ARTICLES

Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I

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Segregation of homologous maternal and paternal centromeres to opposite poles during meiosis I depends on post-replicative crossing over between homologous non-sister chromatids, which creates chiasmata and therefore bivalent chromosomes. Destruction of sister chromatid cohesion along chromosome arms due to proteolytic cleavage of cohesin's Rec8 subunit by separase resolves chiasmata and thereby triggers the first meiotic division. This produces univalent chromosomes, the chromatids of which are held together by centromeric cohesin that has been protected from separase by shugoshin (Sgo1/MEI-S332) proteins. Here we show in both fission and budding yeast that Sgo1 recruits to centromeres a specific form of protein phosphatase 2A (PP2A). Its inactivation causes loss of centromeric cohesin at anaphase I and random segregation of sister centromeres at the second meiotic division. Artificial recruitment of PP2A to chromosome arms prevents Rec8 phosphorylation and hinders resolution of chiasmata. Our data are consistent with the notion that efficient cleavage of Rec8 requires phosphorylation of cohesin and that this is blocked by PP2A at meiosis I centromeres.

During mitosis, sister chromatids are held together by a multisubunit complex, called cohesin, from their creation during DNA replication until their disjunction at anaphase. Cohesin ensures that sister kinetochores attach to microtubules emanating from opposite spindle poles (known as amphitelic attachment). Cleavage of cohesin's α-kleisin subunit (Scc1/Rad21) by a specialized protease called separase destroys sister chromatid cohesion and triggers the metaphase to anaphase transition[1]. Separase is regulated by the binding of an inhibitory chaperone called securin, the destruction of which at the hands of a ubiquitin protein ligase called the anaphase-promoting complex or cyclosome (APC/C) only occurs when all chromosomes have achieved amphitelic attachment[2].

During meiosis, haploid gametes are formed from diploid precursors by two rounds of chromosome segregation after a single round of DNA replication. The first meiotic division differs from mitosis in two key respects. First, crossing over between homologous non-sister chromatids, which takes place after pre-meiotic DNA replication, creates chiasmata and thereby bivalent chromosomes containing four sets of chromatids. Second, owing to the action of meiosis-specific kinetochore proteins called monopolins[3,4], maternal and paternal sister centromere pairs, but not sister centromeres, are pulled in opposite directions by meiosis I microtubules. This creates a novel state of tension, namely between microtubules attempting to pull homologous non-sister centromeres towards opposite poles of the cell and sister chromatid cohesion distal to crossovers that holds bivalents together. This tug of war is resolved by the destruction of sister chromatid cohesion along chromosome arms through cleavage by separase of cohesin's α-kleisin subunit, in this case a meiosis-specific variant called Rec8 (refs 5, 6). This triggers anaphase I and the first meiotic division. Crucially, cohesin holding sister centromeres together is refractory to cleavage by separase during meiosis I and persists at centromeres until a second round of separase activation at the onset of anaphase II, which finally causes disjunction of sister centromeres, splitting of univalents into individual chromatids, and thereby formation of haploid progeny. Although not necessary for meiosis I, persistence of centromeric sister chromatid cohesion is essential for the amphitelic attachment of sister kinetochores during meiosis II[3].

Protection of cohesin from separase at meiosis I depends on a class of proteins called shugoshins[7,8], which are orthologues of the MEI-S332 protein from *Drosophila melanogaster*[9]. Budding yeast *Saccharomyces cerevisiae*[10,11] and *Drosophila* possess only a single orthologue (Sgo1 and MEI-S332, respectively) that is expressed in mitotic as well as meiotic cells. The fission yeast *Schizosaccharomyces pombe* possesses two paralogues[7,8], one (Sgo1) that is expressed solely during meiosis I and is essential for protecting centromeric sister chromatid cohesion, and a second (Sgo2) that is expressed in both mitotic and meiotic cells. In fission yeast, the association of Sgo1 with centromeres depends on Bub1, a protein that is necessary for delaying activation of the APC/C until all chromosomes have come under tension on metaphase plates as well as for protecting centromeric sister chromatid cohesion[12]. Sgo1 also protects centromeric

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sister chromatid cohesion in mammalian tissue culture cells, where phosphorylation of cohesin's Scc3/SA2 subunit causes cohesin to dissociate from chromosomes via a mechanism (known as the prophase pathway) that does not involve cleavage of α-kleisins by separase[13,14]. It is not understood how Sgo1 orthologues protect cohesin either from separase during meiosis or from the prophase pathway during mitosis.

