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Pds5p regulates the maintenance of sister chromatid cohesion and is sumoylated to promote the dissolution of cohesion

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Pds5p and the cohesin complex are required for sister chromatid cohesion and localize to the same chromosomal loci over the same cell cycle window. However, Pds5p and the cohesin complex likely have distinct roles in cohesion. We report that *pds5* mutants establish cohesion, but during mitosis exhibit precocious sister dissociation. Thus, unlike the cohesin complex, which is required for cohesion establishment and maintenance, Pds5p is required only for maintenance. We identified *SMT4*, which encodes a SUMO isopeptidase, as a high copy suppressor of both the temperature sensitivity and precocious sister dissociation of *pds5* mutants. In contrast, *SMT4* does not suppress temperature sensitivity of cohesin complex mutants. Pds5p is SUMO conjugated, with sumoylation peaking during mitosis. *SMT4* overexpression reduces Pds5p sumoylation, whereas *smt4* mutants have increased Pds5p sumoylation. *smt4* mutants were previously shown to be defective in cohesion maintenance during mitosis. These data provide the first link between a protein required for cohesion, Pds5p, and sumoylation, and suggest that Pds5p sumoylation promotes the dissolution of cohesion.

Introduction

In vertebrate cells, sister chromatids are associated along their length from their formation in S phase through metaphase. During mitosis, cohesion between sisters is maintained as chromosomes condense, attach to the mitotic spindle in a bipolar orientation, and congress to the metaphase plate. At the metaphase–anaphase transition, cohesion is dissolved and sisters segregate to opposite poles via microtubule-dependent movements. Finally, the segregated sisters decondense. In budding yeast, individual chromosomes from vegetative cells cannot be visualized by microscopy. However, FISH experiments revealed that sister chromatids are associated along their length until anaphase and undergo cell cycle–dependent condensation (Guacci et al., 1993, 1994). Thus, yeast and vertebrate chromosomes share structural similarities.

Chromosomal proteins essential for sister chromatid cohesion have been identified in yeast and vertebrates (Koshland and Guacci, 2000; Meluh and Strunnikov, 2002). These proteins share homology indicating that the molecular mechanism responsible for cohesion is similar in yeast and vertebrates. Proteins required for cohesion can be functionally divided into those that mediate cohesion (i.e., form the molecular "glue" that physically holds sisters together) and those that promote the establishment of cohesion, but are not part of the glue. The cohesin complex is comprised of four proteins, Mcd1p/Scc1p, Smc1p, Smc3p, and Irr1p/Scc3p, and is thought to comprise at least part of the molecular glue (Guacci et al., 1997; Michaelis et al., 1997; Losada et al., 1998, 2000). This complex is bound to chromosomes from early S phase until the metaphase–anaphase transition, and is required for cohesion throughout this cell cycle window (Guacci et al., 1997; Michaelis et al., 1997; Uhlmann and Nasmyth, 1998). Efficient establishment of cohesion during S phase requires additional proteins that are required for cohesin complex localization to chromosomes before DNA replication (e.g., Scc2p) and that couple cohesion establishment with DNA replication (e.g., Ctf7p/Eco1p; Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000).

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Abbreviations used in this paper: *CEN*, centromere; HU, hydroxyurea; IP, immunoprecipitation; Nz, nocodazole.

The cohesin complex comprises only part of the molecular glue responsible for sister chromatid cohesion. Another protein, Pds5p, was first identified in fungi (*PDS5* in budding yeast, BimD6 in *Aspergillus nidulans*, Spo76 in *Sordaria*) as being important for sister chromatid cohesion, condensation, and chromosome segregation (Denison et al., 1993; van Heemst et al., 1999; Hartman et al., 2000). Pds5p homologues have since been identified in higher eukaryotes (Sumara et al., 2000). Like the cohesin complex, Pds5p localizes to chromosomes from early S phase until the metaphase–anaphase transition (Hartman et al., 2000; Panizza et al., 2000; Sumara et al., 2000). In budding yeast, the cohesin complex and Pds5p bind to the same discrete chromosomal loci, including the centromere (*CEN*) DNA, a known site of cohesion (Megee and Koshland, 1999; Hartman et al., 2000; Panizza et al., 2000). In cells arrested at metaphase by nocodazole (Nz) treatment, Pds5p is required to maintain cohesion at both *CEN*-proximal and -distal loci (Hartman et al., 2000). Finally, Pds5p localization to chromosomes is dependent on the cohesin complex, whereas the cohesin complex still localizes to chromosomes in the absence of Pds5p (Hartman et al., 2000; Tanaka et al., 2001; Wang et al., 2002). These results indicate that Pds5p cooperates with the cohesin complex to mediate sister chromatid cohesion, but that Pds5p and the cohesin complex serve distinct roles in cohesion.

SUMO is a small ubiquitin-related protein that is covalently attached to proteins (Melchior, 2000). In budding yeast, Smt3p (SUMO) and SUMO isopeptidases are important for cell cycle progression and sister chromatid cohesion. *smt3* mutants delay in G2/M phase (Biggins et al., 2001). Smt3p/SUMO is removed from proteins by the SUMO isopeptidases Ulp1p and Smt4p/Ulp2p, and isopeptidase mutants also delay in G2/M phase (Li and Hochstrasser, 1999, 2000). *smt4* mutants have increased levels of SUMO-conjugated proteins and exhibit precocious sister dissociation at *CEN*-proximal loci (Li and Hochstrasser, 2000; Bachant et al., 2002). These results suggest that sumoylation of one or more proteins regulates cohesion. Here, we identify *SMT4* as a suppressor of the temperature sensitivity and the precocious dissociation of sister chromatids in *pds5* mutants. We show that Pds5p is sumoylated and that Smt4p activity modulates this sumoylation. We propose a model to explain the distinct roles that Pds5p and the cohesin complex play in cohesion, and suggest that Pds5p sumoylation promotes the dissolution of cohesion.

Results

PDS5 is required for maintenance of cohesion in mitosis, but not for establishment

Previously, we demonstrated that *pds5* mutants exhibit precocious sister dissociation at *CEN*-proximal and -distal loci when arrested at nonpermissive temperature in mitosis by treatment with Nz (Hartman et al., 2000). Furthermore, we showed that *pds5* mutants retain cohesion if arrested with Nz at permissive temperature, but rapidly lose cohesion when transferred to nonpermissive temperature, indicating that Pds5p is required to maintain cohesion during metaphase. These experiments could not determine whether budding yeast Pds5p is required for establishment. In *Schizosaccharomyces pombe*, *pds5* mutants have normal cohesion after S phase, but exhibit precocious sister dissociation when arrested in G2 phase or mitosis (Tanaka et al., 2001). Thus, fission yeast Pds5p is required for cohesion maintenance, but not for establishment. To test whether this is also the case in budding yeast, we assessed cohesion in synchronously growing *pds5* mutant cells.

Wild-type and *pds5* mutants were grown in YEPD at 23°C, arrested in S phase using hydroxyurea (HU), shifted to 37°C, and then released into fresh 37°C YEPD containing α-factor (see Materials and methods). By this regimen, cells progress synchronously through S phase and mitosis at the nonpermissive temperature for *pds5* mutants, and then arrest in G1 phase. To monitor cohesion, strains contained a Tet operator array integrated at the *URA3* locus and also expressed a Tet repressor–GFP fusion protein. Cell cycle progression was monitored by FACS® analysis and Western blot of Pds1p levels. Pds1p is a negative regulator of the metaphase–anaphase transition, and its destruction can be used as a molecular marker for anaphase onset (Cohen-Fix et al., 1996; Yamamoto et al., 1996a,b). HU-arrested cells at 37°C (t = 0) have unreplicated DNA, so both wild-type and *pds5-2* cells had only one GFP signal (Fig. 1, A and B). Both wild-type and *pds5* cells completed DNA replication by 30 min after release from HU (Fig. 1 B). As expected, few wild-type cells had two GFP signals (separated sisters) because of sister chromatid cohesion. Similarly, few *pds5-2* cells had two GFP signals, indicating that sister chromatid cohesion had been established and was still present on most chromosomes. In contrast, cohesin complex mutants exhibited significant loss of cohesion after S phase (Michaelis et al., 1997). By 45 min, the number of wild-type and *pds5-2* cells with two GFP signals increased significantly (Fig. 1 A). In wild-type cells, the sisters separated as a consequence of normal anaphase, as evidenced by decreased Pds1p levels (Fig. 1 C) and the location of *CEN*-proximal GFP signals at the leading edges of the separating DNA masses (Fig. 1 D, left). In contrast, Pds1p levels remained high in *pds5-2* cells from 45–90 min, even though most cells had two GFP signals (Fig. 1, A and C). The separated GFP signals in these cells remained close together (Fig. 1 D, middle). These results indicate that precocious sister dissociation had occurred in *pds5* cells and had activated the mitotic checkpoint. Finally, by 120 min in *pds5-2* cells, Pds1p levels had decreased and anaphase cells appeared (Fig. 1, A, C, and D; right). Similar results were observed with *pds5-1* cells (unpublished data). Thus, in *pds5* mutants, cohesion is established, but sisters undergo precocious dissociation before anaphase. Therefore, as in fission yeast, budding yeast Pds5p is not required for cohesion establishment, but is required for its maintenance during mitosis.

