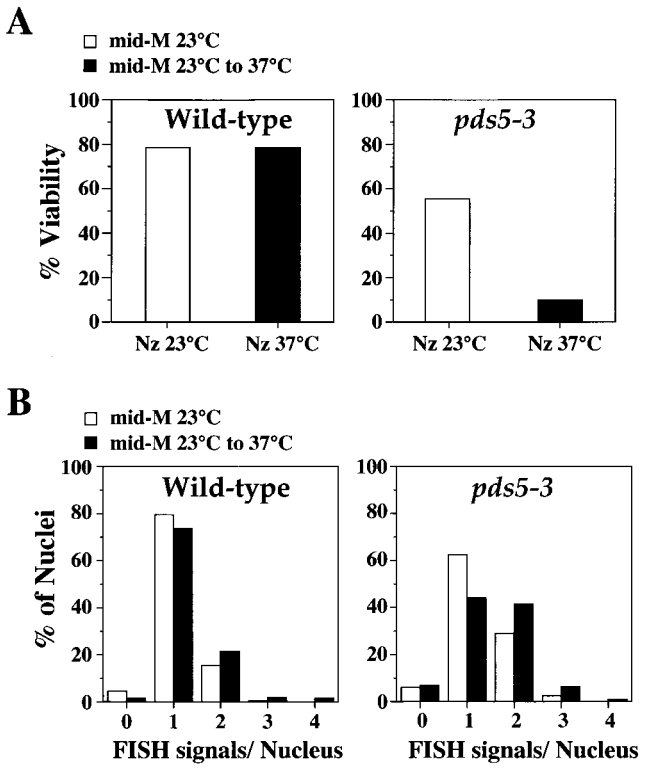

Figure 4. Maintenance of sister chromatid cohesion in wild-type and *pds5* cells arrested in mid-M phase. Wild-type (VG982-6A) and *pds5-3* (VG988-1C) cells were arrested in G1 phase at 23°C, and then released from G1 phase and arrested in mid-M phase at 23°C. Cells were transferred to 37°C for 1 h while still arrested. Aliquots of mid-M phase cells at 23° and 37°C were plated to determine cell viability (A) and processed for FISH (B). (A) Cell viability. Mid-M phase cells at 23° and 37°C were plated onto YEPD and grown for 3 d at 23°C, and the percentage cell viability was calculated (Materials and Methods). Data was generated from two independent experiments. (B) Sister chromatid cohesion. Mid-M phase cells at 23° and 37°C were processed for FISH using a chromosome XVI centromere distal probe (cosmid 70912). The number of FISH signals in each DNA mass was quantitated as described in Fig. 3 B.

(Fig. 3, A and B). In a small number of DNA masses, two FISH signals were detected due to either a low level of precocious sister chromatid dissociation or spurious background (Fig. 3 B). In contrast, most *pds5* cells in mid-M phase at 37°C had two FISH signals, indicating that sister chromatids had precociously dissociated (Fig. 3, A and B). The second FISH signal in mid-M phase *pds5* cells was not due to preexisting aneuploidy since in G1 phase most cells had only one FISH signal (Fig. 3 B). The two FISH signals detected in a few nuclei from G1 phase wild-type and *pds5* cells were likely due to spurious background. Similar results were obtained using probes from *CEN*-proximal regions of chromosomes I, IV, and XVI (data not shown). Thus, Pds5p is required for sister chromatid cohesion at both *CEN*-proximal and distal chromosomal regions.

Cells defective in the mitotic checkpoint precociously dissociate their sister chromatids as a consequence of their failure to arrest in response to spindle damage (Straight et al., 1996; Yamamoto et al., 1996b). However, *pds5* mutants have a functional mitotic checkpoint since nocodazole-treated *pds5* cells do not undergo new rounds of DNA replication or new bud formation (Fig. 3 C, and data not shown). Therefore, the precocious dissociation of sister chromatids in *pds5* cells is not due to a defect in cell-cycle regulation, but reflects a potential role for Pds5p in sister chromatid cohesion.

While we have shown that *pds5* mutants are defective for cohesion, the previous regimen does not distinguish whether the defect is in the establishment or the maintenance of cohesion. We used a different regimen to distinguish between these possibilities by inactivating the mutant Pds5p after cohesion had been established. Wild-type and *pds5-3* haploid cells were arrested in G1 phase at 23°C using α factor, released from G1 phase and arrested in mid-M phase at 23°C by treatment with nocodazole, and then transferred to 37°C to inactivate the mutant Pds5-3p in mid-M phase (Materials and Methods). Aliquots of mid-M phase cells at 23° and 37°C were plated for cell viability and processed for FISH using a centromere-distal probe from chromosome XVI. As expected, most wild-type cells in mid-M phase at 23° and 37°C were viable and had one FISH signal (Fig. 4, A and B). Similarly, most *pds5-3* cells in mid-M phase at 23°C were viable and had one FISH signal. However, upon shift of mid-M phase *pds5-3* cells to 37°C, viability decreased and the number of cells with two FISH signals increased (Fig. 4, A and B). Similar results were obtained using FISH probes from centromere-proximal regions of chromosomes IV and XVI (data not shown). These results demonstrate that Pds5p function is required to maintain sister chromatid cohesion in mid-M phase at centromere proximal and distal chromosomal regions.

Pds5p Is Required for Chromosome Condensation

We had previously shown that the *mcd1* mutant was defective in chromosome condensation as well as in sister chromatid cohesion (Guacci et al., 1997b). Therefore, we used FISH to determine whether *pds5* mutants were also defective for condensation. Wild-type, *pds5-1*, *pds5-2,* and *pds5-3* haploid cells were arrested in G1 phase at 23°C using α factor, incubated at 37°C for 1 h, and then released from G1 phase and arrested in mid-M phase at 37°C using nocodazole and processed for FISH (Materials and Methods). First, we examined chromosome condensation at the rDNA locus, a 500-kb block of repetitive DNA. In G1 phase cells, an amorphous rDNA FISH characteristic of a decondensed chromosome is detected, while in mid-M phase haploid cells, the FISH signal formed a single line, characteristic of a condensed chromosome with paired sister chromatids (Guacci et al., 1994). As expected, in wild-type cells arrested in mid-M phase, the FISH signal formed a single line at the edge of the DNA masses (Fig. 5 A). In contrast, in *pds5-1*, *pds5-2*, and *pds5-3* cells in mid-M phase, an amorphous FISH signal reminiscent of decondensed rDNA was detected (Fig. 5 A, and data not shown). An amorphous FISH signal is not expected if sisters chromatids had simply dissociated since in wild-type cells, precociously dissociated sisters are detected as two line-like signals (Guacci et al., 1997b). Furthermore, in anaphase nuclei from cycling wild-type cells, the rDNA is line-like after sister chromatids have separated and segregated (Guacci et al., 1994). Thus, *pds5* cells in mid-M phase at 37°C have rDNA morphology indicative of a defect in condensation and not merely a dissociation of sister chromatids.

