Pds5 cooperates with cohesin in maintaining sister chromatid cohesion

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Background: Sister chromatid cohesion depends on a complex called cohesin, which contains at least four subunits: Smc1, Smc3, Scc1 and Scc3. Cohesion is established during DNA replication, is partially dismantled in many, but not all, organisms during prophase, and is finally destroyed at the metaphase-to-anaphase transition. A quite separate protein called Spo76 is required for sister chromatid cohesion during meiosis in the ascomycete *Sordaria*. Spo76-like proteins are highly conserved amongst eukaryotes and a homologue in *Aspergillus nidulans*, called BimD, is required for the completion of mitosis. The isolation of the cohesin subunit Smc3 as a suppressor of BimD mutations suggests that Spo76/BimD might function in the same process as cohesin.

Results: We show here that the yeast homologue of Spo76, called Pds5, is essential for establishing sister chromatid cohesion and maintaining it during metaphase. We also show that Pds5 co-localizes with cohesin on chromosomes, that the chromosomal association of Pds5 and cohesin is interdependent, that Scc1 recruits Pds5 to chromosomes in G1 and that its cleavage causes dissociation of Pds5 from chromosomes at the metaphase-to-anaphase transition.

Conclusions: Our data show that Pds5 functions as part of the same process as cohesin. Sequence similarities and secondary structure predictions indicate that Pds5 consists of tandemly repeated HEAT repeats, and might therefore function as a protein–protein interaction scaffold, possibly in the cohesin–DNA complex assembly.

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Background

The accurate transmission of genetic information during cell proliferation depends on chromosome duplication and the subsequent segregation of sister chromatids to the opposite poles of the cell during mitosis. Traction exerted by microtubules emanating from spindle pole bodies and attached to sister kinetochores is responsible for chromatid movement during anaphase. Some kind of physical link between sister chromatids is presumably necessary to ensure that sister kinetochores connect to microtubules that extend to opposite poles [1–4]. Sister chromatid cohesion is established during DNA replication and is retained until the onset of anaphase. It is important for resisting the tendency of microtubules to split sister chromatids once they have attached to kinetochores. The sudden destruction of sister chromatid cohesion has an important role in triggering chromatid segregation at the metaphase-to-anaphase transition [5].

Sister chromatid cohesion is mediated by a protein complex called cohesin, which consists of several subunits that are highly conserved from fungi to humans. Cohesin contains at least four subunits: Scc1/Mcd1, Scc3, Smc1 and Smc3 [6–10]. In yeast, the Scc1 subunit is almost wholly degraded at the end of mitosis and its resynthesis during late G1 leads to the loading of cohesin onto chromosomes shortly before the onset of DNA replication [7]. In vertebrates, a large fraction of cohesin dissociates from chromosomes during mitosis but rejoins them during telophase [8,10,11]. Loading of cohesin onto chromosomes in yeast depends on a separate complex consisting of at least two subunits (Scc2 and Scc4) [12]. Proteins homologous to Scc2 also exist in other organisms, including fission yeast (Mis4) [13], *Coprinus* (Rad9) [14], *Drosophila* (Nipped-B) [15] and humans, and it is therefore possible that such 'cohesin loading' complexes are conserved among eukaryotes.

Sister chromatid cohesion is established during S phase and must be maintained until the onset of anaphase. This suggests that the bridges that connect sisters during G2 and early M phase might be constructed as sisters emerge from replication forks [16]. An additional protein called Eco1 (also known as Ctf7 and Eso1 in fission yeast) [9,17,18] is crucial for establishing cohesion during S phase but not for maintaining it during G2/M. Eco1 is neither associated with soluble cohesin complex nor necessary for

loading cohesin onto chromosomes. Thus, the loading of cohesin onto chromosomes is not *per se* sufficient to establish cohesion. In yeast, sister chromatid cohesion is destroyed at the metaphase-to-anaphase transition by the proteolytic cleavage of the Scc1 subunit, which is mediated by the separin Esp1 [19,20].

A crucial question is whether the process mediated by cohesin constitutes the sole mechanism for holding sister chromatids together. Other proteins hitherto implicated in holding sister chromatids together may be involved in cohesin function. One protein for which this question is pertinent is the product of the *BIMD* gene, which is essential for chromosome segregation and DNA repair in *Aspergillus nidulans*, and interacts genetically with a Smc3-like protein [21,22]. The BimD homologue in *S. macrospora* (Spo76) is required for synaptonemal complex formation and sister chromatid cohesion during meiosis [23]. In *spo76* homozygous mutants, axial elements split during prophase and sister chromatids separate precociously during prometaphase and metaphase of meiosis I. Mitosis in *spo76* mutants is not, however, as severely impaired as meiosis. Despite a delay in prometaphase, during which chromosome compaction is altered, *spo76* mutants do not exhibit any major abnormalities during metaphase or anaphase. It has nevertheless been proposed that BimD/Spo76 has an important role in regulating chromatid cohesion and chromosome compaction in mitotic cells. The Spo76 protein is tightly associated with chromatin for most of the cell cycle but disappears from chromosomes during prometaphase. This distribution resembles that of cohesin in animal cells.