SMT4 suppresses the temperature sensitivity of *pds5* mutants

To gain insight into the role of Pds5p in cohesion, we screened for high copy suppressors of the temperature sensitivity of *pds5-1* cells (see Materials and methods). High copy plasmids bearing the *SMT4* gene (2μ *SMT4*) suppressed the temperature sensitivity of *pds5-1* cells to an extent comparable to *PDS5* itself (Fig. 2 A). High copy *SMT4* also suppressed haploid *pds5-2* and *pds5-3* mutant strains, although suppres-

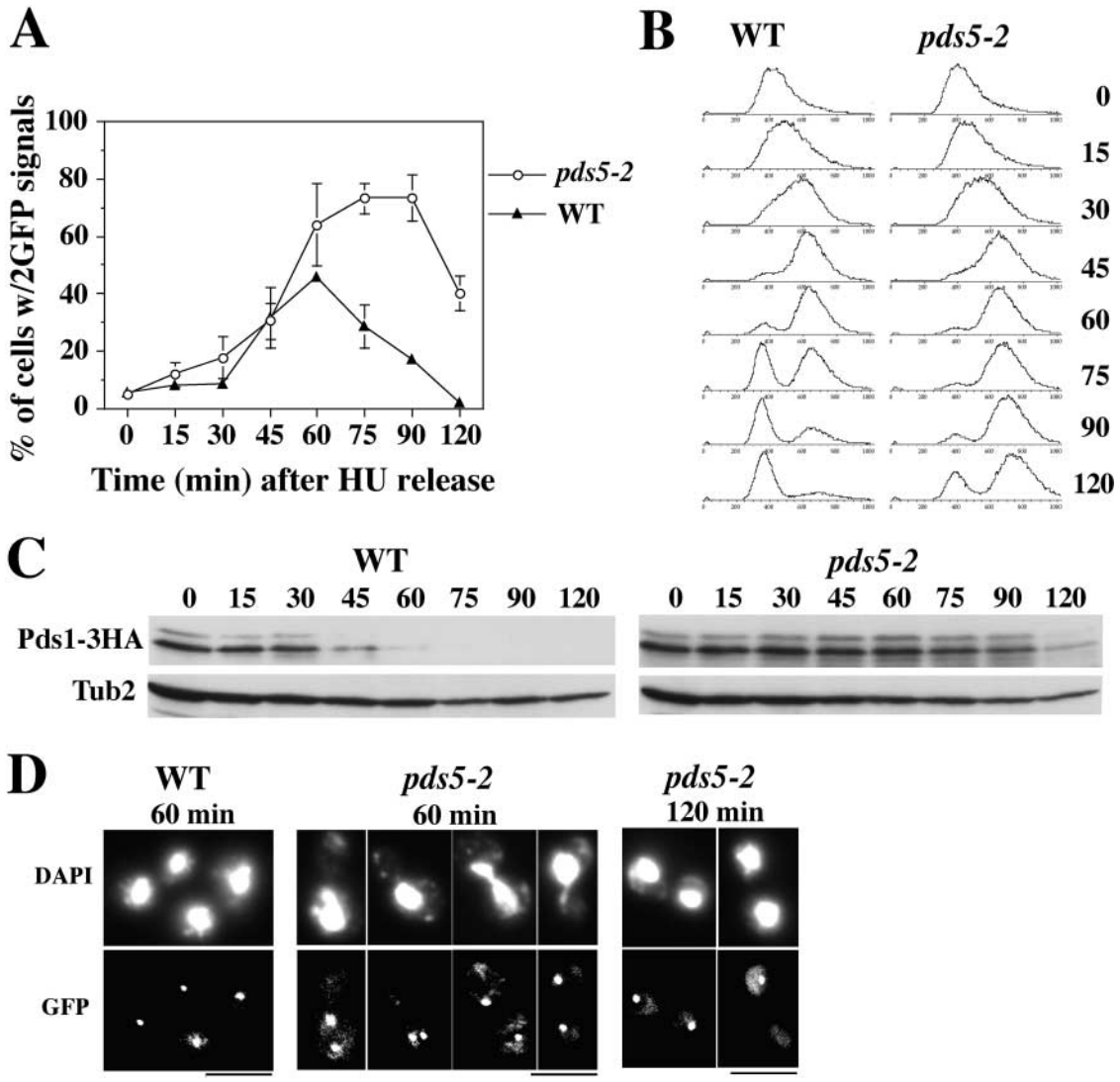

Figure 1. **Characterization of cell cycle and cohesion defects of *pds5* cells.** Wild-type (VG2450-7A and VG2390-37A) and *pds5-2* (VG2456-5C and VG2416-12A) haploids released from S phase (HU arrest) at 37°C. (A) Percentage of cells with two GFP signals. The number of cells with two GFP signals was determined in HU-arrested cells (t = 0) and at various times after release from arrest, and were then plotted as the percentage of total cells (see Materials and methods). (B) DNA content of cells by FACS® analysis. (C) Pds1p levels. Wild-type (VG2450-7A) and *pds5-2* (VG2456-5C) cells subjected to Western blot using anti-HA (Pds1-3HA) and anti-β tubulin (Tub2) antibodies (see Materials and methods). (D) Micrographs of cells with two GFP signals after release from HU arrest at 37°C. Chromosomal DNA (DAPI) and *URA3* locus (GFP) are shown. Bars, 5 μm. Data were generated from two independent experiments for all figures. For A, 100–200 cells were scored at every time point to generate error bars.

sion was weaker in *pds5-3* cells. *SMT4* present on a low copy *CEN* vector also strongly suppressed all three *pds5* alleles (Fig. 2 B). These results demonstrate that the temperature sensitivity of *pds5* mutants is exquisitely sensitive to *SMT4* levels.

The fact that *SMT4* robustly suppressed *pds5* cells suggested that *SMT4* either directly affects a property of the mutant protein or bypasses the need for Pds5p function. To distinguish between these possibilities, we asked whether *SMT4* could suppress a deletion of *PDS5.* Because *PDS5* is an essential gene in budding yeast (Hartman et al., 2000), we used a plasmid shuffle assay to test for suppression of a *pds5*-null allele (*pds5*::*URA3*; see Materials and methods). A haploid *pds5*::*URA3* strain was kept viable by a plasmid-borne copy of *PDS5* (pTH10; *PDS5 CEN LEU2*). We assayed the ability of high copy plasmid pTH40 (2μ *SMT4 TRP1*) to enable plasmid pTH10 loss. After 40 generations of growth in nonselective media, the pTH10 reporter was never lost from cells bearing pTH40 (2μ *SMT4 TRP1*). For positive and negative controls, we assayed high copy plasmids pTH39 (2μ *PDS5 TRP1*) and YEplac112 (2μ *TRP1*), respectively. As expected, the pTH10 reporter plasmid was readily lost from cells bearing pTH39 because it provides Pds5p, but pTH10 was never lost from cells bearing YEplac112. Thus, *SMT4* overexpression cannot suppress a *pds5*-null allele, indicating that Smt4p is likely suppressing a defect of the mutant *pds5* protein.

SMT4 is not a general suppressor of mutants defective in cohesion

Next, we determined whether *SMT4* specifically suppresses *pds5* mutants or if it is a general suppressor of mutants defec-

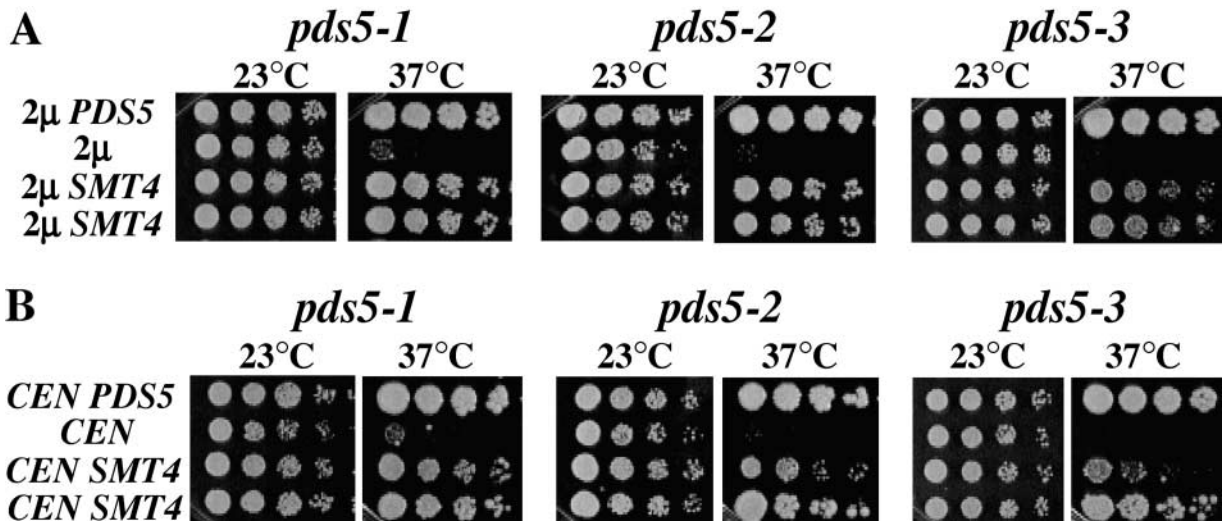

Figure 2. ***SMT4* suppresses *pds5* mutant temperature sensitivity.** (A) Effect of high copy *SMT4* (2μ *URA3* vector) on the temperature-sensitive phenotype of *pds5* mutants. Haploid strains *pds5-1* (VG986-5B), *pds5-2* (VG987-5C), and *pds5-3* (VG988-1C) containing 2μ *PDS5* (pVG175), 2μ (pRS202), or 2μ *SMT4* (pTH5) grown to saturation at 23°C in SC-URA liquid, plated in 10-fold serial dilutions on YEPD, and incubated for 72 h at 23 or 37°C. (B) Effect of low copy *SMT4* (*CEN URA3* vector) on the temperature-sensitive phenotype of *pds5* mutants. Haploid *pds5-1* (VG986-5B), *pds5-2* (VG987-5C), and *pds5-3* (VG988-1C) strains containing *CEN PDS5* (pVG282), *CEN* (YCplac33), or *CEN SMT4* (pTH4) grown and plated as described in A.