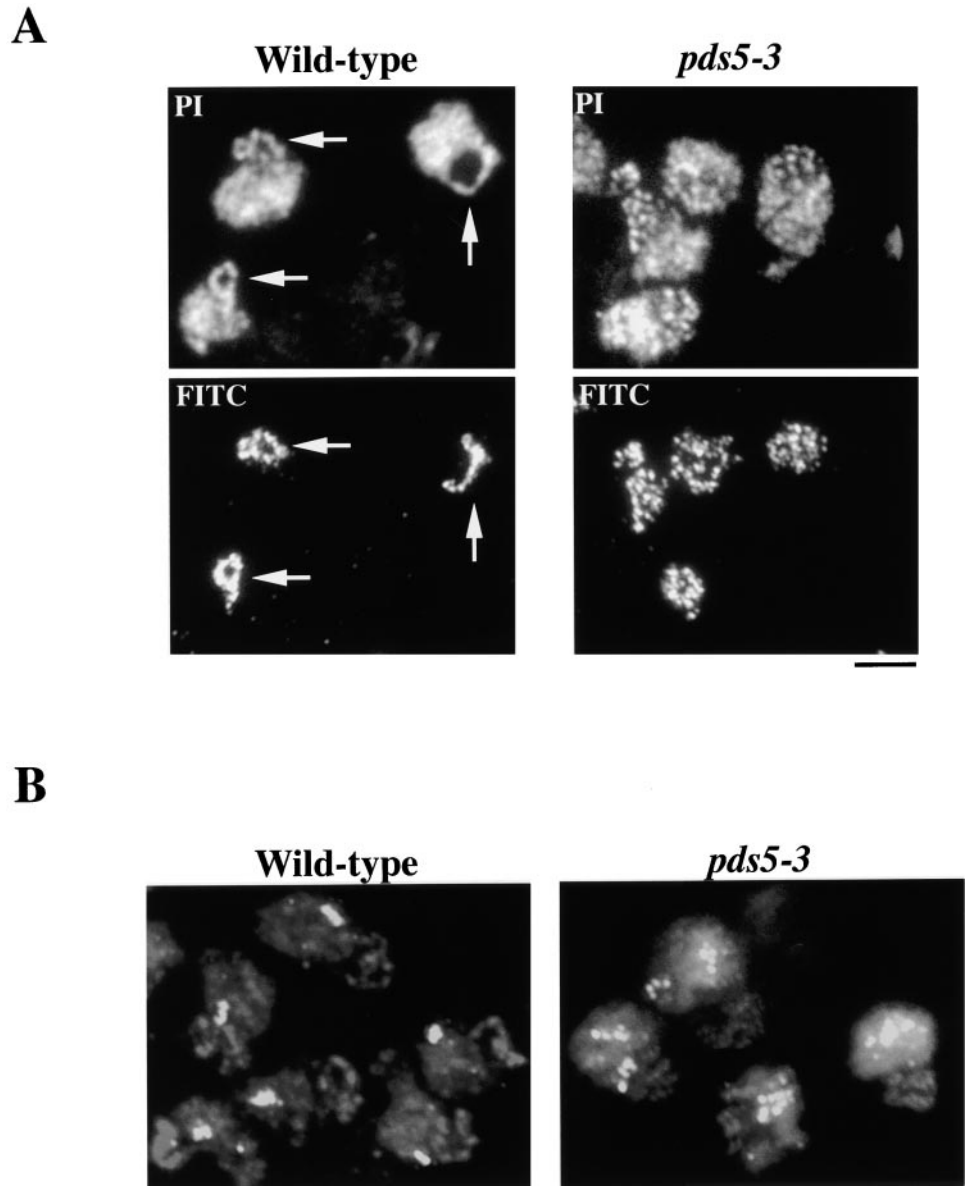

Figure 5. Analysis of chromosome condensation in wild-type and *pds5* cells. Wild-type (VG982-6A) and *pds5-3* (VG988-1C) cells were synchronously arrested in mid-M at 37°C, as described in Fig. 3 A. (A) Micrographs showing FISH of the rDNA. Mid-M phase cells at 37°C subjected to FISH using rDNA as probe. Chromosomal DNA (PI) and hybridized probe (FITC) are shown. Arrows indicate line-like rDNA in wild-type cells. Bar, 5 μm. (B) Micrographs showing FISH of chromosome VIII region. Mid-M phase cells at 37°C subjected to FISH using a mixture of six chromosome VIII probes. Chromosomal DNA (gray) and hybridized probe (white) are shown. Bar, 5 μm.

We also determined whether Pds5p is required for chromosome condensation at unique chromosomal regions. The number and spacing of FISH signals in cells hybridized with a mixture of chromosome VIII or XVI probes provides a qualitative measure of chromosome condensation. In haploid G1 phase cells hybridized with four chromosome XVI probes, four widely spaced FISH signals are often detected per nucleus, whereas in mid-M phase haploid cells a short line-like FISH signal with one or two closely associated FISH signals is detected (Guacci et al., 1994). These patterns indicate a change from a decondensed chromosome in G1 phase to a condensed chromosome with paired sister chromatids in mid-M phase. Wild-type and *pds5-3* haploid cells were arrested in mid-M phase cells at 37°C, as described above for rDNA FISH, and then subjected to FISH using a mixture of probes from either chromosome VIII or XVI (Materials and Methods). In wild-type cells, a single line-like FISH signal or a few closely associated FISH signals were detected in most nuclei (Fig. 5 B, and data not shown). By contrast, in *pds5-3* cells, a larger number of more widely spaced FISH signals were detected in many DNA masses (Fig. 5 B, and data not shown). Some of the increased numbers of FISH signals are expected due to precocious sister chromatid dissociation. However, if sister chromatids remained condensed there should be two tight lines or clusters of FISH signals, one from each separated and condensed sister chromatid. Instead, the dispersed FISH signals are reminiscent of two separated and decondensed chromosomes (Fig. 5 B). Similar results were obtained with *pds5-1* and *pds5-2* cells (data not shown). Taken together, the results from FISH using rDNA and chromosome VIII and XVI probes indicate that *pds5* cells exhibit defects in chromosome condensation as well as sister chromatid cohesion.