Sgo1 binds PP2A at meiosis I

To investigate how Sgo1 protects centromeric cohesin, *S. pombe* and *S. cerevisiae* Sgo1 proteins (called SpSgo1 and ScSgo1, respectively) were purified using a carboxy-terminal tandem affinity purification (TAP) tag[15] from meiosis I cells. Purified material was visualized by SDS–polyacrylamide gel electrophoresis (PAGE) and analysed by tandem mass spectrometry (MS/MS). Notably, for both SpSgo1 and ScSgo1 the most abundant co-purifying proteins were subunits of PP2A (Fig. 1a, b; see also Supplementary Fig. S1a, b and Supplementary Table S1). Importantly, none of these was found associated with 'control' proteins purified in parallel by the very same technique (Fig. 1a, b; see also Supplementary Fig. S1a, b and Supplementary Table S1).

PP2A is mostly found as a hetero-trimeric complex containing catalytic (C), scaffold (A) and regulatory (B, B′, B″ or B‴) subunits. The *S. pombe* genome encodes two isoforms of the C subunit (SpPpa1 and SpPpa2), which have largely overlapping functions[16,17], a single isoform of the A subunit (SpPaa1), one B subunit (SpPab1), and two B′ subunits (SpPar1 and SpPar2)[18,19]. Hereafter, individual subunits of PP2A (or their genes) will be identified as either A, B or C type using the appropriate superscript letter appended to the end of the subunit name. Notably, we detected only a single combination of PP2A subunits associated with SpSgo1, namely $SpPaa1^{A}$–$SpPar1^{B'}$–$SpPpa2^{C}$, and an equivalent combination with ScSgo1, namely $ScTpd3^{A}$–$ScRts1^{B'}$–$ScPph21^{C}$/$ScPph22^{C}$ (refs 20, 21), which we refer to hereafter as SpPP2A-π and ScPP2A-π (from the Greek for protector).

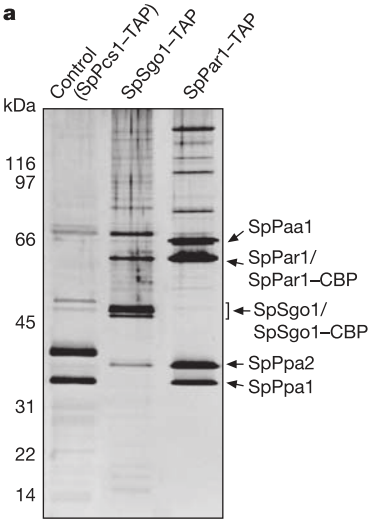
To investigate whether the Sgo1–PP2A-π interaction is conserved in mammals, we expressed a functional TAP-tagged mouse Sgo1 protein at physiological levels in HeLa cells[22]. Mouse Sgo1–TAP, but not a control protein, associated with catalytic, scaffold and all five isoforms of the B′ PP2A-π regulatory subunit of PP2A. Notably, endogenous human Sgo1 also associated with mouse Sgo1–TAP, suggesting that Sgo1 proteins form homo-oligomers (Supplementary Fig. S1a, b and Supplementary Table S1).