tive in cohesion, chromosome structure, or cell cycle progression. For this purpose, we assayed the effect of high copy *SMT4* (plasmid pTH5) on the temperature sensitivity of mutants in cohesin complex subunits (*mcd1*, *smc1*, and *smc3*), a protein required for cohesin complex loading on chromosomes (*scc2*), topoisomerase II (*top2*), a condensin complex subunit (*smc2*), and two genes important for cell cycle progression through mitosis (*pds1* and *esp1*). *SMT4* failed to suppress the cohesion, chromosome topology, or mitotic regulatory mutants, even at semi-permissive temperature (Fig. 3 A, 30 or 34°C; unpublished data). *SMT4* did weakly suppress *smc2-8*, the condensin complex subunit mutant (Fig. 3 A), consistent with a previous report (Strunnikov et al., 2001). Because Pds5p is required for condensation as well as cohesion, the weakness of *smc2-8* suppression may indicate an indirect suppression due to an effect on wild-type Pds5p by *SMT4* or a direct effect on the mutant Smc2p. Thus, *SMT4* suppression is largely specific for *pds5* mutants.

SMT4 encodes a SUMO isopeptidase (i.e., deconjugase), and as shown in Fig. 4, wild-type Pds5p is sumoylated. It is possible that *SMT4* suppresses *pds5* mutants by decreasing SUMO conjugation of mutant Pds5p. Consistent with this idea, a catalytically dead *smt4* allele fails to suppress *pds5* mutants (unpublished data). Moreover, overexpressing other SUMO pathway genes should affect *pds5* mutants in predictable ways. Proteins that promote SUMO deconjugation should suppress *pds5* mutants, whereas proteins that promote SUMO conjugation should be toxic. Therefore, we assayed the effect of high copy plasmids bearing either *ULP1* (another SUMO isopeptidase; Li and Hochstrasser, 2000), or *NFI1/SIZ2* (a SUMO E3 ligase; Johnson and Gupta, 2001) on the temperature sensitivity of *pds5* mutants (Fig. 3 B). High copy *ULP1* exhibited only a weak suppression compared with *SMT4*. In contrast, high copy *NFI1* exacerbated the temperature sensitivity of *pds5-1* cells. Similar effects were observed in *pds5-2* and *pds5-3* mutants (unpublished data). Thus, overexpression of either SUMO isopeptidase suppresses the *pds5* temperature sensitivity, whereas overexpression of a SUMO-conjugating enzyme is toxic. These results support the idea that sumoylation of the mutant Pds5p modulates its activity. Ulp1p localizes to the nuclear periphery, whereas Smt4p localizes to the nucleus (Li and Hochstrasser, 2000). The differences in their ability to suppress may reflect distinct target specificities, localization, or cell cycle control of their activity.

Pds5p is sumoylated in a cell cycle–dependent manner

Given the ability of SUMO pathway genes to either suppress or exacerbate the temperature sensitivity of *pds5* mutants, it seemed likely that Pds5p is modified by SUMO. Sumoylation could alter Pds5p function, and the mutant Pds5p might be especially sensitive to this modification. Alternatively, the effect could reflect an indirect effect of SUMO conjugation to another protein. Therefore, we examined whether wild-type Pds5p is sumoylated. Protein extracts from haploid yeast strains bearing MYC epitope–tagged Pds5p (*PDS5-6MYC* or *PDS5-12MYC*) or untagged Pds5p (*PDS5*) were isolated. MYC-tagged Pds5p was immunoprecipitated using anti-MYC antibodies, and then SUMO conjugation was detected by Western blot (see Materials and methods). Two prominent SUMO cross-reacting bands and a third weaker band of higher mobility can be seen in immunoprecipitations (IPs) from *PDS5-6MYC* and *PDS5-12MYC* strains (Fig. 4 A, left). In contrast, no SUMO bands were detected in the IP from an untagged strain. The sumoylated forms of Pds5p-6MYC had a mol wt ~20 kD smaller than those for Pds5p-12MYC. This correlates well with the size difference between Pds5p-6MYC and Pds5p-12MYC as shown by Western blot using anti-MYC antibodies (Fig. 4 A, right; see Materials and methods). Because

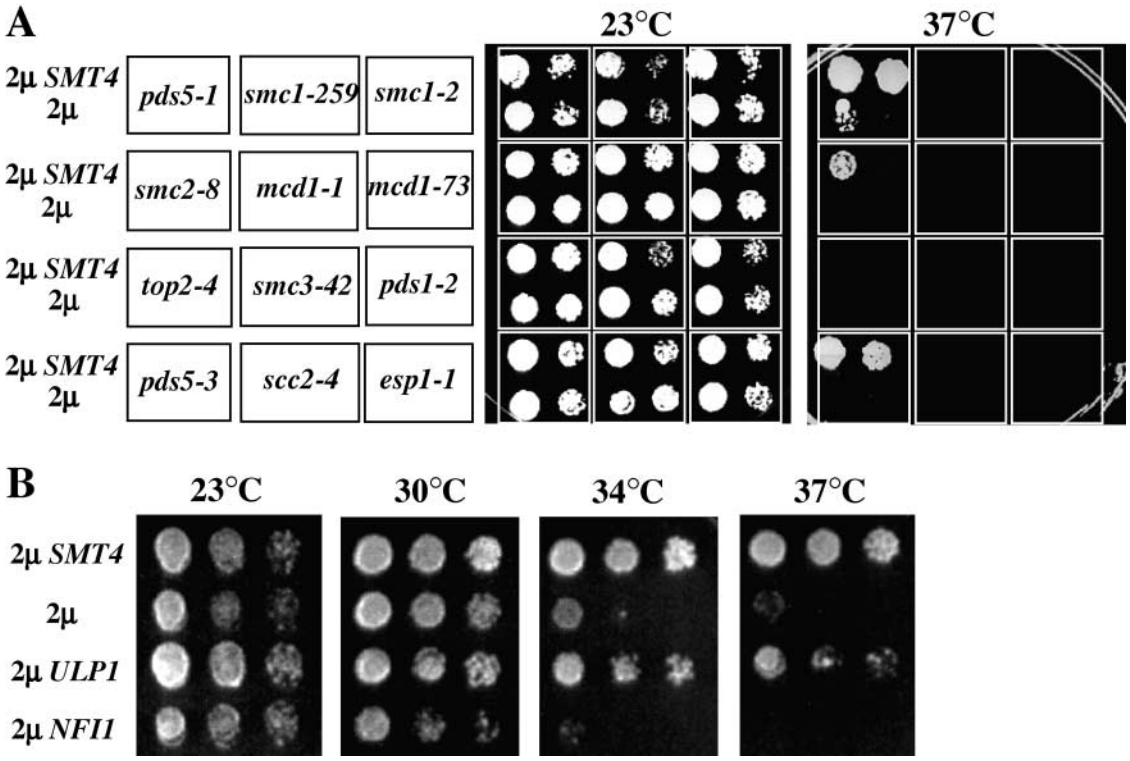

Figure 3. **Specificity of *SMT4* suppression.** (A) Effect of high copy *SMT4* (2μ *URA3* vector) on the temperature-sensitive phenotype of mutants defective in chromosome structure or cell cycle progression. Haploids *pds5-1* (VG986-5B), *pds5-3* (VG988-1C), *mcd1-1* (VG985-7C), *mcd1-73/scc1-73* (K5832), *smc1-2* (VG1360-7C), *smc1-259* (K6013), *smc3-42* (K5824), *scc2-4* (K5828), *smc2-8* (VG2029-7B), *top2-4* (VG2014-4D), *pds1-2* (VG971-1A), and *esp1-1* (2788) containing 2μ *SMT4* (pTH5) and either 2μ (pRS202) or 2μ (YEplac195) were grown to saturation at 23°C in SC-URA liquid, serially diluted fivefold (first well) and 10-fold (second well) on YEPD plates, and were then incubated at 23 or 37°C for 72 h. (B) Effect of high copy plasmids bearing genes in the SUMO pathway on the temperature-sensitive phenotype of *pds5* mutants. Haploid *pds5-1* (VG986-5B), *pds5-2* (VG987-5C), and *pds5-3* (VG988-1C) cells bearing high copy 2μ plasmids *SMT4* (pTH5), *ULP1*(pPM237), *NFI1* (pPM353), or vector only (YEplac195) were grown and plated as described in Fig. 2.

these strains are identical except for the number of MYC tags on Pds5p, these results confirm that Pds5p is sumoylated. As SUMO is added as monomers to target lysines, it appears that three SUMO molecules can be added to Pds5p.