BimD/Spo76 is a highly conserved protein with homologues in most, if not all, eukaryotic cells. Here we address the function and behaviour of the homologous protein in *S. cerevisiae*, which has been called Pds5 (Saccharomyces Genome Database accession number SGDID S0004681). We show that Pds5 is required for sister chromatid cohesion. We also show that Pds5 binds to chromosomes at the same sites as cohesin and that the association and dissociation of Pds5 from chromatin during the cell cycle depends on that of cohesin. We also point out that Pds5 is composed of tandem HEAT repeats, a property shared with the cohesin-loading protein Scc2. These observations imply that Pds5 does not function independently of cohesin but cooperates with it in establishing and maintaining sister chromatid cohesion.

Results

Pds5 dissociates from chromosomes at the onset of anaphase

To analyse the expression of Pds5 we tagged the carboxyl terminus of the endogenous *PDS5* gene with 18 Myc epitopes. The resulting Pds5–Myc fusion protein appeared to be fully functional. Pds5–Myc was concentrated within nuclei throughout the cell cycle (data not shown). Indirect immunofluorescence of chromosome spreads prepared from an asynchronous culture (cycling cells) demonstrated that Pds5–Myc was tightly associated with chromosomes in many but not all cells (data not shown).

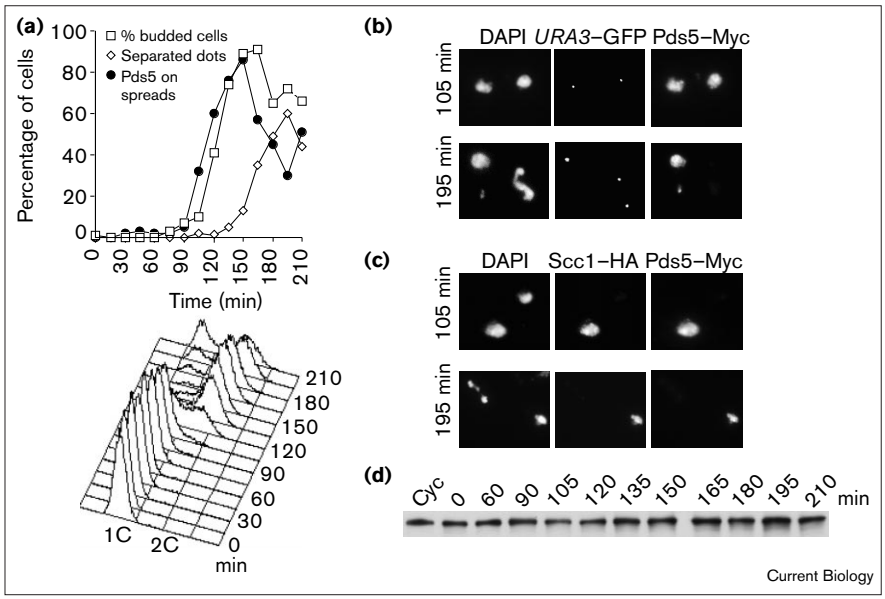
To investigate whether the association between Pds5–Myc and chromosomes was cell-cycle regulated, we analysed chromosome spreads prepared from a synchronous culture obtained by centrifugal elutriation. Sequences at the *URA3* locus (which is 35 kb from *CEN5*) were marked by the binding of a fusion protein of the Tet repressor and green fluorescent protein (TetR–GFP) to a tandem array of Tet operators, in order to monitor sister chromatid separation [7]. Pds5 was absent from chromosomes for most of G1 phase but was found associated with them from the beginning of the S phase until the onset of sister chromatid separation during anaphase (Figure 1a). During G2 and M phases, Pds5–Myc was invariably associated with chromosomes which had not yet separated sisters, that is spreads with unseparated GFP dots, but largely absent from chromosomes which had just separated sisters, that is spreads containing two GFP dots (Figure 1b). These data suggest that Pds5 associates with chromosomes during late G1 and dissociates from them at the onset of anaphase.

The pattern of Pds5 association with chromosomes resembles that of the cohesin Scc1 subunit. To compare the distribution of these two proteins more directly, we analysed in the same experiment the distribution of Pds5–Myc and haemagglutinin (HA)-tagged Scc1 (Scc1–HA) in chromosome spreads and found that their presence and absence on chromosomes correlated very tightly. In particular, mitotic chromosomes from which Scc1 had not yet dissociated invariably still contained Pds5–Myc, whereas 'anaphase' chromosomes from which Scc1 had disappeared also lacked Pds5–Myc (Figure 1c). We also measured Pds5–Myc protein levels by western blotting and found that its abundance did not change significantly during the cell cycle (Figure 1d).