The SpSgo1–PP2A-π interaction was confirmed by purifying a TAP-tagged $SpPar1^{B'}$ subunit. Most $SpPar1^{B'}$ was associated with A and C subunits of PP2A, but a minor fraction associated with SpSgo1 (Fig. 1a, b; see also Supplementary Table S1). Interaction of Sgo1 and PP2A-π was reproduced *in vitro*. When synthesized in reticulocyte lysates, $SpPar1^{B'}$ and $SpPaa1^{A}$, but not $SpPpa2^{C}$, associated with a maltose binding protein (MBP)–SpSgo1 fusion protein immobilized on an affinity matrix but not with MBP alone (Supplementary Fig. S1c). This experiment was repeated using MBP fusions containing different parts of SpSgo1, which revealed that $SpPar1^{B'}$ and $SpPaa1^{A}$ bound most tightly to SpSgo1's amino-terminal coiled-coil region (Supplementary Fig. S1d).

PP2A-π protects sister chromatid cohesion in *S. pombe*

To test the role of PP2A-π during meiosis, we deleted the genes for $SpPpa2^{C}$ and $SpPar1^{B'}$ as well as their two closest paralogues in the fission yeast genome, $SpPpa1^{C}$ and $SpPar2^{B'}$. Sequences close to *cen1*, marked by a *lac* operator array that binds GFP–LacI (*lys1*–GFP), segregated with high fidelity to all four meiotic products in $Spppa1^{C}\Delta$ and $Sppar2^{B'}\Delta$ cells, mis-segregated modestly in $Spppa2^{C}\Delta$ cells, and mis-segregated massively in $Sppar1^{B'}\Delta$ cells (Supplementary Fig. S2a). Although we did not detect $SpPpa1^{C}$ associated with SpSgo1 (Fig. 1a, b; see also Supplementary Table S1), it is possible that this isoform can partially replace $SpPpa2^{C}$, as in vegetative growth[17].

To analyse chromosome segregation in more detail, we observed segregation of *lys1*–GFP when homozygous in h^{90} cells or heterozygous in h^{+}/h^{-} cells at anaphase I and II. Deletion of $Sppar1^{B'}$ or $Spppa2^{C}$, like that of *Spsgo1* but not *Spsgo2* (ref. 7), had no effect on meiosis I segregation (Fig. 2a). In contrast, $Sppar1^{B'}$ deletion, like that of *Spsgo1*, caused random segregation of sister *lys1*–GFP sequences at meiosis II. $Spppa2^{C}$ deletion had a more modest but nevertheless significant effect (Fig. 2b). Mis-segregation in *S. pombe* is often accompanied by lagging chromosomes during anaphase. Lagging chromosomes were frequently observed at anaphase I in *Spsgo2* mutant cells but rarely in *Spsgo1*, $Spppa2^{C}$ or $Sppar1^{B'}$



b

MS/MS data:

SpSgo1–TAP purification:

Proteins identified	Score	Peptide coverage	Molecular mass	Description (SU=subunit)
SpSgo1	2,031	84%	38 kDa	Kinetochore protein
SpPar1	470	28%	63 kDa	PP2A B′ regulatory SU
SpPaa1	357	23%	67 kDa	PP2A scaffold SU
SpPpa2	173	31%	37 kDa	PP2A catalytic SU

SpPar1–TAP purification:

Proteins identified	Score	Peptide coverage	Molecular mass	Description (SU=subunit)
SpPaa1	24,367	83%	67 kDa	PP2A scaffold SU
SpPpa2	17,044	91%	37 kDa	PP2A catalytic SU
SpPar1	13,376	54%	63 kDa	PP2A B′ regulatory SU
SpPpa1	9,028	68%	36 kDa	PP2A catalytic SU
SpSgo1	447	42%	38 kDa	Kinetochore protein