Next, we asked whether Pds5p sumoylation is cell cycle regulated by examining a synchronous population of wild-type (*PDS5-6MYC*) haploid cells released from S phase at 23°C into YEPD containing α-factor (see Materials and methods). Every 15 min, cell aliquots were processed for isolation of total protein and FACS® analysis. Pds5p SUMO conjugation was detected by MYC IP and Western blot (see Materials and methods). Pds5p sumoylation was low in HU-arrested cells (t = 0), then increased after release from arrest (Fig. 4 B, top). Increased SUMO conjugation was seen at 15 min, which is before bulk DNA replication (Fig. 4, B and D). Replication was detected at 30 min and completed at 45 min (Fig. 4 D). It was difficult to ascertain when Pds5p sumoylation achieves a maximum because Pds5p levels decrease after release from HU arrest (Fig. 4 B, bottom; Fig. 4 C). Therefore, we performed densitometry to calculate the SUMO/Pds5p ratio. To plot relative sumoylation, we compared the SUMO/Pds5p ratio for HU-arrested cells to that from each time point after release. The ratio was set at one for HU-arrested cells (Fig. 4 E). SUMO conjugation increased on release, and reached a maximum at 75 min. Cells in G2 phase and M phase before anaphase have a single round DNA mass, whereas those in anaphase or telophase have an elongated DNA mass or two separated DNA masses, respectively. We scored DNA morphology and found no evidence for anaphase onset through 60 min, but by 75 min, 20% of cells are in anaphase or telophase. By 105 min, 64% are anaphase or telophase, and 9% have reached G1 phase (unpublished data). Thus, Pds5p SUMO conjugation begins before DNA replication and reaches its maximum at 75 min, when anaphase onset is first detected (Fig. 4 E).

Finally, we assessed Pds5p sumoylation in cells arrested in either G1 phase (α-factor), S phase (HU), metaphase (Nz), and telophase (*cdc15*) as described for Fig. 4 F. As before, densitometry of the SUMO/Pds5p ratio was normalized to HU-arrested cells. Pds5p sumoylation was similar in HU- and Nz-arrested cells, but was almost absent in telophase or G1 cells (Fig. 4 G). Thus, in cycling cells, Pds5p sumoylation increases from S phase through mitosis, but in cells arrested in S phase or metaphase, sumoylation is low. These results indicate that Pds5p sumoylation increases when chromosomes are undergoing dynamic morphological changes, including cohesion establishment, condensation, and dissolution of cohesion.

Smt4p and Nfi1p regulate the levels of Pds5p sumoylation

SMT4 suppresses the temperature sensitivity of *pds5* mutants and Pds5p is sumoylated, indicating a connection between Smt4p activity and Pds5p function. Therefore, we assayed

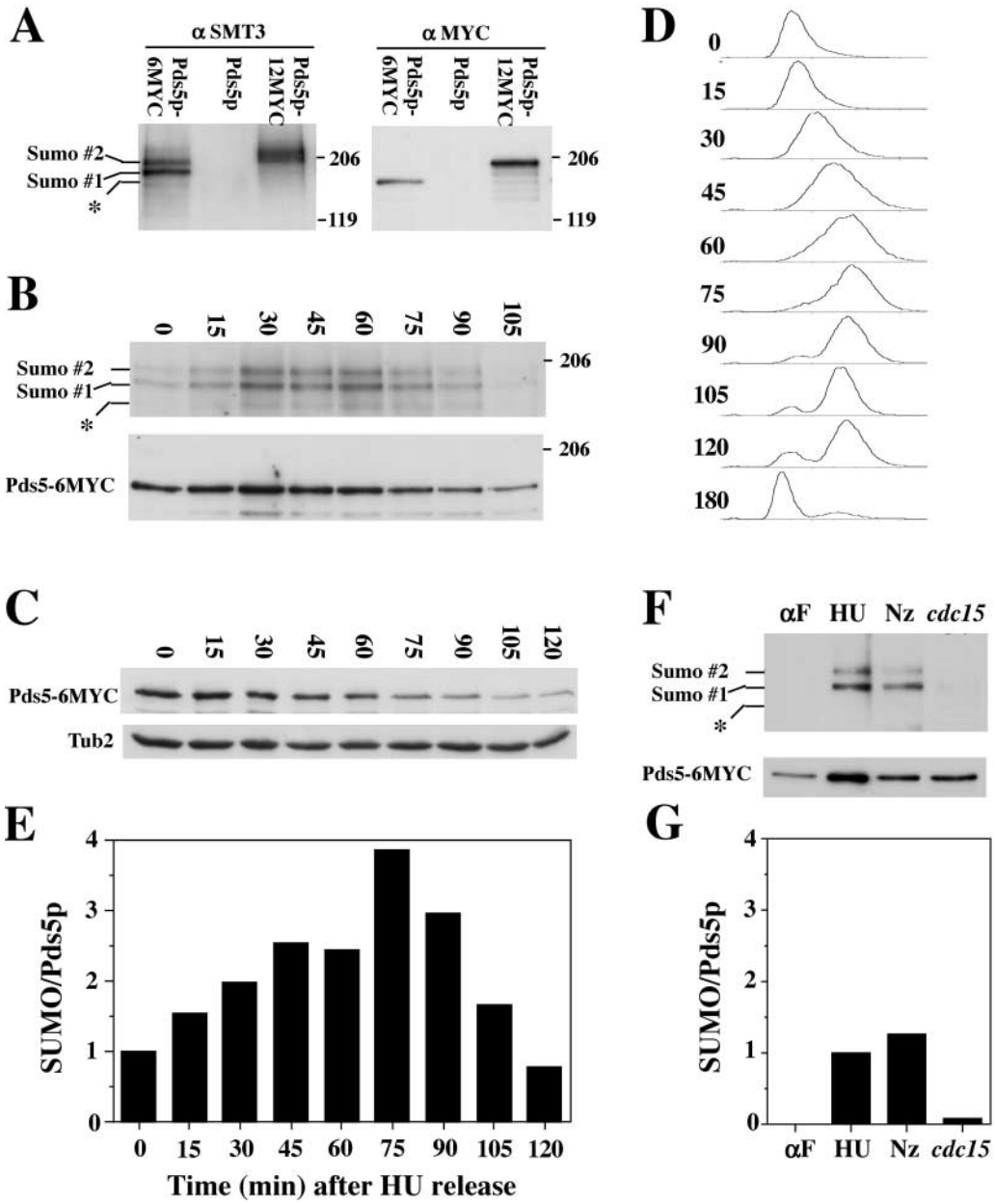

Figure 4. **Sumoylation of Pds5p.** (A) IP Western blot of Pds5p from asynchronous cells. *PDS5-6MYC* (VG2066-7B), *PDS5-12MYC* (VG2067-2B), and *PDS5* (VG982-6A) haploids were grown in YEPD at 23°C, and total protein extracts were made. Pds5p was immunoprecipitated using anti-MYC antibodies, and SUMO conjugation was detected by Western blot using anti-*SMT3* antibody (left gel). The position of two prominent Sumo bands is marked for the Pds5-6MYCp IP. The blot was stripped and probed with anti-*MYC* antibody to detect Pds5p (right gel). As a reference, the position where unsumoylated Pds5p (asterisk) would migrate is shown on the SUMO blot. (B–E) Synchronous populations of *PDS5-6MYC* (VG2066-7B) cells released from S phase arrest (HU). (B) IP Western blot of Pds5p. MYC-tagged Pds5p was immunoprecipitated, then SUMO and Pds5p were detected as described in A. Pds5p sumoylation (top gel). Two prominent Sumo bands are marked, as is the position where unsumoylated Pds5p (asterisk) migrates. The blot was stripped and probed to detect Pds5p (bottom gel). (C) Western blot of Pds5p. Total protein extracts were analyzed using anti-MYC (Pds5-6MYC) or anti-tubulin (Tub2) antibodies. (D) DNA content of cells by FACS® analysis. (E) Relative sumoylation of Pds5p. The SUMO/Pds5p ratio was determined at each time point by densitometry of Western blot in B. (F and G) Pds5p sumoylation in arrested cells. Cells bearing *PDS5-6MYC* were arrested in G1 phase (αF), S phase (HU), metaphase (Nz) using haploid VG2066-7B, or telophase (*cdc15*) using haploid VG2424-4C. (F) IP Western blot to detect Pds5p sumoylation. (G) Relative sumoylation of Pds5p. The SUMO/Pds5p ratio was determined by densitometry.

whether Smt4p activity modulates Pds5p sumoylation. Wild-type haploid (*PDS5-6MYC*) strains bearing either *SMT4* under control of a galactose-inducible promoter (*pGAL-SMT4*) or the *pGAL* promoter alone integrated at *TRP1*, were grown asynchronously at 23°C in YEP raffinose, and then galactose was added to induce the *pGAL* promoter (see Materials and methods). Cell aliquots were processed for isolation of total protein and FACS® analysis. Pds5p SUMO conjugation was detected by MYC IP and Western blot (see Materials and methods). Two Pds5p-SUMO bands were seen in raffinose-grown cells (Fig. 5 A, top). In contrast, 1 h after galactose addition, sumoylated Pds5p was absent in the