Association of Pds5 with arm and centromere sequences depends on cohesin

To test whether Pds5 associates with cohesin on chromosomes, we compared the distribution of Pds5–Myc and Scc1–HA_6 in chromosome spreads and found that many foci of staining along the chromatin coincided (data not shown). Chromatin immunoprecipitation experiments have shown that cohesin specifically binds to centromeres and to particular sequences along chromosome arms [24–26]. We therefore used this technique to test whether Pds5 binds to these same sites, and if so whether its binding is dependent on cohesin. To do this, we used a Pds5–Myc strain in which *SCC1* is expressed exclusively from the *GAL1-10* promoter. Small unbudded G1 cells lacking Scc1 were isolated by centrifugal elutriation of a culture that

Figure 1

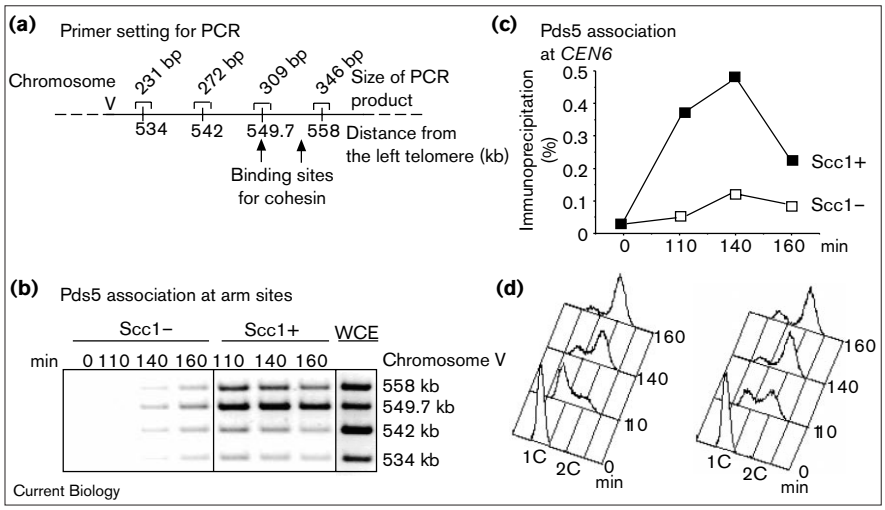


Pds5 association with chromosomes is regulated during the cell cycle. Small G1 cells of a strain expressing Pds5–Myc, Scc1–HA_6 and sequences at the *URA3* locus marked by GFP (K8982) were collected by centrifugal elutriation and released into YEPD at 25°C. Samples were collected every 15 min and **(a)** the percentage of budded cells (open squares), cells with separated sister chromatids (open diamonds), cells with Pds5 associated with chromosomes (filled circles), and DNA content (graph below) were determined. **(b)** Chromosome spreads showing Pds5–Myc association to chromatin before and after sister chromatid separation (upper and lower panels, respectively). **(c)** Chromosome spreads stained for Pds5–Myc and Scc1–HA_6, in cells in early S phase (upper panel) and anaphase (lower panel). **(d)** Pds5–Myc levels were determined by western blot analysis of protein extracts from samples taken at indicated time points and from cycling cells (Cyc).

had previously (90 minutes) been transferred to medium lacking galactose. This synchronous culture was split in half and each half incubated in the presence and absence of galactose.

The binding of Pds5–Myc to four different sites on the left arm of chromosome V was measured using a chromatin immunoprecipitation assay [26] as cells proceeded through the cell cycle (Figure 2a). Pds5–Myc associated strongly with sequences at 549.7 kb and weakly with those at 558 kb (as cells entered S phase) in cells that express Scc1 as a result of incubation with galactose (Figure 2b). This pattern of association was very similar to that observed for the cohesin subunits Scc1, Scc3, Smc1 and Smc3 [26]. The association of Pds5–Myc with chromatin was strongly reduced in cells that fail to express Scc1, that is in cells incubated in the absence of galactose. Pds5 also associated with centromere sequences from chromosome VI in the presence of Scc1, but poorly if at all in its absence (Figure 2c). This reduction in Pds5 association in the absence of Scc1 is not due to a general alteration of chromosome structure, because Scc1 depletion had no effect on the association with centromeres of the kinetochore protein Ndc10 [26]. These data suggest that Pds5 binds to chromosomes at the same sites as cohesin and does so in a manner that depends on cohesin.