Pds5p Localizes to Chromosomes in a Cell Cycle–dependent Manner and Its Binding to Chromosomes Is Dependent on Mcd1p Function

The defects in mitotic chromosome structure detected in *pds5* mutants are consistent with a role for Pds5p as a chromosomal structural protein. To determine whether Pds5p

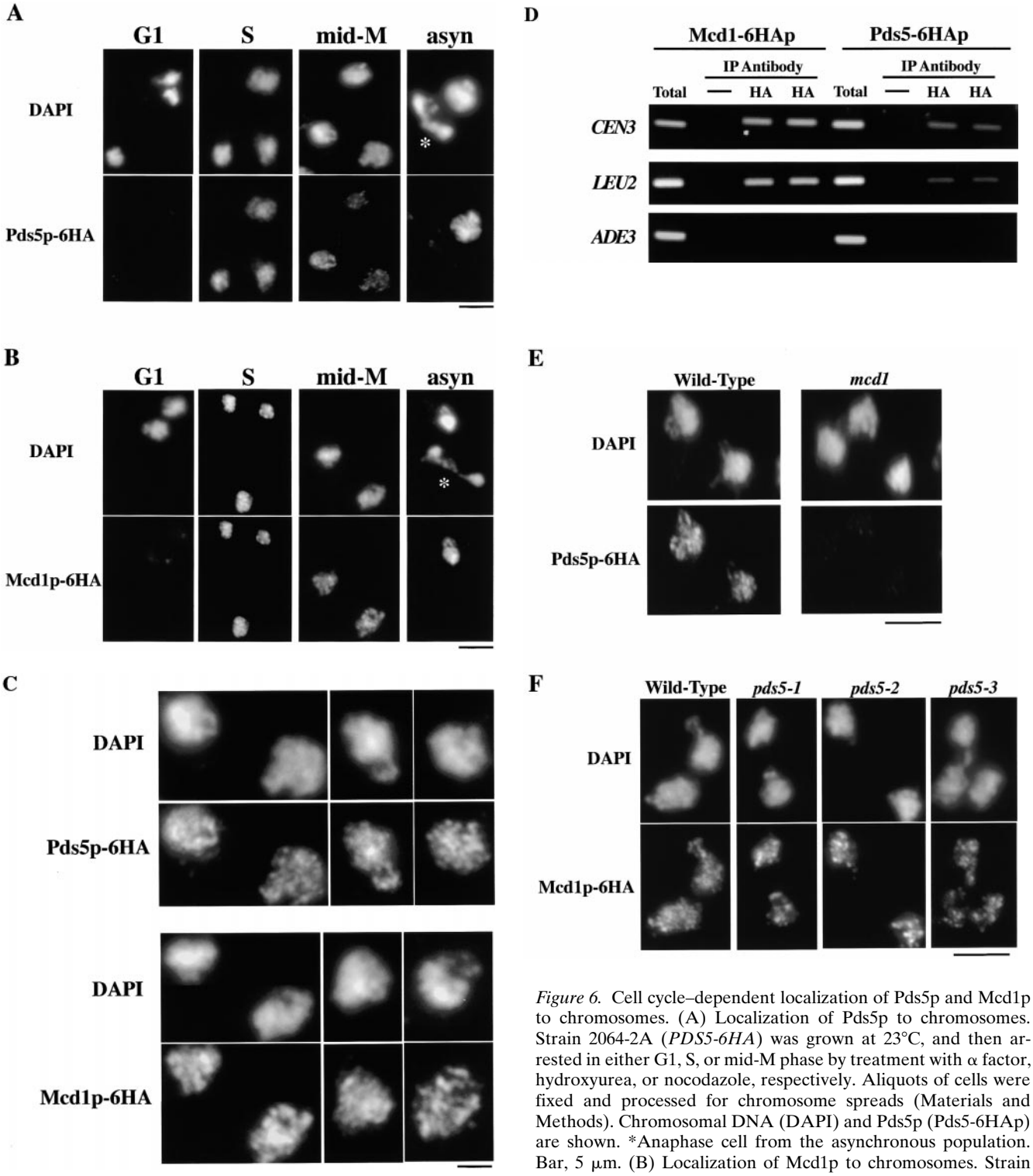

Figure 6. Cell cycle–dependent localization of Pds5p and Mcd1p to chromosomes. (A) Localization of Pds5p to chromosomes. Strain 2064-2A (*PDS5-6HA*) was grown at 23°C, and then arrested in either G1, S, or mid-M phase by treatment with α factor, hydroxyurea, or nocodazole, respectively. Aliquots of cells were fixed and processed for chromosome spreads (Materials and Methods). Chromosomal DNA (DAPI) and Pds5p (Pds5-6HAp) are shown. *Anaphase cell from the asynchronous population. Bar, 5 μm. (B) Localization of Mcd1p to chromosomes. Strain VG2050-1A (*MCD1-6HA*) was grown and processed for chromosome spreads as described in A. Chromosomal DNA (DAPI) and Mcd1p (Mcd1-6HAp) are shown. *Anaphase cell from the asynchronous population. Bar, 5 μm. (C) Enlargement of mid-M phase cells from cultures in A and B. Bar, 1.25 μm. (D) Binding of Pds5p and Mcd1p to *CEN3* and *LEU2* loci. Strains in A were grown to mid-log phase and subjected to ChIP using the anti–HA antibody C12A5, and then analyzed by PCR for the presence of *CEN3*, *LEU2,* and *ADE3* chromosomal loci (Materials and Methods). (Total) PCR of total chromatin equivalent to 1% that used for ChIP; positive control for PCR. (E) Assay to determine whether Mcd1p function is required for Pds5p localization to chromosomes. Wild-type (VG2064-2A) and *mcd1-1* mutant (VG2072-2D) strains containing Pds5p-6HA were arrested in G1 phase at 23°C, transferred to 37°C, and then arrested in mid-M phase at 37°C and processed for chromosome spreads (Materials and Methods). Chromosomal DNA (DAPI) and Pds5p (Pds5-6HAp) are shown. Bar, 5 μm. (F) Assay to determine whether Pds5p function is required for Mcd1p localization to chromosomes. Wild-type (VG2050-1A) and *pds5* mutant ({VG2073-7D; *pds5-1*}, {VG2074-6C; *pds5-2*}, and {VG2075-6D; *pds5-3*} strains bearing Mcd1p-6HA were treated as described in E. Chromosomal DNA (DAPI) and Mcd1p (Mcd1-6HAp) are shown. Bar, 5 μm.

binds to chromosomes, an asynchronous culture of haploid strain VG2064-2A was arrested in G1, S, and mid-M phases. This strain contains a *PDS5-6HA* allele under control of the *PDS5* promoter as the sole source of Pds5p, which enabled us to assess the chromosomal localization of Pds5p using a chromosome "spread" technique (Materials and Methods). In G1 phase cells, little Pds5p was detected on chromosomes (Fig. 6 A). In contrast, a bright Pds5p signal was detected in both S and mid-M phase cells (Fig. 6 A). In asynchronous cells, there are two general types of DNA masses, elongated dumbbell-shaped DNA masses from late anaphase cells and roughly spherical DNA masses from cells at all other stages of the cell cycle. Pds5p was absent from the elongated DNA masses of late anaphase cells (Fig. 6 A). For spherical DNA masses, 42% had prominent Pds5p staining, while the remaining had little or no Pds5p staining, consistent with a cell cycle–dependent chromosomal binding. Finally, in cells arrested in telophase by the *cdc15* mutation, little Pds5-6HAp was detected on chromosomes (data not shown). Mcd1p, like Pds5p, is also required for both sister chromatid cohesion and condensation (Guacci et al., 1997b). Therefore, we analyzed the localization of Mcd1p using haploid strain VG2050-1A, which contains Mcd1-6HAp as the sole source of Mcd1p, and compared it with that of Pds5p (Materials and Methods). Mcd1p bound to chromosomes in a cell cycle–dependent manner indistinguishable from that of Pds5p (Fig. 6 B). The staining for both Pds5p and Mcd1p appeared to be brighter on chromosomes in S than in mid-M phase. Interestingly, on mid-M phase cells, the Pds5p and Mcd1p staining was less diffuse than the DAPI and appeared line-like in many cells (Fig. 6 C). This pattern is suggestive of a staining along the chromosomal axis. These results demonstrate that Pds5p localizes to chromosomes in a cell cycle–dependent manner identical to that of Mcd1p.