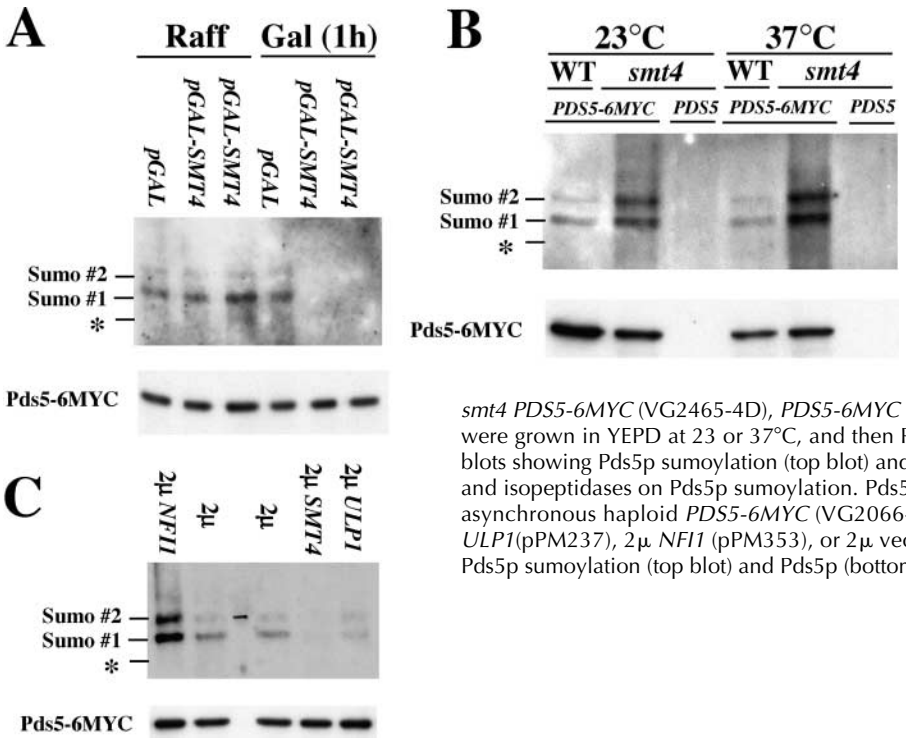

Figure 5. **Modulation of Pds5p sumoylation by SUMO pathway genes.** Pds5p sumoylation determined by IP Western blot as described in Fig. 4 A. (A) Effect of *SMT4* overexpression. Haploids containing *pGAL-SMT4* (VG2525-2B) or *pGAL* (VG2524-1A) were grown in YEP raffinose at 23°C, and then galactose was added for 1 h. Aliquots from raffinose- and galactose-grown cells were processed for IP Western blot analysis of Pds5p. SUMO conjugation (top blot) and Pds5p (bottom blot). IPs from two cultures of *pGAL-SMT4* cells are shown. (B) Pds5p sumoylation in *smt4* mutants. Haploids *smt4 PDS5-6MYC* (VG2465-4D), *PDS5-6MYC* (VG2066-7B), and *smt4 PDS5* (VG2463-1D) were grown in YEPD at 23 or 37°C, and then Pds5p was immunoprecipitated. IP Western blots showing Pds5p sumoylation (top blot) and Pds5p (bottom blot). (C) Effect of SUMO E3 and isopeptidases on Pds5p sumoylation. Pds5p was immunoprecipitated from extracts of asynchronous haploid *PDS5-6MYC* (VG2066-7B) cells containing 2μ *SMT4* (pTH5), 2μ *ULP1*(pPM237), 2μ *NFI1* (pPM353), or 2μ vector (YEplac195). IP Western blots showing Pds5p sumoylation (top blot) and Pds5p (bottom blot).

strain bearing *pGAL-SMT4*, but remained in the *pGAL*-bearing strain. Total Pds5p levels were unaffected by induction of *pGAL-SMT4* (Fig. 5 A, bottom). Thus, *SMT4* overexpression rapidly decreases Pds5p sumoylation.

Next, we assayed Pds5p sumoylation in *smt4* mutants. Wild-type and *smt4* mutant strains bearing Pds5p-6MYC were grown asynchronously in YEPD at 23°C, and were then incubated for 3 h at 37°C, the nonpermissive temperature for the *smt4* mutant. Aliquots of cells from 23 and 37°C were processed to assess Pds5p sumoylation and FACS® analysis (see Materials and methods). Pds5p sumoylation was greatly increased in *smt4* mutants (Fig. 5 B). Finally, we compared wild-type (Pds5p-6MYC) cells containing high copy plasmids bearing *NFI1*, *SMT4,* or *ULP1*. Overexpression of *NFI1* greatly increased Pds5p sumoylation and *SMT4* nearly eliminated it, whereas *ULP1* moderately decreased sumoylation (Fig. 5 C). These results indicate that Pds5p sumoylation is regulated mainly by Nfi1p and Smt4p activity, and is consistent with the idea that sumoylation modulates Pds5p function.

Overexpression of *SMT4* during a single cell cycle window suppresses the inviability and precocious sister dissociation of *pds5* mutants

Continuous *SMT4* overexpression suppressed *pds5* mutants. To map the cell cycle stage where suppression occurs, we overexpressed *SMT4* during a single cell cycle window from S phase through mitosis. *pds5-2* cells containing *PDS1-3HA* and either *pGAL-SMT4* or *pGAL* alone were arrested in S phase at 23°C in YEP raffinose. Galactose was added to induce the *pGAL* promoter (30 min), cells were incubated at 37°C (30 min), and were then released from S phase into fresh 37°C YEP raffinose + galactose containing α-factor (see Materials and methods). By this regimen, the cohesin complex and mutant Pds5-2p are presumed to load normally onto chromosomes in early S phase at permissive temperature, and then *SMT4* is overexpressed at the time when a small fraction of Pds5p is sumoylated (Fig. 4, B and D). Incubation at 37°C inactivates mutant Pds5-2p, and release at 37°C enables cells to progress through mitosis until rearrest in G1 phase with *SMT4* overexpressed at the nonpermissive temperature. Cell aliquots were plated for viability or fixed to monitor cell cycle progression (FACS®, cell morphology, and Pds1p levels) and sister chromatid cohesion (see Materials and methods).

For *pds5-2* cells with *pGAL* alone, viability decreased between 45 and 75 min after release (Fig. 6 A). *SMT4* overexpression largely suppressed this decreased viability (Fig. 6 A). DNA replication was completed by 30 min in *pds5* cells, regardless of *SMT4* overexpression, and in wild-type cells (Fig. 6 B), revealing that the decreased viability of *pds5-2* cells with *pGAL* alone occurs after replication. One possible explanation for this suppression was that *SMT4* overexpression abrogates the cell cycle delay of *pds5* mutants at 37°C (Fig. 1, B and C). However, FACS® profiles and Pds1p Western blots showed that *pds5* cells overexpressing *SMT4* still delayed in mitosis as compared with wild-type cells (Fig. 6, B and C). To assess when anaphase onset normally occurs, we scored DNA morphology in wild-type cells by counting anaphase (stretched DNA) or telophase (two separated DNA masses) cells. At 30 min, few anaphase cells were detected, but by 45 min, 25% of cells were in anaphase or telophase, and such cells increased to 60% by 60 min (Fig. 6 D). Thus, *pds5* cells lose viability around the time of anaphase onset, and *SMT4* overexpression prevents this viability decrease.

We assayed sister separation in *pds5* cells and by 30 min, few cells with two GFP signals are detected, indicating that cohesion exists regardless of *SMT4* expression (Fig. 7 A). By 45 min, separated sisters were seen in 36% of *pGAL* cells, but in only 23% of *pGAL-SMT4* cells. By 60 min, separated sisters