Figure 2



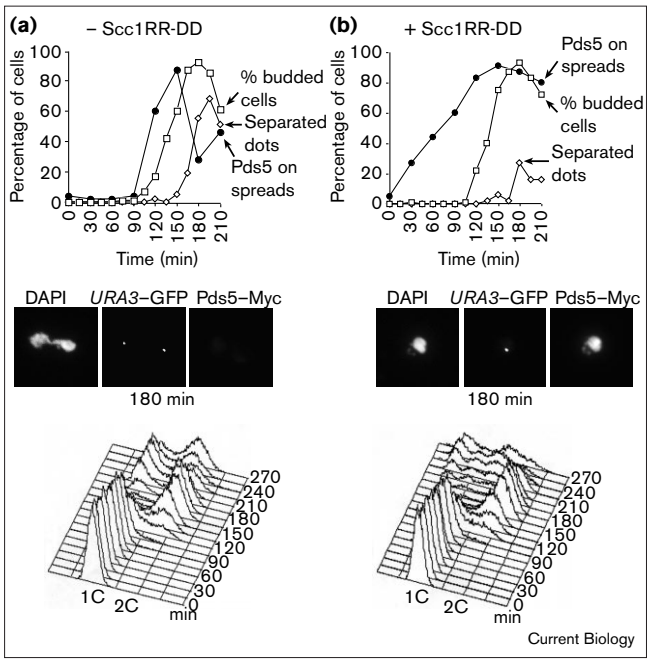
Association of Pds5 with chromosomes is dependent on cohesin and occurs at the same site as cohesin. Cells from the strain K9177 (*GAL–SCC1 scc1Δ PDS5–Myc18*) grown in YEPRaffgal were transferred into YEPRaff for 90 min. Then, G1 unbudded cells were collected by centrifugal elutriation and incubated in YEPD (– Scc1) or YEPRaffgal (+ Scc1) at 25°C (time 0 min). Association of Pds5–Myc to arm sites was detected by chromatin immunoprecipitation assay. **(a)** Map of genomic intervals amplified by PCR. **(b)** PCR was performed on DNA from immunoprecipitates (obtained using anti-Myc antibody) or whole-cell extract (WCE). **(c)** Association of Pds5–Myc with *CEN6*. The percentage of total DNA immunoprecipitated was calculated [26]. **(d)** DNA content measured by FACS.

We also investigated whether the association of Pds5 protein with chromosomes was dependent on Eco1. We found little or no difference in the amount of Pds5–Myc associated with chromosome spreads in wild type and *eco1-1* mutant cells at the restrictive temperature (data not shown).

Scc1 cleavage triggers dissociation of Pds5 from chromosomes at the onset of anaphase

The dependence on Scc1 of the association of Pds5 with chromosomes suggests that its dissociation from chromatin at the onset of anaphase might be induced by proteolytic cleavage of Scc1. To investigate this, we followed association of Pds5 with spread chromosomes as G1 cells progressed through the cell cycle in the presence and absence of the mutant Scc1RR-DD protein, which cannot be cleaved in a separin-dependent manner because of the mutation of P1 arginines at both sites of cleavage to aspartic acid [19]. Unbudded G1 cells from a strain expressing HA-tagged *SCC1RR-DD* from the *GAL* promoter were isolated by centrifugal elutriation and incubated in the presence and absence of galactose. In the absence of galactose (no Scc1RR-DD expression), Pds5–Myc associated with chromosomal DNA at the G1/S boundary and dissociated at the time of sister chromatid separation (Figure 3a). In the presence of galactose, Pds5–Myc not only associated with chromosomes long before the initiation of DNA replication but also failed to dissociate from them when sisters should have separated (Figure 3b). These results imply that the precocious accumulation of Scc1RR-DD protein during early G1 (that is before the inactivation of separin by securin accumulation during late G1) is sufficient to recruit Pds5 to chromosomes (*SCC1* expression from the *GAL* promoter does not *per se* lead to Scc1 accumulation in G1 and delay in metaphase). Detection of HA epitopes on chromosome spreads confirmed the accumulation of Scc1RR-DD protein on chromosomes in early G1 (data not shown). Our results also show that Scc1 cleavage triggers the dissociation of Pds5 from chromosomes at the metaphase-to-anaphase transition.