Pds5p and Mcd1p localize to chromosomes at the same stages of the cell cycle and are both required for cohesion and condensation. This suggests that these two proteins may function by binding to the same chromosomal sites. To test this possibility, we compared the binding of Pds5p and Mcd1p at three discrete chromosomal loci. Asynchronous cultures of VG2064-2A (Pds5-6HAp) and VG2050-1A (Mcd1-6HAp) were subjected to ChIP using anti–HA antibodies. PCR analysis of the ChIP revealed that Pds5p and Mcd1p bound to *CEN3* DNA and a sequence near the *LEU2* gene, while neither protein bound to a region near the *ADE3* gene (Fig. 6 D, and Megee et al., 1999). Thus, Pds5p and Mcd1p bind to the same two specific chromosomal loci, which suggests that they function at common chromosomal sites to mediate sister chromatid cohesion.

We then determined whether the chromosomal binding of Pds5p was dependent on Mcd1p function. Asynchronously growing cultures of strains VG2072-2D (*mcd1-1* Pds5-6HAp) and VG2064-2A (Pds5-6HAp) were arrested in G1 phase at 23°C, transferred to 37°C to inactivate the mutant mcd1p, and then released from G1 and arrested in mid-M phase at 37°C (Materials and Methods). Mid-M phase cells at 37°C were subjected to chromosome spreads cells to detect Pds5p (Materials and Methods). As expected, Pds5p was widely distributed on chromosomes in wild-type cells (Fig. 6 E). In contrast, Pds5p failed to bind to any chromosomal regions in the *mcd1* mutant in mid-M phase at 37°C (Fig. 6 E). When the *mcd1* mutant was grown asynchronously at 23°C, Pds5p bound to chromosomes in 42% of cells, similar to the wild-type, where it bound in 43% of cells. Thus, the localization of Pds5p to chromosomes is dependent on Mcd1p function. We then assessed whether Mcd1p localization to chromosomes was dependent on Pds5p function. Wild-type VG2050-1A (Mcd1p-6HA) and *pds5* mutant strains VG2073-7D (*pds5-1* Mcd1p-6HA), VG2074-6C (*pds5-2* Mcd1p-6HA) and VG2075-6D (*pds5-3* Mcd1p-6HA) were arrested in mid-M phase at 37°C and processed for chromosome spreads to Mcd1p as described above. Mcd1p showed a strong chromosomal localization in both wild-type and *pds5* mutant strains, indicating that Pds5p function is not required for Mcd1p binding to chromosomes (Fig. 6 F). However, there was a slight difference in the patterns since Mcd1p staining was more punctate in the *pds5* mutant strains than in wild-type cells. This subtle difference could be a consequence of the condensation defect in *pds5* mutants or, alternatively, may reflect a decrease in Mcd1p binding at specific sites. We are conducting experiments to determine which possibility is correct. These results demonstrate that the global localization of Pds5p to chromosomes is dependent on wild-type Mcd1p function. In contrast, the bulk Mcd1p localization to chromosomes does not depend on Pds5p function.

The chromosome spreads revealed that there is little Pds5p on chromosomes in G1 phase, late anaphase, or telophase cells. We wanted to determine whether this was due to an absence of Pds5p in cells or an exclusion of Pds5p from chromatin. To address this question, an asynchronous culture of haploid strain VG2063-5C (*PDS5-GFP*) was arrested in G1, S, and mid-M phases and the localization of Pds5p directly determined in intact cells by fluorescence microscopy (Materials and Methods). Pds5-GFPp was detected as a single prominent spherical signal in G1, S, and mid-M phase cells (Fig. 7 A). The intensity of Pds5p fluorescence was lower in G1 phase cells than in either S or mid-M phase cells. When DNA in cells was stained with DAPI, the Pds5p was found to localize in the same region as the DNA, indicating that Pds5p was nuclear (data not shown). An asynchronous culture of haploid strain VG2069-1B (*PDS5-GFP cdc15-2*) was grown at 23°C, and then transferred to 37°C for 3 h to arrest cells in telophase due to the *cdc15* mutation (Pringle and Hartwell, 1981). Two spherical Pds5-GFPp signals were observed at opposite ends of each cell (Fig. 7 A). When DNA in cells was stained with DAPI, the Pds5p localized in the same region as the DNA masses at opposite ends, indicating it was nuclear in these cells (data not shown). We examined Mcd1p by arresting haploid strain VG2049-3A (*MCD1-GFP*) in G1, S, and mid-M phase (Materials and Methods). Mcd1-GFPp was not detected in G1 phase cells, but formed a single prominent round signal in S and mid-M phase cells (Fig. 7 B). When haploid strain VG2061-1D (*MCD1-GFP cdc15-2*) was arrested in telophase as described above, a very faint Mcd1-GFPp signal was detected, indicating that most of the Mcd1p was absent from cells. Western blot analysis of total protein from cells bearing either Pds5-6HAp or Mcd1-6HAp confirmed the cell cycle–dependent changes in Pds5p and Mcd1p levels obtained with the GFP-tagged proteins (data not shown). These results demonstrate that Pds5p and Mcd1p are bound to chromosomes at similar stages of the cell cycle. However, Pds5p is present in the nucleus at all stages