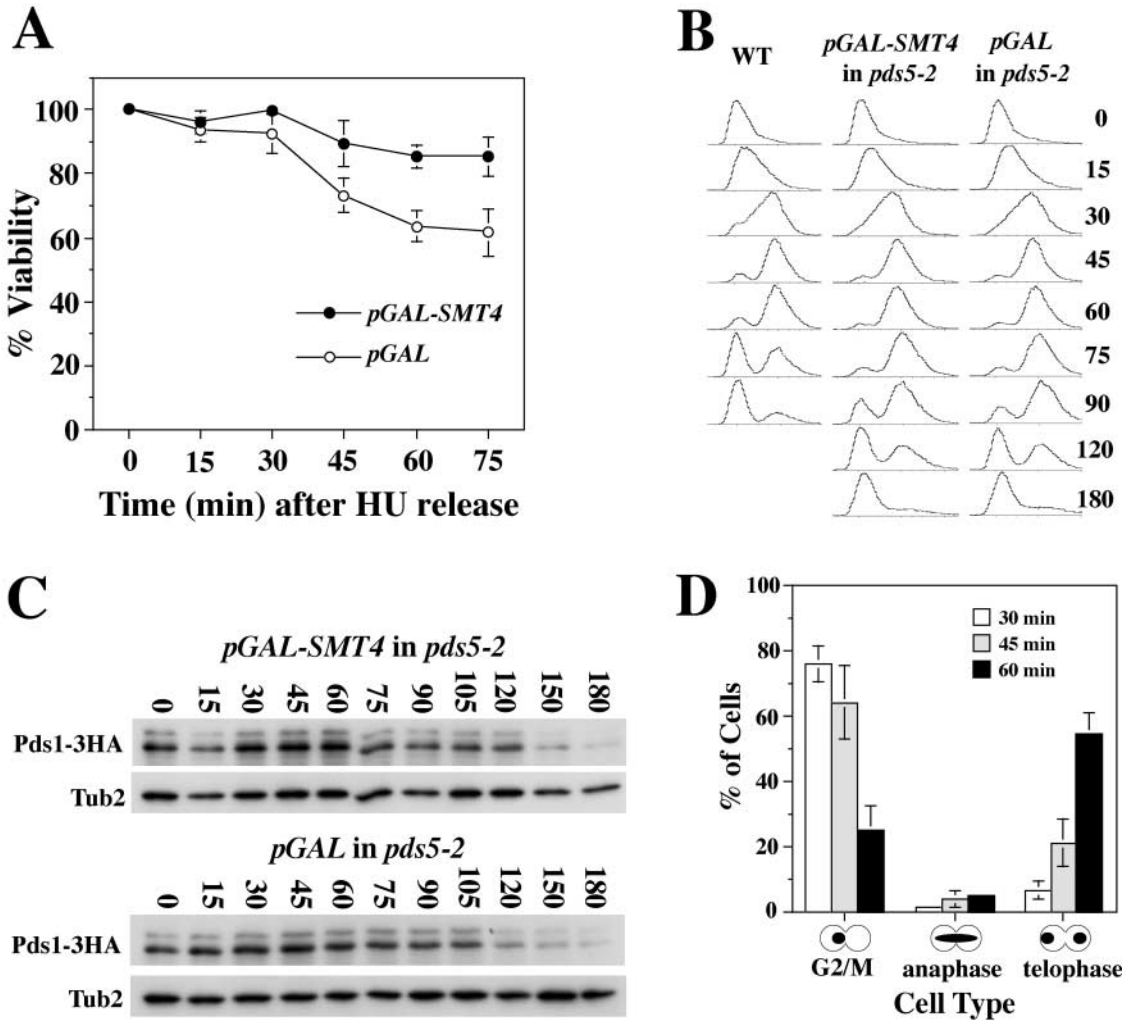

Figure 6. **Effect of *SMT4* overexpression on viability and cell cycle progression of *pds5* cells.** Mutant *pds5-2* cells bearing either *pGAL-SMT4* (VG2445-5B) or *pGAL* (VG2446-6A) and wild-type cells (VG2452-7A) were grown in YEP raffinose at 23°C, were arrested in S phase, galactose was added, and then cells were transferred to 37°C (t = 0). Cells were released from arrest at 37°C, and aliquots were processed every 15 min for FACS® analysis or were plated on YEPD to determine viability. (A) Relative viability. Percentage of cell viability was determined at each time point and normalized to viability in HU-arrested cells. Three independent experiments were performed to generate data and error bars. (B) DNA content of cells by FACS® analysis. (C) Pds1p levels. Mutant *pds5-2* cells bearing PDS1-3HA and either *pGAL-SMT4* (VG2485-3B) or *pGAL* (VG2486-14A) were grown as described above. Western blots of total proteins using anti-HA (Pds1-3HA) or anti-β tubulin (Tub2) antibodies. One of two independent experiments is shown. (D) Time of anaphase execution in wild-type cells. Large budded cells from haploid strain VG2452-7A in A were scored for DNA morphology to determine when anaphase occurs. 100 cells at each time point from three independent experiments were scored to generate data and error bars.

were detected in 62% of *pGAL* cells, but in only 38% of *pGAL-SMT4* cells. Separated sisters remained close together in *pds5* cells regardless of *SMT4* expression (Fig. 7 B). In contrast, in wild-type cells, separated sisters are far apart due to normal anaphase chromosome segregation. Pds1p levels remain high in *pds5* cells with *pGAL-SMT4*, so there must still be some precocious dissociation of one or more chromosomes, which activates the spindle damage checkpoint and inhibits anaphase spindle elongation. The most simple explanation is that *SMT4* overexpression prevents cell inviability in *pds5* mutants by reducing precocious sister dissociation during mitosis.

Discussion

Pds5p and the cohesin complex are important for sister chromatid cohesion, and bind to chromosomes from S-phase until the onset of anaphase, when cohesion is dissolved to enable sister segregation (Hartman et al., 2000; Koshland and Guacci, 2000). There has been great emphasis on identifying changes in cohesin complex subunits that are required to dissolve cohesion. However, the role of Pds5p in cohesion is not well defined. Recent evidence suggests that Pds5p and the cohesin complex serve distinct roles in cohesion. In this paper, we provide further evidence for this distinction. We identify *SMT4* as a suppressor of *pds5* mutants and provide evidence that Pds5p sumoylation promotes the dissolution of cohesion.

Pds5p is a regulator of cohesion maintenance and is functionally distinct from the cohesin complex

Existing data are consistent with the idea that the cohesin complex functions as the molecular glue responsible for sister

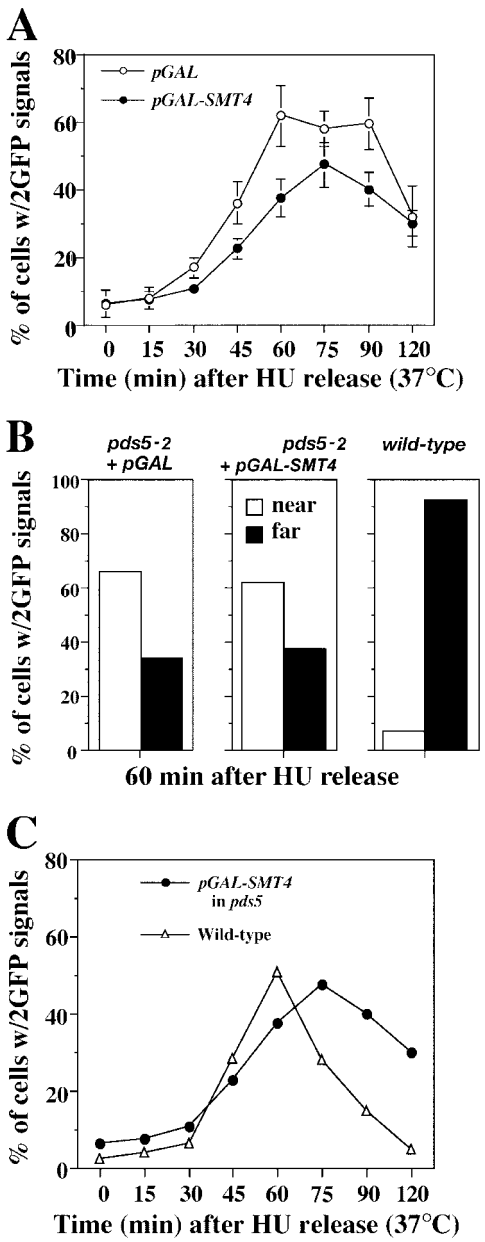

Figure 7. ***SMT4* overexpression suppresses precocious sister chromatid dissociation in *pds5* cells.** Mutant *pds5-2* cells bearing either *pGAL-SMT4* (VG2445-5B) or *pGAL* (VG2446-6A) grown as described in Fig. 6 were fixed to monitor cohesion near *URA3*. (A) Percentage of *pds5* cells with separated sister chromatids. The number of cells with one or two GFP signals was determined. Cells with separated sisters (two GFP signals) were plotted as a percentage of total cells. Three independent experiments were performed, and 200–300 cells at each time point in each experiment were scored to generate data and error bars. (B) Relative distance between separated sisters. In cells containing two GFP signals, the distance between GFP signals was scored as near (separated by less than one bud length) and far (separated by more than one bud length). Three independent experiments were performed. (C) Comparison of sister separation in *pds5* cells overexpressing *SMT4* and wild-type cells. Mutant *pds5-2* cells bearing *pGAL-SMT4* (VG2445-5B) and wild-type cells (VG2452-7A) were grown and scored for sister separation as described above.

chromatid cohesion (Koshland and Guacci, 2000; Meluh and Strunnikov, 2002). Several observations suggest that Pds5p is a regulator of cohesion. Pds5p colocalizes with the cohesin complex on chromosomes, and physically associates with the cohesin complex in yeast and vertebrate cells (Hartman et al., 2000; Sumara et al., 2000; Tanaka et al., 2001; Wang et al., 2002). Pds5p localization to chromosomes is dependent on the cohesin complex, whereas the cohesin complex still localizes to chromosomes in the absence of Pds5p (Hartman et al., 2000; Tanaka et al., 2001; Wang et al., 2002). The cohesin complex is required for cohesion at all times when cohesion exists (Koshland and Guacci, 2000; Meluh and Strunnikov, 2002). In contrast, Pds5p in budding and fission yeasts is required for cohesion maintenance, but not for its establishment (Tanaka et al., 2001; this paper). Finally, homologues of cohesin complex subunits and Pds5p are found in all eukaryotes, but cohesin complex subunits appear to always be essential, whereas *PDS5* is not always an essential gene (Denison et al., 1993; Hartman et al., 2000; Koshland and Guacci, 2000; Tanaka et al., 2001; Meluh and Strunnikov, 2002; Wang et al., 2002). These data indicate that Pds5p is a regulator that helps maintain cohesion via its interaction with the cohesin complex on chromosomes.

SUMO conjugation of Pds5p plays a role in cohesion dissolution

There is growing evidence that SUMO conjugation affects sister chromatid cohesion in budding yeast. The centromere is a site where cohesion exists and mutants in a kinetochore protein (Mif2p) are suppressed by *SMT4* overexpression (Meluh and Koshland, 1995; Megee and Koshland, 1999). *smt4* mutants have normal cohesion after replication, but exhibit precocious dissociation of *CEN*-proximal loci when arrested in G2/M phase (Bachant et al., 2002). *smt4* mutants have increased SUMO conjugation of many proteins, exhibit increased chromosome loss rates, and are sensitive to microtubule inhibitors (Li and Hochstrasser, 2000). Chromatin IP and fractionation experiments indicate that most of this increased SUMO conjugation occurs on chromosomally associated proteins (unpublished data). Finally, *smt3* (SUMO) mutants delay in G2/M phase (Biggins et al., 2001). These observations suggest a link between SUMO conjugation of one or more proteins and the dissolution of cohesion.