Figure 3



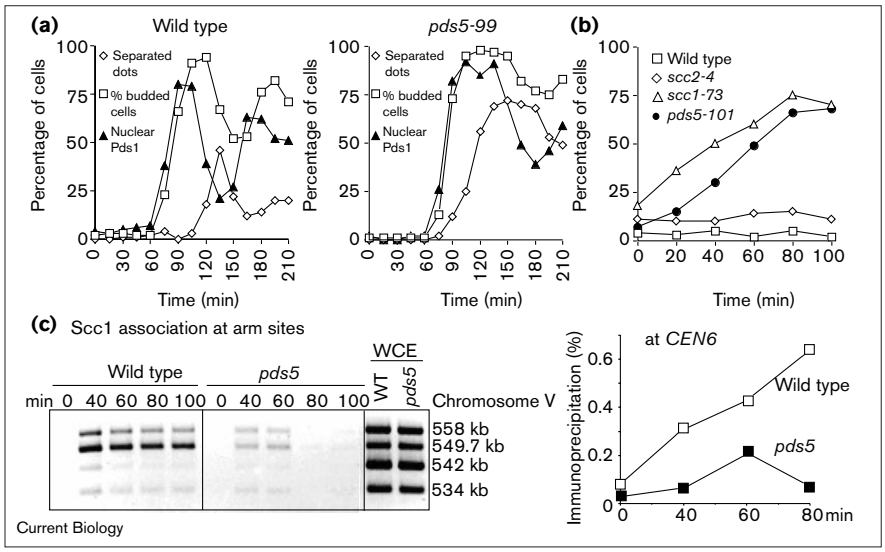
Pds5 dissociation from chromosomes is dependent on Scc1 cleavage. G1 cells of strain K9180 (*GAL–SCC1RR–DD PDS5–Myc18 URA3::tetOs tetR–GFP*) were collected by centrifugal elutriation and incubated in **(a)** YEPD (–Scc1RR–DD) or in **(b)** YEPRaffgal (+Scc1RR–DD) at 25°C (time 0 min). DNA content and the percentage of budded cells (open squares), cells with separated sister chromatids (open diamonds) and cells with Pds5 on chromosomes (filled circles) were determined (top panels). Chromosome spreads from cells analyzed after 180 min of incubation, showing DNA stained by DAPI, URA3–GFP dots and Pds5–Myc stained by Cy3-conjugated-antibody (middle panels). DNA content was measured by FACS (bottom panels).

Pds5 is required for sister chromatid cohesion in mitotic cells

Tetrad dissection of spores derived from a diploid heterozygous for a *PDS5* deletion showed that *PDS5* is an essential gene. Spores lacking *PDS5* stopped proliferating after undergoing one or two divisions following germination. To further characterize the mitotic function of Pds5, we generated temperature-sensitive (ts) alleles of *PDS5* using a mutagenic PCR strategy. We isolated eight independent alleles, each of which permitted growth at 25°C and caused rapid cessation of cell division after a shift to 37°C (data not shown). To test whether Pds5 is required for sister chromatid cohesion, small unbudded G1 cells of the ts *pds5-99* strain were collected by centrifugal elutriation and incubated at 37°C. Sister chromatid separation was scored by counting the fraction of cells with two separate GFP dots [7]. In wild-type cells, *URA3*/GFP dots never separate before degradation of the Esp1 separin inhibitor, the securin Pds1 [27]. In cohesin mutants with defective sister chromatid cohesion, GFP dots separate precociously and do so while Pds1 securin is still abundant [9,12]. The phenotype of *pds5-99* cells was similar to that of cohesin mutants. Sister chromatids separated somewhat earlier than wild type (compare left and right panels in Figure 4a). Meanwhile, Pds1 degradation was delayed. As a result, sister chromatids separated in the presence of Pds1 in a large fraction of *pds5-99* cells (Figure 4a). We concluded that Pds5 is necessary for sister chromatid cohesion.

The previous experiment did not address whether Pds5 is required to maintain sister chromatid cohesion during G2 or M phase or is merely needed to establish cohesion during S phase. To investigate this, we generated a ts *pds5* mutant strain (*pds5-101*) whose *CDC20* gene is expressed exclusively from the *GAL1-10* promoter (*GAL–CDC20*). Cdc20 is an activator of the anaphase-promoting complex (APC) and is needed for the destruction of securin and

Figure 4



Pds5 is required for sister chromatid cohesion and for association of cohesin with chromosomes. **(a)** Pds5 prevents premature sister chromatid separation. G1 cells of a strain K9179 (*pds5-99 PDS1–Myc18 URA3::tetOs tetR–GFP*) were collected by centrifugal elutriation and incubated in YEPD at 37°C (time, 0 min). Samples were collected every 15 min and percentage of budded cells (open squares), cells with two separated dots (open diamond) and cells with nuclear Pds1–Myc staining (filled triangles) were determined (right). Previously published data [7] is shown as a control (left). **(b)** Pds5 is required to maintain sister chromatid cohesion in metaphase-arrested cells. Strains K8465 (*cdc20::Leu2 GAL–CDC20*), K9181 (*cdc20::LEU2 GAL–CDC20 pds5-101*), K8466 (*cdc20::LEU2 GAL–CDC20 scc2-4*), and K8468 (*cdc20::LEU2 GAL–CDC20 scc1-73*) containing the URA3–GFP dots were grown in YEPRaffgal at 21°C and then transferred into YEPD for 3.5 h, to repress *CDC20* expression. Cells were then placed in YEPD prewarmed to 35.5°C and samples were collected every 20 min (time 0 min). Arrested cells showed 2C DNA content (data not shown). Sister chromatid separation was scored by counting the fraction of cells with two GFP dots. **(c)** Cells of K8038 (*SCC1–Myc18 PDS5*) and K9178 (*SCC1–Myc18 pds5-99*) strains were arrested in a G1-like state by treatment with α-factor (1.5 μg/ml) for 140 min. Cells were harvested by filtration and incubated (time 0 min) in YEPD-containing nocodazole (15 μg/ml) at 33°C, which is a restrictive temperature for *pds5-99* cells. Scc1 association with chromatin was investigated at both arm and centromere (CEN6) sites (map as in Figure 2a).