Figure 1 | Sgo1 binds to PP2A-π *in vivo*. **a**, **b**, Pre-starved *S. pombe* h^{-}/h^{-} diploids, expressing either TAP-tagged SpSgo1 or SpPar1, were induced to enter synchronous meiosis triggered by Pat1 inactivation[46] and cells were harvested just before undergoing the first meiotic division. Protein complexes associated with SpSgo1 and SpPar1 were isolated by tandem affinity purification (TAP). Co-purified proteins were separated by SDS–PAGE, visualized by silver-staining (**a**) and identified by tandem mass spectrometry (MS/MS). Proteins that specifically associate with Sgo1 or Par1 are shown (**b**). The specificity of co-purified proteins was determined by comparison to a 'control' protein SpPcs1, which was isolated by TAP in parallel. Scores shown in the tables were derived from Mascot software. They are measures of the significance of protein identifications. (For more details concerning Mascot or Sequest scores, see Supplementary Information.) All identified proteins shown are highly significant hits. For a full list of co-purified proteins see Supplementary Table S1.

mutants. In contrast, they were frequently observed at anaphase II in *Spsgo1* and *Sppar1*$^{B'}$ mutants but less frequently in *Spppa2*^{C} or *Spsgo2* mutants (Fig. 2c). These data imply that loss of SpPar1$^{B'}$ and SpSgo1 have similar effects on meiotic chromosome segregation, namely random segregation of sister centromeres at meiosis II after a normal meiosis I. *Sppar1*$^{B'}$ mutants resembled *Spsgo1* mutants in another crucial respect, namely a failure of most cells to retain SpRec8 at centromeres after meiosis I (Fig. 2d). A similar defect was also observed in a large fraction of *Spppa2*^{C} mutant cells. In contrast, SpPP2A-π mutants had no noticeable effect on the expression, nuclear accumulation or association with centromeres of SpRec8 before anaphase I (Supplementary Fig. S3a–c).

PP2A-π protection of centromeric cohesin is conserved

To investigate the role of PP2A-π in *S. cerevisiae*, we analysed the effect of eliminating genes encoding either of its two catalytic subunits (*ScPPH21*^{C} and *ScPPH22*^{C}) or its single B′ regulatory subunit (*ScRTS1*$^{B'}$) from diploid strains in which the *URA3* locus close to the centromere of chromosome V was marked by a *tet* operator array that binds tetR–GFP (*URA3*–GFP). Homozygous deletion of *ScPPH21*^{C} or *ScPPH22*^{C} had little or no effect on meiotic chromosome segregation; however, deletion of *ScRTS1*$^{B'}$ caused massive mis-segregation (Supplementary Fig. S2b). Deletion of either *ScSGO1* (refs 10, 11) or the spindle checkpoint gene *ScBUB1* (ref. 23) (Supplementary Fig. S2b) caused a greater severity of defects, mainly because these mutations affect meiosis I as well as meiosis II (see Fig. 3c). ScPP2A-π can contain either ScPph21^{C} or ScPph22^{C}, and presumably either protein is sufficient to prevent meiosis II mis-segregation. Elimination of both causes a severe growth defect[20]. A more detailed analysis of chromosome segregation in *Scrts1*$^{B'}$*Δ* cells at anaphase I and II (in diploids either homozygous or heterozygous for *URA3*–GFP) revealed that sister centromeres invariably segregate to the same pole at anaphase I but frequently separate precociously (Fig. 3a), as found in *Scsgo1Δ* (refs 10, 11) and *Scbub1Δ* mutants. This precocious separation is followed by (and presumably causes) high rates of non-disjunction at meiosis II (Fig. 3b). Disjunction of homologous centromeres at meiosis I was normal in the *Scrts1*$^{B'}$*Δ* mutant, which contrasts with frequent non-disjunction in *Scsgo1Δ* (23%) and *Scbub1Δ* cells (46%) (Fig. 3c). This implies that ScRts1$^{B'}$ participates in some but not all functions of ScSgo1.