Pds5p and Top2p are SUMO-conjugated proteins implicated in sister chromatid cohesion (Bachant et al., 2002; this paper). However, if sumoylation regulates cohesion, Pds5p is likely the more relevant target. *pds5* and *smt4* mutants establish cohesion, but exhibit precocious dissociation of sisters during mitosis, indicating both have a defect in cohesion maintenance (Tanaka et al., 2001; Bachant et al., 2002; this paper). *pds5* and cohesin complex mutants arrested in mitosis have similar high levels of precocious sister dissociation, whereas *top2* mutants exhibit very low levels of sister separation in arrested cells (Guacci et al., 1997; Michaelis et al., 1997; Hartman et al., 2000; Bachant et al., 2002; Bhalla et al., 2002). Pds5p is intimately involved in cohesion via its physical interaction with the cohesin complex on chromosomes. There is no evidence that Top2p binds to the cohesin complex in vitro or on chromosomes. In addition, cohesion exists between sisters in the absence of DNA catenation

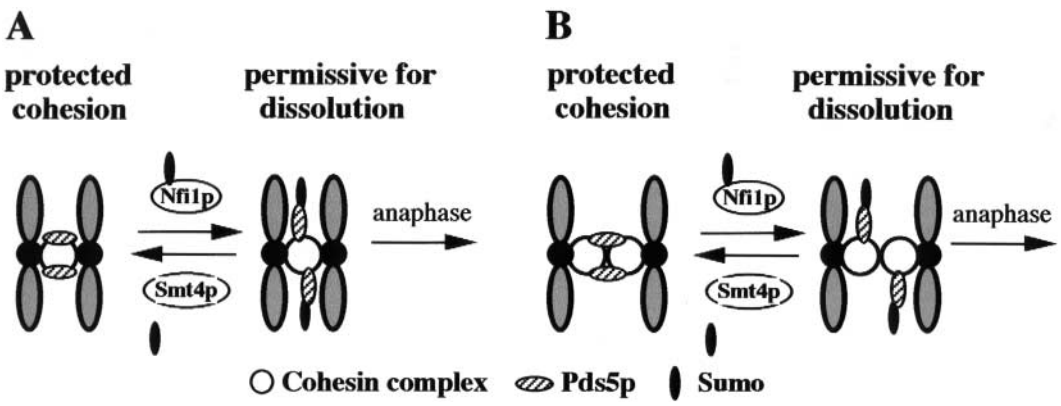

Figure 8. **Controlled access model for sister chromatid cohesion.** Shown are Pds5p (hatched ovals), the cohesin complex (open circles), SUMO (black ovals), and Nfi1p and Smt4p (open ovals). Ubc9p binds SUMO and transfers it to targets, but Nfi1p provides target specificity. For simplicity, we show only Nfi1p.

(Koshland and Hartwell, 1987; Guacci et al., 1994). These data suggest that Top2p plays an indirect role in cohesion. Smt4p overexpression efficiently suppresses the precocious dissociation in *pds5* mutants such that sister separation kinetics are similar to wild-type cells (Fig. 7 C). Furthermore, *pds5 smt4* double mutants are either synthetically sick or lethal, and a significantly higher fraction of Pds5p is sumoylated as compared with Top2p (unpublished data). Together, these results suggest that Pds5p sumoylation is more strongly connected to regulation of cohesion than is Top2p sumoylation.

Controlled access model whereby Pds5p regulates the cohesin complex

In our model, Pds5p bound to the cohesin complex on chromosomes controls the access of other factors to the complex. Models for sister chromatid cohesion envision one or two cohesin complexes at each cohesion site (Guacci et al., 1997; Anderson et al., 2002; Haering et al., 2002). Our model is consistent with either version (Fig. 8, A and B). We place the cohesin complex between sisters as the primary molecular glue because it is required for both cohesion establishment and maintenance. In contrast, Pds5p is positioned across the cohesin complex(es) and can promote cohesion maintenance in two ways. First, Pds5p could provide additional strength to the molecular glue. Second, Pds5p could protect the underlying cohesin complex from factors that trigger cohesion dissolution. In this configuration, sister chromatid cohesion is in a protected state.

To promote sister chromatid separation, Pds5p is sumoylated, which either changes its conformation or positioning to expose the underlying cohesin complex (Fig. 8, A and B; right side). Alternatively, sumoylation could drive Pds5p from chromosomes, but we favor a conformational change because this enables reversibility. Modulation of Pds5p sumoylation by *NFI1* and *SMT4* implicate them as the primary SUMO E3 ligase and SUMO isopeptidase for Pds5p, respectively. One attractive feature of the configuration proposed in Fig. 8 B is that the junction between two cohesin complexes would provide a target for regulating cohesion distinct from that controlling cohesin complex binding to chromosomes. Sumoylation of Pds5p would not trigger cohesion dissolution, but rather, would render the cohesin complex accessible to other factors that actually dissolve the molecular glue. Thus, our model proposes a two-step process. First, sister chromatid cohesion is made permissible for dissolution by Pds5p sumoylation. Second, the cohesin complex is acted upon to dissolve cohesion. The second step may involve Polo and Aurora kinases, the Esp1p protease, or other as yet unidentified factors (Uhlmann et al., 1999; Alexandru et al., 2001; Losada et al., 2002; Sumara et al., 2002).

The dissolution of cohesion is a crucial event for proper chromosome segregation. One popular model proposes that Mcd1p cleavage by Esp1p causes cohesin complex dissociation from chromosomes, such that cleavage is necessary and sufficient for cohesion dissolution (Uhlmann et al., 1999; Meluh and Strunnikov, 2002). However, several observations from budding yeast support alternative interpretations. In *smt4* mutants, neither Mcd1p cleavage nor dissociation from chromosomes is detected in G2/M-arrested cells, yet precocious sister dissociation occurs over a domain extending out at least 35 kb from the *CEN* DNA (Bachant et al., 2002). In *cdc5* (i.e., polo) mutants, Mcd1p cleavage is significantly reduced and Mcd1p remains at centromeres, yet sister separation at loci at least through 35 kb from the *CEN* DNA is similar to that in wild-type cells (Alexandru et al., 2001). Finally, in *cdc5 pds1* double mutants, Mcd1p cleavage is essentially eliminated and there is no apparent loss of Mcd1p from chromosomes, yet sister separation is normal at 1.4 kb from the *CEN* DNA, and occurs, but is delayed 35 kb from the *CEN* DNA (Alexandru et al., 2001). The cohesin complex is highly enriched over a 50-kb region flanking the *CEN* DNA (Blat and Kleckner, 1999; Megee et al., 1999), yet these three examples indicate that neither Mcd1p cleavage nor dissociation from chromosomes is necessary for sister separation at *CEN*-proximal loci. Microtubule-dependent forces can cause a separation or "breathing" of sisters at loci up to 9 kb from the *CEN* DNA (Goshima and Yanagida, 2000; He et al., 2000). One possibility is that this breathing region is expanded in *smt4* and *cdc5* mutants. However, sister separation in *smt4* mutants occurs in Nz-arrested cells, so it is independent of microtubule forces (Bachant et al., 2002). Alternatively, it is possible that the *smt4* and *cdc5* mutant analyses reveal an unrecognized early stage in sister separation, which does not entail either Mcd1p cleavage or cohesin complex dissociation

from chromosomes. Such a putative initial step could have been missed if the time frame of its execution was brief.

Sumoylation could modulate other aspects of chromosome dynamics

Pds5p is required for maintenance of cohesion, but not for establishment (Tanaka et al., 2001; this paper). Our data are consistent with a model showing that Pds5 sumoylation promotes cohesion dissolution during mitosis (Fig. 8). Fission yeast Pds5p may also have a role as an inhibitor of establishment (Tanaka et al., 2001). Our data do not preclude budding yeast Pds5p from playing a similar role. If it does, then sumoylation could also be involved because Pds5p sumoylation increases during S phase.

Pds5p sumoylation levels are low in S phase– or metaphase-arrested cells, whereas in cycling cells, Pds5p sumoylation increases during replication through mitosis. This suggests that sumoylation could play other roles in chromosome morphogenesis. Indeed, sumoylation is implicated in multiple aspects of chromosome structure in budding yeast. Smt4p activity and SUMO conjugation have been connected to proteins involved in cohesion (Pds5p), condensation (Smc2p), and DNA topology (Top2p) (Strunnikov et al., 2001; Bachant et al., 2002; this paper). Pds5p and the cohesin complex are also important for condensation (Hartman et al., 2000; Koshland and Guacci, 2000). Finally, correct temporal ordering of cohesin complex and condensin complex activity is important for proper chromosome condensation (Lavoie et al., 2002). These results indicate that there may be a relationship between the processes of cohesion, condensation, and DNA topology. One intriguing possibility is that SUMO conjugation could help regulate mitotic chromosome structure and dynamics by coordinately modulating the activity of Pds5p, Top2p, and Smc2p.

Materials and methods

Reagents and media

Reagents and media were as described previously (Hartman et al., 2000), except that YEP raff and YEP raff gal were identical to YEPD, and that dextrose was omitted and 2% raffinose or galactose was added. Yeast strains are listed in Table I.

Identification of suppressors of *pds5* temperature sensitivity

Haploid *pds5-1* (VG986-5B) was transformed with a pRS202 (2μ *ARS URA3*) high copy vector–based yeast genomic library (provided by P. Hieter, University of British Columbia, Vancouver, Canada) as described previously (Hartman et al., 2000). 23,000 transformants were screened, and five contained plasmids that suppressed lethality at 37°C. Four contained a 6.1-kb fragment bearing the *SMT4* ORF. The other had a distinct insert not the subject of this paper. The *SMT4* ORF (3.9-kb SphI/NheI fragment), cloned into YEplac195 (2μ *URA3*) to form pTH5, still suppressed the *pds5-1* temperature sensitivity (Fig. 1 A). pTH5 was linearized by BglII digestion, the 5′ overhang was filled in using Klenow, and the plasmid was recircularized to form pTH6. This truncates the *SMT4* ORF by introducing a frame shift after 114 amino acids and abolished suppression, confirming *SMT4* as the suppressor.