B-type cyclins [28,29]; the transfer of *GAL–CDC20* cells to medium lacking galactose causes cells to arrest in metaphase. Wild type and *pds5-101 GAL–CDC20* cells growing at 21°C were uniformly arrested in metaphase by incubation in medium lacking galactose for 3.5 hours and then shifted to 35°C, which is a restrictive temperature for this allele. This caused sister chromatids to separate in a high fraction of *pds5-101* cells but in few if any wild-type cells (Figure 4b). As expected, under the same conditions sister chromatids separated in a high fraction of *scc1-73* cells, whereas almost no separation was observed in *scc2-4* cells [12]. This implies that Pds5 is required to maintain cohesion between sister chromatids during metaphase.

Pds5 is necessary for the stable association of Scc1 with chromosomes

We have found that Pds5 co-localizes with cohesin on chromosomes, that it is recruited to chromatin by cohesin, and that it is needed for maintaining sister chromatid cohesion during metaphase. This suggests that Pds5 might be part of the same mechanism and/or structure by which cohesin connects sisters. If so, Pds5 might be needed for the stable association of cohesin with chromosomes. To test this, we used chromatin immunoprecipitation (CHIP) to compare the association of Scc1 protein (tagged with Myc epitopes) with arm and centromere sites, after wild-type and *pds5-99* mutant cells were released from an alpha-factor-induced G1 arrest at 33.5°C, in the presence of nocodazole. Association of Scc1 with both arm and centromere sequences was greatly reduced in the *pds5-99* cells (Figure 4c). This suggests that Pds5 is necessary for the stable association of cohesin with chromosomes and raises the possibility that the loss of sister chromatid cohesion in *pds5* mutants might be due, at least partly, to defects in the association of cohesin with chromosomes. We also detected a reduction in the amount of Scc1 associated with spread chromosomes in *pds5-99* cells (data not shown).

Pds5 is composed of HEAT repeats

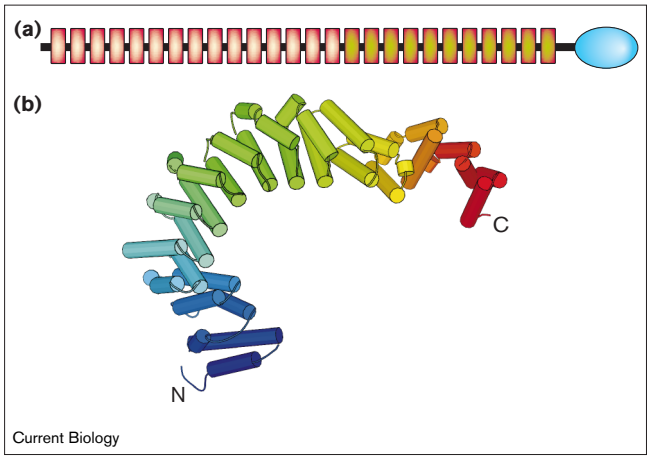
Pds5 is an evolutionarily well-conserved protein [23]. Protein database searches with the Pds5 sequence revealed homologues in other fungi (*Sordaria macrospora*, *A. nidulans*, *Schizosaccharomyces pombe*) and in higher eukaryotes (*Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana* and humans). We analysed the secondary and tertiary structures of Pds5 predicted from its amino acid sequence. We found a very low glycine content, which indicates reduced backbone flexibility and, generally, correlates with increased helix content. There is also a highly charged carboxy-terminal region 1190–1277 (with 44/88 charged residues (type DEKR)). Secondary structure content predictions [30,31] suggest an α-helical content of more than 60% of all residues and very little β-structure. A hidden Markov model (HMM) of the HEAT-repeat domain [32] produces positive scores (> 2.2)

in the three regions 277–312, 356–391, and 396–431 and a fourth marginally positive score for segment 793–828.