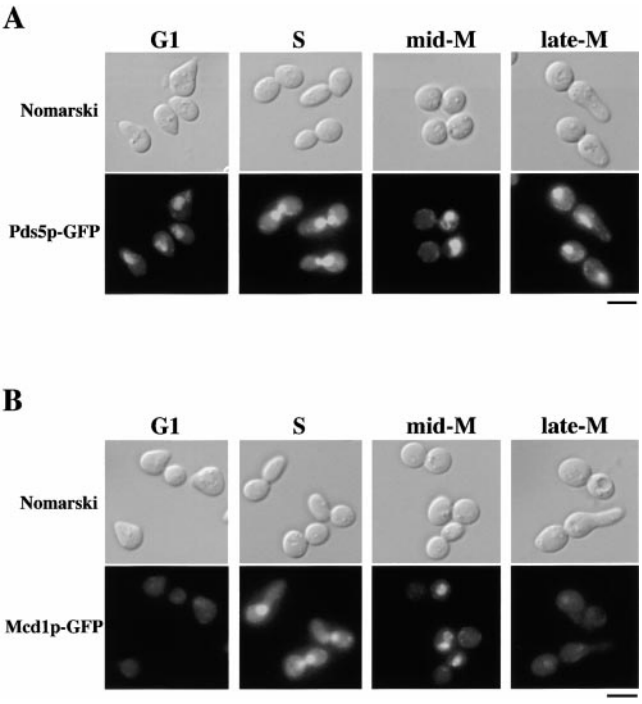

Figure 7. Localization of Pds5p and Mcd1p in intact yeast cells. Haploid strains VG2063-5C (*PDS5-GFP*) and VG2049-3A (*MCD1-GFP*) were grown to mid-log phase at 23°C, and then arrested in G1, S, or mid-M phase. Haploid strains VG2069-1B (*cdc15-1 PDS5-GFP*) and VG2061-1D (*cdc15-2 MCD1-GFP*) were grown to mid-log phase at 23°C, and then arrested in telophase by incubation at 37°C for 3 h. Arrested cells were fixed and the localization of GFP-tagged proteins determined in intact cells by fluorescence microscopy (Materials and Methods). Localization of (A) Pds5p and (B) Mcd1p in whole cells at different cell cycle stages.

of the cell cycle, while Mcd1p is at prominent levels only at times when it is bound to chromosomes. The fact that Pds5p only binds to chromosomes at times when Mcd1p is present is consistent with our data showing Pds5p binding to chromosomes is dependent on Mcd1p function.

Discussion

We have executed a cytogenetic screen of temperature sensitive mutants to identify those that exhibited precocious dissociation of sister chromatids. This screen led to the identification of the *pds1* and *mcd1* mutants (Yamamoto et al., 1996a,b; Guacci et al., 1997b). Here we identified and characterized *PDS5*, another gene identified by this screen. *PDS5* is an essential gene for vegetative growth. Pds5p is required for sister chromatid cohesion at centromere proximal and centromere distal regions. Pds5p is also required for chromosome condensation at both repetitive and unique chromosomal regions. *Pds5* mutant cells have a functional mitotic checkpoint, which demonstrates their defects in cohesion and condensation are not due to a failure in cell cycle arrest. The cell cycle stages when Pds5p is required for viability and loaded onto chromosomes provides insights into its role in cohesion. Pds5p function is important for viability either during or soon after S phase until anaphase and localizes to chromosomes during this cell cycle window, which encompasses the times when sister chromatid cohesion exists. Furthermore, Pds5p is required to maintain cohesion as late as mid-M phase. In contrast, Ctf7p, Scc2p, and Scc4p, which are necessary for the establishment of cohesion, are required for sister chromatid cohesion and cell viability in S phase but not in mid-M phase (Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000). Thus, Pds5p has the same properties as the cohesin complex members Mcd1p, Smc1p, Smc3p, and Scc3p. These results demonstrate that Pds5p is a component of the molecular glue that mediates sister chromatid cohesion and that the maintenance of cohesion requires more than the cohesin complex proteins.

What is the relationship between Pds5p and the cohesin complex? First, we have shown that Pds5p and Mcd1p localize to chromosomes at the same cell cycle stages. Interestingly, the localization of Pds5p and Mcd1p in mid-M phase cells was tighter than the DAPI, suggesting that these proteins may be at the core of the chromosomal axis (this study). Second, chromatin immunoprecipitation of Pds5p reveals that it binds to chromosomal DNA sites in *CEN3* and near *LEU2,* but not near *ADE3*. This binding pattern is identical to that of Mcd1p (Megee et al., 1999). In addition, the global localization of Pds5p to chromosomes depends on Mcd1p function, while Mcd1p localization to chromosomes is independent of Pds5p function. Third, *mcd1* and *pds5* mutants are synthetically lethal (V. Guacci, unpublished results). Moreover, the bimD6 mutant is suppressed by a mutation in SudA gene, which encodes the *A. nidulans* homologues of Pds5p and Smc3p, respectively (Holt and May, 1996). These results reveal that Pds5p and the cohesin complex proteins functionally interact. Furthermore, the fact that Mcd1p binding to chromosomes is independent of Pds5p function suggests that the cohesin complex may serve to load Pds5p onto chromosomal sites, which enables Pds5p to mediate sister chromatid cohesion.

Two homologues of Pds5p, the BimD6 protein of *A. nidulans* and the Spo76 protein of *Sordaria*, have been shown to be essential for mitotic and meiotic chromosome segregation, respectively. BimD6 mutant cells exhibited lethal defects in mitotic chromosome segregation and had fewer kinetochore microtubules than wild-type cells (Denison et al., 1993). The Spo76p localizes to meiotic chromosomes along their length, while the *spo76-1* mutant arrests in meiosis and exhibits defects in sister chromatid cohesion and condensation (van Heemst et al., 1999). During mitosis, the *spo76-1* mutant had only a transient defect in both sister chromatid cohesion and condensation, but exhibited no growth abnormalities. These results indicate that the Spo76p may serve a less prominent or nonessential function during mitosis or that the *spo76-1* allele has almost normal mitotic function. Alternatively, there may be another Pds5p homologue in *Sordaria* that performs the essential mitotic chromosomal function. Thus, Pds5p homologues also play important roles in chromosome structure and function in other eukaryotes.

Previously, we proposed that cohesion and condensation are linked because *mcd1* mutants had defects in both cohesion and condensation (Guacci et al., 1997b). The fact that all three *pds5* alleles also have defects in cohesion and condensation confirms this link. Thus, the link between cohesion and condensation is a general property of yeast chromosomes. Since, Pds5p and Mcd1p have homologues

in other eukaryotes, it is likely that the cohesion–condensation link would also be conserved. Support for this idea comes from *Sordaria*, where the *spo76-1* mutant exhibited defects in both cohesion and condensation (van Heemst et al., 1999). Unlike in budding yeast, the mitotic chromosomes of *Sordaria* can be detected as individual rod-like chromosomes reminiscent of other eukaryotic chromosomes. The conservation of chromosome structure in eukaryotes is not surprising since chromosomal proteins involved in condensation are conserved throughout eukaryotes. This conservation likely reflects that a common chromosomal architecture is required to execute the structural changes that eukaryotic chromosomes undergo during mitosis.

It has been proposed that cohesion and condensation are distinct chromosomal processes because the soluble condensin and cohesin complexes in yeast, *Xenopus*, and mammals do not copurify or temporally coexist on chromosomes (Hirano et al., 1997; Losada et al., 1998; Schmiesing et al., 1998; Toth et al., 1999). Furthermore, immunodepletion of cohesin complex members in interphase *Xenopus* egg extracts resulted in cohesion defects when chromosomes entered mitosis but rod like chromosomes still formed, which suggested that condensation was unaffected (Losada et al., 1998). These results seem to contradict the link between cohesion and condensation revealed by analysis of budding yeast and *Sordaria* mutants. There are several ways to explain the seemingly contradictory results.