To address whether non-disjunction of sister centromeres at meiosis II in *Scrts1*$^{B'}$*Δ* cells could be caused by a failure to protect ScRec8, we analysed its localization in anaphase I spreads. In wild-type cells, a cluster of centromeric ScRec8 foci is invariably associated with spindle poles. These clusters were entirely absent in 80% of

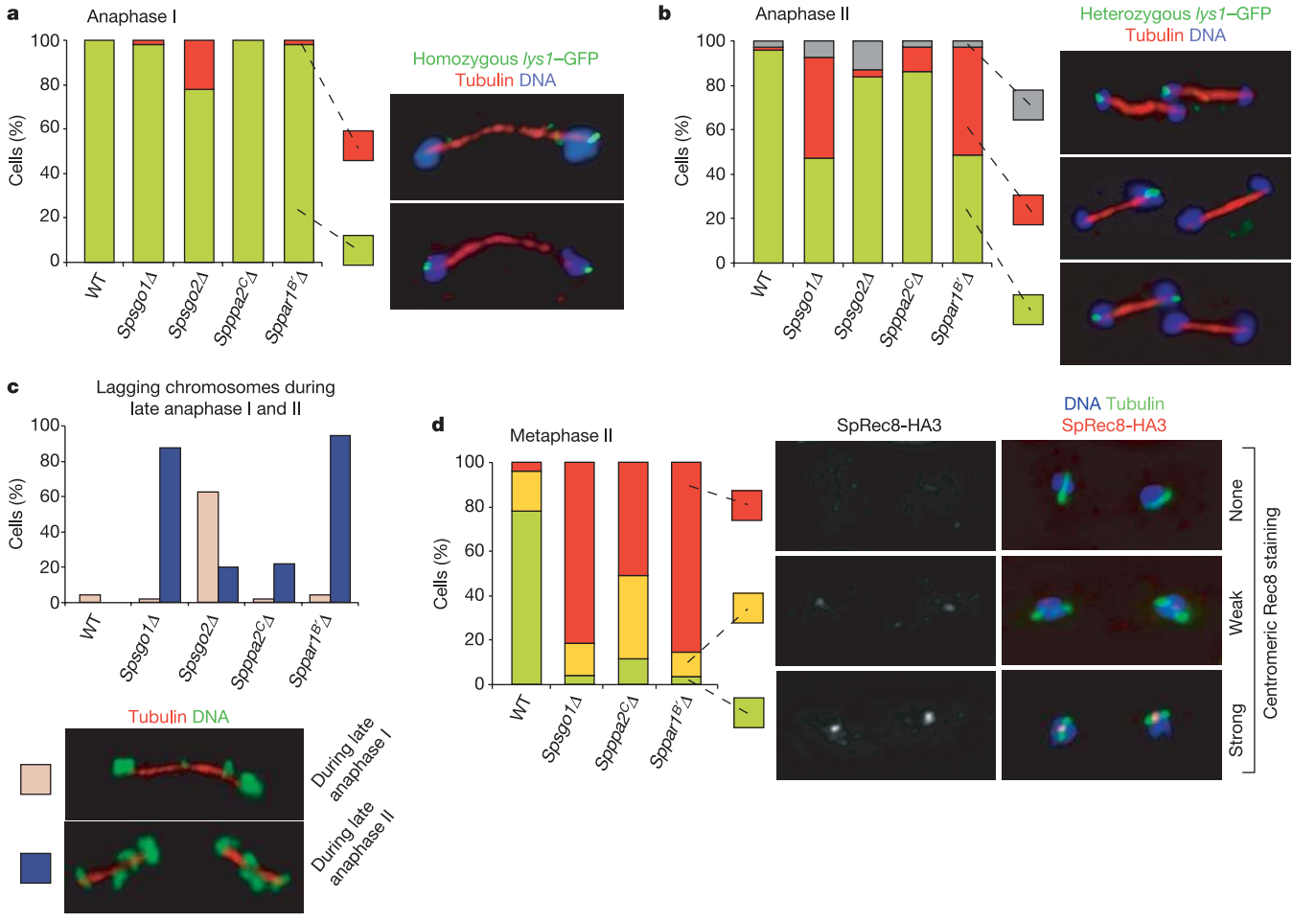


Figure 2 | PP2A-π is required to protect centromeric sister chromatid cohesion at meiosis I in *S. pombe*. **a**, **b**, Segregation of homozygous (**a**) or heterozygous (**b**) *lys1*–GFP in late anaphase I (**a**) or anaphase II (**b**) cells. h^{90} (**a**) or h^{+}/h^{-} diploid (**b**) wild-type, *Spsgo1Δ*, *Spsgo2Δ*, *Spppa2*^{C}*Δ* and *Sppar1*$^{B'}$*Δ* cells were sporulated for 18 h in liquid medium and samples were taken for *in situ* immunofluorescence microscopy ($n = 100$). Late anaphase I or anaphase II cells were identified as binucleates or tetranucleates containing one or two bipolar spindles of more than 7 or 4.5 μm in length, respectively. **c**, The same cells analysed in **b** were scored for the presence of lagging chromosomes in late anaphase I and anaphase II ($n = 100$). **d**, h^{90} wild-type, *Spsgo1Δ*, *Spppa2*^{C}*Δ* and *Sppar1*$^{B'}$*Δ* cells, expressing SpRec8-HA3, were sporulated for 20 h in liquid medium and samples were taken for *in situ* immunofluorescence analysis of SpRec8 at metaphase II centromeres ($n = 100$). Metaphase II cells were identified as binucleates containing two bipolar spindles.