Iterative profile searches with PSI-BLAST [33] (inclusion condition $p < 0.003$) find numerous hits of HEAT-repeat-containing proteins (among which are the regulatory subunit A of protein phosphatase 2A, importin β, karyopherin, and known tertiary structures [34–36] in the region 1–625. We suggest the sequential locations of 15 HEAT repeats each including 35–45 residues with starts at sequence positions 6, 48, 87, 140, 185, 230, 275, 314, 354, 393, 435, 475, 516, 553 and 595 (Figure 5a).

We can also make reasonable suggestions for most of the remaining protein. If only the sequence segment 600–1277 is subjected to an iterative PSI-BLAST database search (three rounds), a hit to the regulatory subunit A of protein phosphatase 2A from *S. pombe* appears with $p = 0.29$ for the Pds5 region 720–1026 (seven potential HEAT-repeat segments). Although the statistical significance is low, this finding is in agreement with secondary structure content and secondary structure predictions as well as with the fourth HEAT-repeat HMM hit at positions 793–828. In addition, there are four long helices predicted in the region 620–720 (representing two possible HEAT repeats) and another four in the region 1040–1130 (yet another two potential repeats). Thus, we may suppose the existence of another 11 (= 2 + 7 + 2) HEAT repeats in the sequence range 630–1130 (Figure 5a).

Figure 5



Domains and tertiary structure of Pds5. **(a)** Sequence similarity considerations indicate that the region 1–625 of Pds5 consists of two-helix HEAT units which are tandemly repeated (15 domains shown in light colour). There is other but less significant evidence that the sequence 625–1130 is composed of another 11 HEAT repeats (dark colour). The carboxy-terminal segment 1190–1277 (blue) is heavily charged. **(b)** The structure of the regulatory subunit A of the protein phosphatase PP2A (PDB entry 1b3u [34]) may serve as a model for the tertiary structure of the first 15 domains as its sequence is the closest to that of Pds5 (image produced with Molscript [46]). We propose that Pds5 is a band of HEAT-repeat units with an inner surface interacting with target proteins. The helices forming the outer surface are sometimes subdivided into two subhelices mostly because of prolines in the sequence.

Our sequence analysis suggests that Pds5 is a HEAT-repeat protein (segment 1–1130, totally ~26 repeats) with a highly charged carboxy-terminal end (1190–1277). Pds5 therefore appears to be a large scaffold protein with numerous sites for binding globular domains of other proteins. It may function as an organizer of protein–protein interactions, presumably with cohesin. The charged carboxyl terminus may have a role in binding charged macromolecules, for example DNA.

In the course of our analyses, we realised that Scc2, like Pds5, also contains multiple HEAT repeats. The HMM of the HEAT repeats [32] revealed eight HEAT repeats in Scc2 within a region of amino acid 250–1281 (A. Schleiffer, personal communication). Although the existence of HEAT repeats in Pds5 and Scc2 does not necessarily imply that they can bind to the same domains, we speculate that the HEAT-repeat structures present in both proteins mediate an interaction with cohesin.

Discussion

The Spo76 protein in *Sordaria* has an important role in maintaining sister chromatid cohesion during meiosis [23]. Most eukaryotic genomes encode homologous proteins. In this paper we have addressed whether Spo76-like proteins are needed for sister chromatid cohesion during mitosis and, if so, whether they function in the same process as cohesin. We show that the Spo76 homologue in *S. cerevisiae*, which is called Pds5, is needed for maintaining cohesion during G2 and M phase, and that cohesin and Pds5 co-localize on chromosomes in a mutually interdependent manner. These results imply that Pds5/Spo76-like proteins have a crucial role in holding sister chromatids together and that they are an important component of the cohesin system. The notion that Pds5 acts as part of the cohesin system is consistent with the previous finding that mutations in the homologous protein in *Aspergillus*, which is called BimD, are suppressed by mutations in the *SUDA* gene, which encodes an Smc3-like protein [22]. On the basis of protein sequence similarity and secondary structure analysis, we show that Pds5 consists of HEAT repeats over most of its sequence. In analogy to known structures of HEAT-repeat proteins, the tertiary structure of Pds5 appears to be a superhelical band of two-helix repeats [37]. Typically, the inner surface of this band offers interaction regions with globular target proteins. Pds5 appears to be a large scaffold protein having numerous, possibly similar or even uniform, sites for binding specific globular domains of other proteins. The HEAT-repeat structures would serve as a platform for multiple protein–protein interactions, either with cohesin subunits or with other chromosomal proteins involved in DNA repair and chromosome

structure. However, we have little or no idea at present how Pds5/ Spo76-like proteins contribute to holding sisters together. An attractive possibility is that binding to Pds5 is a requirement for cohesin loading onto chromosomes. In this case, Pds5 would mediate cohesin/DNA complex assembly.

BimD and Spo76 are also required for DNA repair and both mutants are hypersensitive to exposure to DNA-damaging agents [21,38]. Preliminary data indicate that *pds5-99* mutants are also partially defective in DNA repair after irradiation with γ-rays (Camilla Sjogren and K.N., unpublished results).