First, it could be that chromosome structure is fundamentally different in yeast and higher eukaryotes. Several lines of evidence make this unlikely. The proteins required for cohesion and condensation are conserved throughout eukaryotes (reviewed in Koshland and Strunnikov, 1996; Koshland and Guacci, 2000). In addition, cohesin complex members are present on chromosomes at similar cell cycle stages in budding yeast, *Xenopus,* and human cells. Cohesin complex members become bound to chromosomes during interphase and remain on chromosomes, albeit at lower levels, during mitosis (Losada et al., 1998, 2000; Schmiesing et al., 1998; I. Waizenegger, S. Hauf, and J.M. Peters, unpublished observations). A similar pattern is seen in budding yeast (see below). Budding yeast and mammalian chromosomes undergo cell cycle–dependent condensation, although yeast exhibits a smaller level of condensation (Guacci et al., 1994). Cohesin binding sites have been mapped over large chromosomal regions in budding yeast and recent PCR-based chromosomal walking has revealed that these sites occur every 8–9 kb in noncentromeric regions (Blat and Kleckner, 1999; Laloraya, S., V. Guacci, and D. Koshland, manuscript submitted for publication). Cohesin binding sites have not been mapped in higher eukaryotes, but it seems likely that such sites are more widely spaced since metaphase chromosomes appear to contain 50–250-kb DNA loops (Earnshaw and Laemmli, 1983; Gasser et al., 1986). Thus, it is likely that cohesin binding sites are distributed along eukaryotic chromosomes, but have different spacing in budding yeast and higher eukaryotes. Such a decreased spacing (i.e., higher density) of cohesion sites was proposed to explain the lower levels of chromosome condensation in budding yeast (Guacci et al., 1997b). In summary, chromosomes from budding yeast and higher eukaryotes have significant, fundamental similarities in structure as well as the mechanism of cohesion and condensation.

Second, interactions between cohesin and condensin complexes might exist, but have been missed. The cell cycle window when condensin and cohesin complex members interact could be very short or involve a small amount of the total protein. In addition, the putative interactions may occur only on proteins that are bound to chromosomes. Finally, interactions between the soluble complexes could be too weak to be preserved during immunoprecipitations or fractionations. In fact, biochemical evidence in yeast supports an interaction between cohesin and condensin complex members. Smc1p and Smc2p, members of the cohesin and condensin complexes, respectively, co-immunoprecipitate when both are overexpressed (Strunnikov et al., 1995). In addition, two other yeast proteins, Trf4p and Hec1p, coimmunoprecipitate with both Smc1p and Smc2p (Castano et al., 1996; Zheng et al., 1999). These results show that interactions between condensin and cohesin members occur and could be mediated through a common component(s).

Finally, the discrepancy may simply reflect that different aspects of chromosome condensation were monitored. To explain the cohesion–condensation link, we proposed that Mcd1p-containing cohesin complexes at one cohesion site associate with those at the next cohesion site along the chromosome (Guacci et al., 1997b). This would loop out the intervening DNA from the chromosomal axis and shorten chromosomes. The DNA between adjacent cohesion sites (looped-out regions) must also be packaged, presumably by the condensin complex. Thus, fully condensed mitotic chromosomes would require the interaction of cohesion proteins at adjacent cohesion sites along the chromosome and condensin-mediated packaging of the intervening DNA loops (Fig. 8 A). The association of adjacent cohesion sites could occur before loop packaging or after the packaging of loops has brought adjacent cohesion sites in close proximity. The mechanism is different, but the end result, the shortening of the chromosomal axis, is the same. If Mcd1p or Pds5p function is perturbed, the interactions between adjacent cohesion sites along the chromatid would be altered and result in less axial shortening (Fig. 8 B). However, the loops would be packaged by the condensin complex or other factors so that rod-shaped chromosomes should form but would be longer than normal metaphase chromosomes. We used FISH to assess the length and spacing of DNA sequences along individual chromosome arms and found that the overall length and spacing was increased in *pds5* and *mcd1* mutants (this study and Guacci et al., 1997b). In contrast, the formation of rod-like chromosomes was taken as evidence that condensation was unaffected by immunodepletion of the cohesin complex in the *Xenopus* extracts (Losada et al., 1998). However, the axial length of individual chromosomes was not measured, which leaves open the possibility that axial shortening of chromosomes was actually perturbed by the immunodepletion. Clearly, many questions must be answered before the cohesion–condensation link can be fully understood.

In *Xenopus*, the cohesin complex members are present at high levels on chromosomes in interphase, but decrease to low levels on chromosomes in mitosis (Losada et al., 1998,

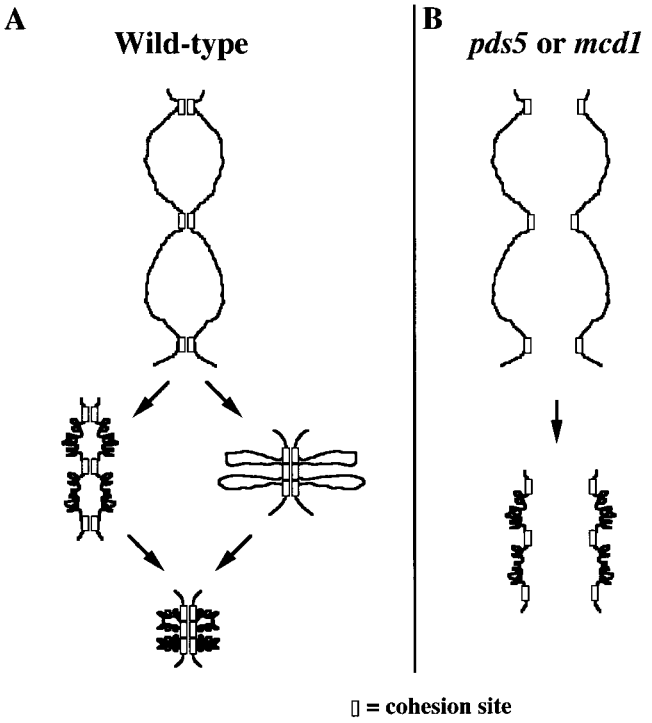

Figure 8. Model for chromosome condensation. (A) Condensation in wild-type cells. (Top) Decondensed chromosome with sister chromatid cohesion. Chromosome condensation begins by either packaging of DNA between cohesion sites (middle left) or by the association of adjacent cohesion sites (middle right). The fully condensed mitotic chromosome (bottom) requires both the association of adjacent cohesion sites and the packaging of the DNA between cohesion sites. (B) Condensation in *pds5* or *mcd1* mutants. (Top) Decondensed chromosome with dissociated sister chromatids. When chromosomes undergo condensation, the DNA between cohesion sites is still packaged, but the adjacent cohesion sites cannot associate.