Plasmids

PDS5 (5.8-kb ClaI fragment) in pRS202 (*2μ URA3*) forms pVG175. *PDS5* (5.9-kb KpnI/XbaI fragment) in YEplac112 (*2μ TRP1*), YCplac111 (*CEN LEU2*), and YCplac33 (*CEN URA3*) forms pTH39, pTH10, and pVG282, respectively. *SMT4* (3.9-kb SphI/NheI fragment) in YCplac33 (*CEN URA3*) and YEplac112 (*2μ TRP1*) forms pTH4 and pTH40, respectively. Plasmid pPM237 has *ULP1* (2.8-kb fragment) in pRS202 (*2μ URA3*). *NFI1* (3.8-kb fragment) in pRS426 (*2μ URA3*) forms pPM353.

Plasmid shuffle assay

Diploid VG1359-1 has a truncation of the *PDS5* ORF by insertion of URA3 (*pds5*::*URA3*) to form a null allele on one homologue (Hartman et al., 2000). Plasmid pTH10 (*PDS5 CEN LEU2*) was transformed into VG1359-1, the transformants were sporulated, and tetrads were dissected. Haploid spore 2259-6B contained *pds5*::*URA3* and pTH10, and was used as the parent strain for plasmid shuffle assays. Because *PDS5* is an essential gene (Hartman et al., 2000), pTH10 cannot be lost from 2259-6B cells as it contains the sole copy of *PDS5*, so pTH10 is a reporter for test plasmid suppression of the *pds5*-null allele. High copy plasmids pTH40 (2μ *SMT4 TRP1*), pTH39 (2μ *PDS5 TRP1*), and YEplac112 (2μ *TRP1*) were transformed into 2259-6B as test plasmids for plasmid shuffle assays. Two transformants were used for each test plasmid. Cells were grown to saturation in SC-tryptophan (TRP) and leucine (LEU) to select for test and reporter plasmids (t = 0). Cells were grown for 40 generations in SC-TRP to retain test plasmids but to allow pTH10 loss, plated on YEPD, incubated for 3 d at 23°C, and then replica plated to SC-LEU to score presence (LEU+) or absence (LEU−) of pTH10.

COOH-terminal MYC tagging of Pds5p

Haploids with Pds5p COOH-terminal tagged (Pds5p-6MYC or Pds5p-12MYC) under control of the *PDS5* promoter (sole Pds5p source) were made as described previously for Pds5p-6HA (Hartman et al., 2000), except BamHI fragments with either 6 or 12 MYC epitopes were inserted after the last amino acid of the *PDS5* ORF. Strains grew at rates similar to the wild type, indicating tags did not compromise Pds5p function.

IP of MYC-tagged proteins

Yeast cells (10 OD_{600}) in 400 μl lysis buffer (50 mM Tris-HCL, pH 7.4, 5 mM EDTA, pH 8.0, 100 mM NaCl, and 1% SDS), 6 μl protease inhibitor cocktail (catalogue no. P8215; Sigma-Aldrich), and 2 mM *N*-ethylmaleimide (catalogue no. E1271; Sigma-Aldrich) were broken using glass beads. Samples were boiled and centrifuged for 10 min at 14 K. For IP, 100 μl protein extract was added to 900 μl IP dilution buffer (167 mM NaCl, 16.7 mM Tris, pH 8.0, 1.1% Triton X-100, 1.2 mM EDTA, and 1% BSA), 5 μl protease inhibitor, and 15 μl 9E10 mouse monoclonal anti-MYC antibody (Santa Cruz Biotechnology, Inc.), and were incubated overnight at 4°C. 60 μl protein G beads (per IP) were incubated in IP dilution buffer overnight at 4°C. Protein G beads were pelleted (2 K for 1 min at 4°C), supernatant was removed, protein extract antibody and 2 μl protease inhibitor were added, and beads were incubated for 4 h at 4°C. Beads were pelleted, supernatant was aspirated, and beads were washed in cold buffer with 2 μl protease inhibitor (1× with IP dilution buffer, 2× with TNT250 [250 mM NaCl, 50 mM Tris, pH 8.0, and 0.5% Triton X-100], 2× with TNT500 [500 mM NaCl, 50 mM Tris, pH 8.0, and 0.5% Triton X-100]). Beads were pelleted, supernatant was aspirated, and beads were resuspended in 65 μl 2× Laemmli Buffer, boiled, and then pelleted at 12 K. 15 μl IP was used for detection of SUMO.

Yeast cell culture conditions

Cells were grown in YEPD or YEP raffinose at 23°C to mid-log phase, and then treated as follows: for arrested cells; G1, S, or mid-M phase cells were arrested using α-factor, HU, or Nz and in telophase (*cdc15*) as described previously (Hartman et al., 2000). For synchronous populations of cycling cells released from S phase; mid-log phase cells were arrested in S phase at 23°C (when required, incubated for 30 min at 37°C in S phase), released into media containing α-factor to allow cell progression through mitosis, and arrested in G1 phase as described previously (Hartman et al., 2000). To induce the *pGAL* promoter, galactose (2% final concentration) was added to cells arrested in S phase in YEP raffinose, and cells were then incubated for 30 min.

Cell viability

Percentage of viable cells was determined as described previously (Yamamoto et al., 1996b).

Antibodies

Monoclonal mouse anti-MYC antibody 9E10 (Santa Cruz Biotechnology, Inc.) was used for IPs and detection of MYC-tagged proteins (1:1,000). Monoclonal mouse anti-HA antibody 12CA5 (1:5,000; Boehringer) and polyclonal rabbit antibody C257-2 (1:20,000) was used to detect HA-tagged or SUMO-conjugated proteins, respectively.

Monitoring sister chromatid cohesion at the *URA3* locus

Tandem array of Tet operator sequences integrated at *URA3* (35 kb from *CEN5* on chromosome V) and cohesion monitored by Tet repressor GFP fusion protein binding to the operator (Michaelis et al., 1997). Images were collected and recorded as described previously (Hartman et al., 2000).

Table I. **Yeast strains**

Strain	Genotype
VG982-6A[a]	*Mat**a** trp1 ura3 bar*
VG986-5B[a]	*pds5-1*
VG987-5D[a]	*pds5-2*
VG988-1C[a]	*pds5-3*
VG985-7C[a]	*mcd1-1*
VG2029-7B[a]	*smc2-8*
VG2014-4D[a]	*top2-4*
VG971-1A[a]	*pds1Δ::LEU2 leu2*
VG2128-2D[a]	*pds5-1 his3*
VG2066-7B[a]	*PDS5-6MYC*
VG2424-4C[a]	*PDS5-6MYC cdc15-2*
VG2067-2B[a]	*PDS5-12MYC lys2*
VG1360-7C	*Matα smc1-2:LEU2 ura3 leu2 bar1*
K5832[b]	*Mat**a** mcd1-73/scc1-73 ade2 can1 leu2 his3 ura3*
K6013[b]	*smc1-259 trp1*
K5824[b]	*smc3-42 trp*
K5828[b]	*scc2-4 trp1*
2788	*Mat**a** esp1-1 ura3 leu2 can1 lys2*
VG2524-1A	*Mat**a** PDS5-6MYC pGAL-TRP1:trp1 leu2 ura3 his3 bar1 lys2*
VG2525-2B	*Mat**a** PDS5-6MYC pGAL-SMT4-TRP1:trp1 leu2 ura3 lys2 his3 bar1*
VG2465-4D	*Mat**a** PDS5-6MYC smt4::HIS3 leu2 his3 bar1*
VG2463-1D	*Mat**a** smt4::HIS3 leu2 his3 bar1*
VG2390-37A[c]	*Mat**a** TETGFP-LEU2:leu2 TetOx224-URA3:ura3 trp1 bar1*
VG2416-12A[c]	*pds5-2*
VG2452-7A[c]	*pGAL-TRP1:trp1*
VG2450-7A[c]	*PDS1-3HA-KAN*
VG2456-5C[c]	*pds5-2 PDS1-3HA-KAN*
VG2445-5B[c]	*pds5-2 pGAL-SMT4-TRP1:trp*
VG2446-6A[c]	*pds5-2 pGAL-TRP1:trp1*
VG2452-7A[c]	*pGAL-TRP1:trp1*
VG2485-3B[c]	*pds5-2 pGAL1-SMT4-TRP1:trp1 PDS1-3HA-KAN*
VG2486-14A[c]	*pds5-2 pGAL1-TRP1:trp1 PDS1-3HA-KAN*
VG2259-6B	*Mat**a** pds5::URA3 trp1 leu2 ura3 + pTH10 (PDS5 CEN LEU2)*

[a]Strains were identical to VG982-6A except for genotypes shown.
[b]Strains were identical to K5832 except for genotypes shown (*mcd1-73*/*scc1-73* only in K5832), and were provided by K. Nasmyth (Research Institute of Molecular Pathology, Vienna, Austria).
[c]Strains were identical to VG2390-37A except for genotypes shown.

Flow cytometry

Flow cytometry was described previously (Yamamoto et al., 1996a).

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