Scbub1Δ or *Scrts1*$^{B'}$*Δ* cells and very faint in the remaining 20% (Fig. 3d). Whole-cell staining of ScRec8 in *Scrts1*$^{B'}$*Δ* cells revealed a similar picture. Few if any *Scrts1*$^{B'}$*Δ* metaphase II binucleates contained centromeric ScRec8 (Supplementary Fig. S3d). Notably, deletion of *ScRTS1*$^{B'}$ had no effect on the distribution of ScRec8 on chromosomes before meiosis I (Supplementary Fig. S3e, f).

Persistence of centromeric sister chromatid cohesion can be directly measured in *S. cerevisiae* using monopolin (for example, *Scmam1*) mutants, which attempt to pull sister centromeres to opposite poles at meiosis I but are prevented from disjoining them by cohesin that has resisted attack by separase[3]. This causes accumulation of uninucleate cells with bipolar spindles and low levels of securin (ScPds1). Deletion of *ScRTS1*$^{B'}$ (but not *ScSPO11*) suppresses this meiosis I division defect (Fig. 3e) as does deletion of *ScSGO1* (ref. 10). However, owing to the presence of chiasmata, which still enables co-orientation of some homologous centromeres even in the absence of monopolin, sister centromeres segregated to opposite poles in only 40% of cells (Fig. 3f). Elimination of crossing over by deleting *ScSPO11* enabled 85% of *Scrts1*$^{B'}$*Δ Scmam1Δ* cells to undergo a fully equational division. These results imply that PP2A-π actively maintains centromeric sister chromatid cohesion between meiotic divisions in *S. cerevisiae*.

Association of PP2A-π with centromeres depends on Sgo1

Unlike SpSgo1, most SpPP2A-π is not associated with chromosomes and is distributed throughout cells undergoing meiosis I (data not shown), which is consistent with our finding that most SpPP2A-π is not actually associated with SpSgo1 (Fig. 1a, b; see also Supplementary Table S1). This suggests that SpSgo1 mediates protection of centromeric sister chromatid cohesion by recruiting a small but