2000). Pds5p and Mcd1p are present at high levels on yeast chromosomes in S and mid-M phase (this study; Michaelis et al., 1997). Our qualitative analysis of Pds5p and Mcd1p levels by chromosome spreads suggests that the levels of these proteins bound to chromosomes may also decrease from S to mid-M phase. While this result must be confirmed by quantitative methods, it suggests that in both yeast and *Xenopus*, chromosomal levels of proteins that mediate sister chromatid cohesion decrease during mitosis. Since chromosomes become condensed in mitosis, the decrease in cohesin complex correlates with condensation. It is also possible that the decrease is required for proper chromosome condensation. Yeast chromosomes undergo less condensation during mitosis than mammalian chromosomes (Guacci et al., 1994). Therefore, it is possible that the more dramatic decrease of the cohesin complex in *Xenopus* may be a consequence of the higher level of chromosome condensation than occurs in budding yeast.

Pds5p is bound to chromosomes in mid-M phase cells (nocodazole arrested), when sister cohesion is present, but is absent from chromosomes in late anaphase, when sisters are separated. Similar results are observed with Mcd1p (this study; Michaelis et al., 1997; Uhlmann et al., 1999). It has been suggested that Mcd1p/Scc1p is cleaved and departs from chromosomes at the metaphase to anaphase transition, which results in the dissolution of cohesion (Michaelis et al., 1997; Uhlmann et al., 1999). It is also tempting to speculate that the loss of Pds5p occurs at the metaphase to anaphase transition and is crucial for the dissolution of cohesion. However, chromosomes also decondense in late anaphase and both Pds5p and Mcd1p are required for condensation (this study; Guacci et al., 1994, 1997b). Therefore, it is essential to correlate the precise time that Pds5p and Mcd1p depart from chromosomes with the state of chromosome condensation. Unfortunately, in budding yeast, the metaphase to anaphase transition cannot be easily detected because individual chromosomes are not visible by conventional microscopy. Yeast cells from S phase until midmitosis have a round DNA mass and a short spindle. Just after the metaphase to anaphase, centromere proximal sequences of sister chromatids rapidly reach opposite poles before any prominent elongation of the DNA mass or spindle occurs in yeast (Guacci et al., 1997a; Straight et al., 1997). It is only until well after the metaphase to anaphase transition, when cells have elongated their spindles and DNA masses, that one can be sure that sister chromatids have separated. It was in these late anaphase cells that most of the Pds5p and Mcd1p was not detected on chromosomes (this study; Michaelis et al., 1997; Uhlmann et al., 1999). Therefore, it is not known whether the departure of Pds5p and Mcd1p occurs at the precise time when sister cohesion is dissolved (the metaphase to anaphase transition) or whether it occurs later in mitosis when chromosomes decondense (late anaphase or telophase). Until the precise timing is determined for the departure of Pds5p and Mcd1p from chromosomes, the effect that their departure has on chromosome structure remains an open question.

Our finding that Pds5p is part of the molecular glue responsible for the maintenance of sister chromatid cohesion demonstrates that the cohesin complex proteins represent only a part of the mechanism that mediates cohesion. Further characterization of Pds5p and its relationship to the cohesin complex will provide valuable insights into how cohesion is maintained and ultimately dissolved. Towards this end, we are examining whether Pds5p is a new member of the cohesin complex, and whether it loads onto chromosomes before the complex or is added afterwards. We are also testing for genetic interactions between Pds5p and cohesin and condensin complex members in yeast to help dissect the link between cohesion and condensation.

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References

Blat, Y., and N. Kleckner. 1999. Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell.* 98:249–259.