Two complexes have thus far been implicated in sister chromatid cohesion: cohesin, which contains the Smc1, Smc3, Scc1 and Scc3 proteins [9,8] and a separate complex containing the Scc2 and Scc4 proteins [12]. Whether these two complexes are really separate *in vivo* is still unclear, because at least some Scc2 has been found associated with cohesin. Though essential for cohesin's association with chromosomes, neither Scc2 nor Scc4 co-localize with cohesin on chromatin. Furthermore, neither protein appears to be required for maintaining cohesion between sisters during metaphase. Pds5 therefore shares many more properties with cohesin subunits than it does with Scc2 or Scc4. We found that a substantial fraction of cohesin is associated with Pds5 in immunoprecipitates (data not shown). However, unlike the interaction between individual cohesin subunits, the Pds5–cohesin interaction is salt sensitive and we cannot exclude the possibility that it is mediated through other chromatin-bound components. Vertebrate Pds5 homologues have also been shown to co-fractionate with cohesin subunits in a salt-dependent manner [11].

It is therefore doubtful whether Pds5 should be considered a *bona fide* cohesin subunit, as it is not clear whether it interacts stably with soluble cohesin. Cleavage of cohesin's Scc1 subunit has recently been shown to trigger the onset of anaphase in yeast [19]. Cohesin therefore clearly has a crucial role in holding sister chromatids together, but whether it constitutes the actual bridge connecting sister chromatids has yet to be resolved. Future work should clarify whether Pds5/Spo76 like proteins regulate bridges mediated by cohesin.

Materials and methods

Yeast strains and growth conditions

All strains are derivatives of W303. Epitope tagging and disruption of *PDS5* at the genomic locus were performed with a PCR-based method, taking advantage of homologous recombination [39]. Strains carrying a tagged version of the gene did not show any differences in cell growth and proliferation, when compared to the wild type.

YEP medium [40] was supplemented with 2% raffinose (YEPRaff), 2% raffinose and 2% galactose (YEPRaffgal), or 2% glucose (YEPD). To obtain synchronous cultures, cells were grown in YEPRaff medium at 23°C, and small G1 cells were isolated by centrifugal elutriation [41]. For release from G1-like phase, Mat**a** cells were grown at 23°C, treated with α-factor for 2.5 h (0.5 μg/ml at 0, 1 and 2 h) and then filtered. For G2/M-like arrest, nocodazole was added to 15 μg/ml.

Construction of conditional alleles of PDS5

A *PDS5/Δpds5::HIS3* diploid strain (K9175) was transformed with a centromeric plasmid containing the wild-type gene (YCplac33PDS5) and allowed to sporulate. After dissection of tetrads, a *Δpds5::HIS3* haploid strain kept alive by the presence of the plasmid YCplac33*PDS5* (K9176) was used to isolate temperature-sensitive alleles of *PDS5*. To achieve this, *PDS5* was mutagenized by PCR [42,43] and the strain K9176 was transformed with these PCR products and with the YCplac111 plasmid carrying a gapped version of *PDS5* lacking most of the gene. Transformants grown on Leu$^-$ YEPD plates were replica plated on plates containing 5-fluoro-orotic acid (5-FOA), to isolate those that lost the YCplac33PDS5 plasmid. Colonies growing in the presence of 5-FOA were then replica plated on phloxine B-containing YEPD plates (1 mg phloxine B per 1 l medium) at 25°C and 37°C. Roughly 200 temperature-sensitive transformants were streaked for single colonies and checked for ts phenotype. Out of many that showed tight temperature sensitivity at 37°C, eight independent strains were chosen and mutated *PDS5* rescued from all of them were recloned into the YIplac128 integrative plasmid. These constructs were integrated at the *LEU2* genomic locus in K9176, and transformants that lost the YCplac33PDS5 plasmid were selected on 5-FOA-containing plates. Two independent strains were transformed back with the YCplac33PDS5 plasmid, to verify the rescue of the ts phenotype, and were then used for further studies.

Other techniques

A FACSscan (Becton-Dickinson) was used for flow cytometric quantification of cellular DNA content [44]. Visualization of sequences at 35 kb from centromere on chromosome V with the TetR–GFP/tetO system and chromosome spreading were performed as already described [7]. Myc-epitope-tagged Pds5 was detected using anti-Myc mouse antibody 9E10 with anti-mouse Cy3-conjugated goat antibody (Amersham). For double staining of Pds5–Myc and Scc1–HA, anti-Myc rabbit antibody with anti-rabbit Cy3-conjugated antibody and 16B12 anti-HA mouse antibody (Babco) with goat anti-mouse ALEXA 488 antibody (Molecular Probes) were used.

Chromatin immunoprecipitation (CHIP) was performed as previously described [45].

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