Castano, I., P. Brzoska, B. Sadoff, H. Chen, and M. Christman. 1996. Mitotic

chromosome condensation in the rDNA requires TRF4 and DNA topoisomerase I in *Saccharomyces cerevisiae*. *Genes Dev*. 10:2564–2576.
Chuang, P.T., D.G. Albertson, and B.J. Meyer. 1994. DPY-27: a chromosome condensation protein homolog that regulates *C. elegans* dosage compensation through association with the X chromosome. *Cell*. 79:459–474.
Ciosk, R., M. Shirayama, A. Shevchenko, T. Tanaka, A. Toth, A. Shevchenko, and K. Nasmyth. 2000. Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. *Mol. Cell*. 5:243–254.
Denison, S.H., E. Kafer, and G.S. May. 1993. Mutation in the bimD gene of *Aspergillus nidulans* confers a conditional mitotic block and sensitivity to DNA damaging agents. *Genetics*. 134:1085–1096.
Donze, D., C.R. Adams, J. Rine, and R.T. Kamakaka. 1999. The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. *Genes Dev*. 13:698–708.
Earnshaw, W.C., and U.K. Laemmli. 1983. Architecture of metaphase chromosomes and chromosome scaffolds. *J. Cell Biol*. 96:84–93.
Funabiki, H., H. Yamano, K. Kumada, K. Nagao, T. Hunt, and M. Yanagida. 1996. Cut2 proteolysis required for sister chromatid separation in fission yeast. *Nature*. 381:438–441.
Furuya, K., K. Takahashi, and M. Yanagida. 1998. Faithful anaphase is ensured by Mis4, a sister chromatid cohesion molecule required in S phase and not destroyed in G1 phase. *Genes Dev*. 12:3408–3418.
Gasser, S.M., T. Laroche, J. Falquet, E. Boy de la Tour, and U.K. Laemmli. 1986. Metaphase chromosome structure. Involvement of topoisomerase II. *J. Mol. Biol*. 188:613–629.
Guacci, V., E. Hogan, and D. Koshland. 1994. Chromosome condensation and sister chromatid pairing in budding yeast. *J. Cell Biol*. 125:517–530.
Guacci, V., E. Hogan, and D. Koshland. 1997a. Centromere position in budding yeast: evidence for anaphase A. *Mol. Biol. Cell*. 8:957–972.
Guacci, V., D. Koshland, and A. Strunnikov. 1997b. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae*. *Cell*. 91:47–57.
Guacci, V., A. Yamamoto, A. Strunnikov, J. Kingsbury, E. Hogan, P. Meluh, and D. Koshland. 1993. Structure and function of chromosomes in mitosis of budding yeast. *Cold Spring Harbor Symp. Quant. Biol.* 58:677–685.
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*. 89:511–521.
Hirano, T., and T.J. Mitchison. 1994. A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. *Cell*. 79:449–458.
Holt, C.L., and G.S. May. 1996. An extragenic suppressor of the mitosis-defective bimD6 mutation of *Aspergillus nidulans* codes for a chromosome scaffold protein. *Genetics*. 142:777–787.
Jessberger, R., B. Riwar, H. Baechtold, and A.T. Akhmedov. 1996. SMC proteins constitute two subunits of the mammalian recombination complex RC-1. *EMBO (Eur. Mol. Biol. Organ.) J*. 15:4061–4068.
Kerrebrock, A., D. Moore, J. Wu, and T. Orr-Weaver. 1995. Mei-S332, a *Drosophila* protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. *Cell*. 83:247–256.
Kimura, K., and T. Hirano. 1997. ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation. *Cell*. 90:625–634.
Kimura, K., V.V. Rybenkov, N.J. Crisona, T. Hirano, and N.R. Cozzarelli. 1999. 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. *Cell*. 98:239–248.
Koshland, D., and A. Strunnikov. 1996. Mitotic chromosome condensation. *Annu. Rev. Cell Dev. Biol*. 12:305–333.
Koshland, D.E., and V. Guacci. 2000. Sister chromatid cohesion: the beginning of a long and beautiful relationship. *Curr. Opin. Cell Biol*. 12:297–301.
Kurlandzka, A., J. Rytka, R. Gromadka, and M. Murawski. 1995. A new essential gene located on *Saccharomyces cerevisiae* chromosome IX. *Yeast*. 11: 885–890.
Lavoie, B.D., K.M. Tuffo, S. Oh, D. Koshland, and C. Holm. 2000. Mitotic chromosome condensation requires brn1p, the yeast homologue of barren. *Mol. Biol. Cell*. 11:1293–1304.
Lawrence, J.B., C.A. Villnave, and R.H. Singer. 1988. Sensitive, high-resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. *Cell*. 52:51–61.
Losada, A., M. Hirano, and T. Hirano. 1998. Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. *Genes Dev*. 12: 1986–1997.
Losada, A., T. Yokochi, R. Kobayashi, and T. Hirano. 2000. Identification and characterization of SA/Scc3p subunits in the *Xenopus* and human cohesin complexes. *J. Cell Biol*. 150:405–416.
Megee, P.C., and D. Koshland. 1999. A functional assay for centromere-associated sister chromatid cohesion. *Science*. 285:254–257.
Megee, P.C., C. Mistrot, V. Guacci, and D. Koshland. 1999. The centromeric sister chromatid cohesion site directs Mcd1p binding to adjacent sequences. *Mol. Cell*. 4:445–450.
Michaelis, C., R. Ciosk, and K. Nasmyth. 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell*. 91:35–45.
Moore, D.P., A.W. Page, T.T. Tang, A.W. Kerrebrock, and T.L. Orr-Weaver. 1998. The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. *J. Cell Biol*. 140:1003–1012.
Ouspenski, I.I., O.A. Cabello, and B.R. Brinkley. 2000. Chromosome condensation factor brn1p is required for chromatid separation in mitosis. *Mol. Biol. Cell*. 11:1305–1313.
Pringle, J.R., and L.H. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. *In* The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance. J.N. Strathern, E.W. Jones, and J.R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 97–142.
Saitoh, N., I.G. Goldberg, E.R. Wood, and W.C. Earnshaw. 1994. ScII: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. *J. Cell Biol*. 127: 303–318.
Saka, Y., T. Sutani, Y. Yamashita, S. Saitoh, M. Takeuchi, Y. Nakaseko, and M. Yanagida. 1994. Fission yeast cut3 and cut14, members of the ubiquitous protein family, are required for chromosome condensation and segregation in mitosis. *EMBO (Eur. Mol. Biol. Organ.) J*. 13:4938–4952.
Schmiesing, J.A., A.R. Ball, H.C. Gregson, J.M. Alderton, S. Zhou, and K. Yokomori. 1998. Identification of two distinct human SMC protein complexes involved in mitotic chromosome dynamics. *Proc. Natl. Acad. Sci. USA*. 95:12906–12911.
Selig, S., K. Okumura, D.C. Ward, and H. Cedar. 1992. Delineation of DNA replication time zones by fluorescence *in situ* hybridization. *EMBO (Eur. Mol. Biol. Organ.) J*. 11:1217–1225.
Skibbens, R., L. Corson, D. Koshland, and P. Hieter. 1999. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes Dev*. 13:307–319.
Straight, A., A. Belmont, C. Robinett, and A. Murray. 1996. GFP tagging of budding yeast chromosomes reveals that protein–protein interactions can mediate sister chromatid cohesion. *Curr. Biol*. 6:1599–1608.
Straight, A.F., W.F. Marshall, J.W. Sedat, and A.W. Murray. 1997. Mitosis in living budding yeast: anaphase A but no metaphase plate. *Science*. 277:574–578.
Strunnikov, A.V., E. Hogan, and D. Koshland. 1995. SMC2, a *Saccharomyces cerevisiae* gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. *Genes Dev*. 9:587–599.
Sutani, T., and M. Yanagida. 1997. DNA renaturation activity of the SMC complex implicated in chromosome condensation. *Nature*. 388:798–801.
Sutani, T., T. Yuasa, T. Tomonaga, N. Dohmae, K. Takio, and M. Yanagida. 1999. Fission yeast condensin complex: essential roles of non-SMC subunits for condensation and Cdc2 phosphorylation of Cut3/SMC4. *Genes Dev*. 13: 2271–2283.
Tanaka, K., T. Yonekawa, Y. Kawasaki, M. Kai, K. Furuya, M. Iwasaki, H. Murakami, M. Yanagida, and H. Okayama. 2000. Fission yeast Eso1p is required for establishing sister chromatid cohesion during S phase. *Mol. Cell. Biol*. 20:3459–3469.
Tanaka, T., M. Cosma, K. Wirth, and K. Nasmyth. 1999. Identification of cohesion association sites at centromeres and along chromosome arms. *Cell*. 98: 847–858.
Toth, A., R. Ciosk, F. Uhlmann, M. Galova, A. Schleiffer, and K. Nasmyth. 1999. Yeast cohesin complex requires a conserved protein, Eco1p (Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes Dev*. 13:307–319.
Trask, B., D. Pinkel, and G. van den Engh. 1989. The proximity of DNA sequences in interphase cell nuclei is correlated to genomic distance and permits ordering of cosmids spanning 250 kilobase pairs. *Genomics*. 5:710–717.
Uhlmann, F., and K. Nasmyth. 1998. Cohesion between sister chromatids must be established during DNA replication. *Curr. Biol.* 8:1095–1101.
Uhlmann, F., F. Lottspeich, and K. Nasmyth. 1999. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature*. 400:37–42.
van Heemst, D., F. James, S. Poggeler, V. Berteaux-Lecellier, and D. Zickler. 1999. Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programs. *Cell*. 98:261–271.
Wang, Z., I.B. Castano, A. De Las Penas, C. Adams, and M.F. Christman. 2000. Pol kappa: a DNA polymerase required for sister chromatid cohesion. *Science*. 289:774–779.
Wilson, E.B. 1925. The Cell in Development and Heredity. The Macmillan Co., New York.
Yamamoto, A., V. Guacci, and D. Koshland. 1996a. Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*. *J. Cell Biol*. 133:85–97.
Yamamoto, A., V. Guacci, and D. Koshland. 1996b. Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). *J. Cell Biol*. 133:99–110.
Zheng, L., Y. Chen, and W.H. Lee. 1999. Hec1p, an evolutionarily conserved coiled-coil protein, modulates chromosome segregation through interaction with SMC proteins. *Mol. Cell. Biol*. 19:5417–5428.