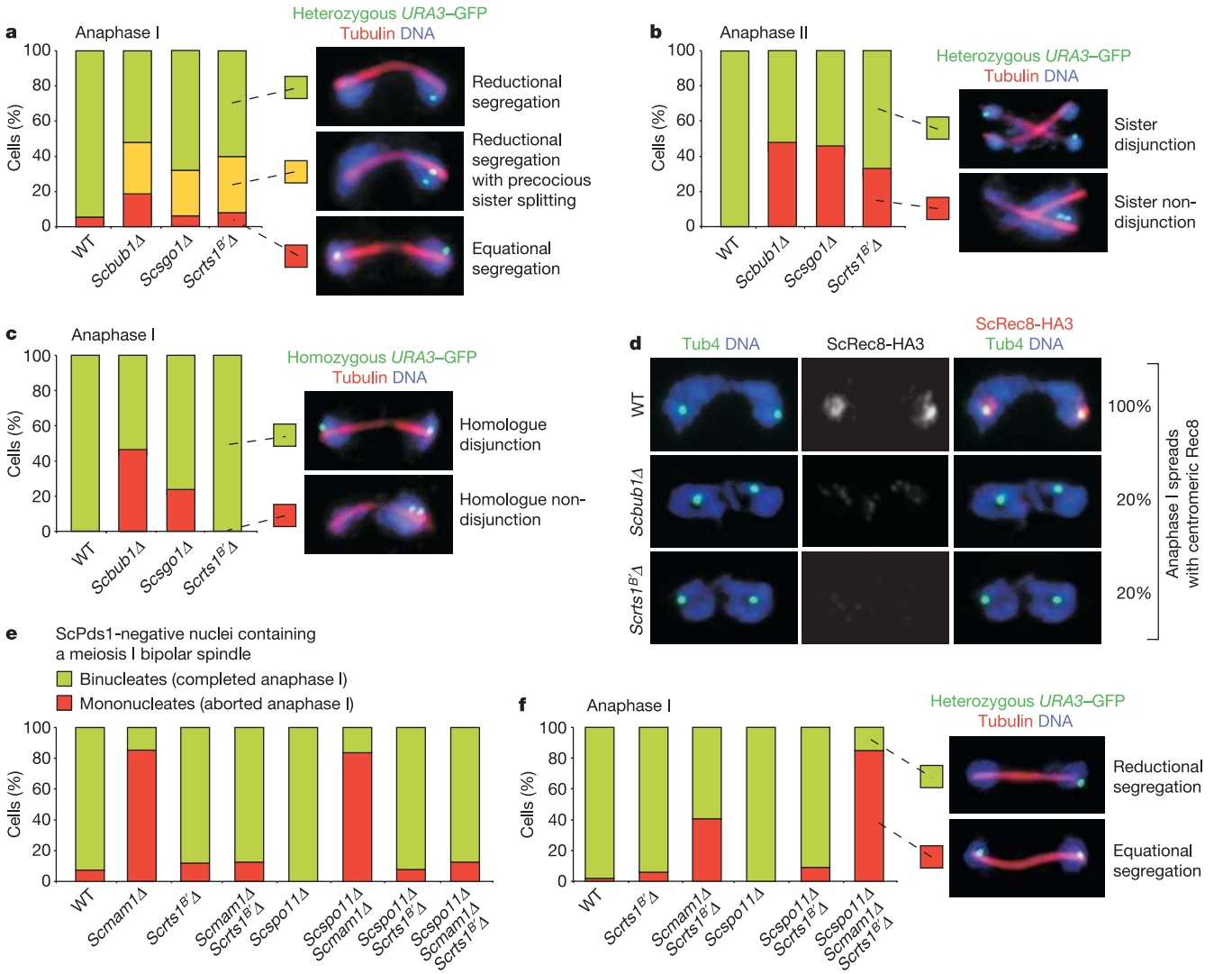


Figure 3 | PP2A-π prevents premature loss of centromeric sister chromatid cohesion at meiosis I in *S. cerevisiae*. **a**, **b**, Homozygous diploid wild-type, *Scbub1Δ*, *Scsgo1Δ* and *Scrts1*$^{B'}$*Δ* cells, heterozygous for *URA3*–GFP and containing ScPds1-myc18, were sporulated in liquid culture for 6 h and samples taken for *in situ* immunofluorescence staining. The segregation of *URA3*–GFP at anaphase I (**a**) or anaphase II (**b**) was monitored in cells containing low nuclear levels of ScPds1-myc18 and either one (**a**) or two (**b**) bipolar spindles in binucleates or tetranucleates, respectively. **c**, As in **a**, except that wild-type, *Scbub1Δ*, *Scsgo1Δ* and *Scrts1*$^{B'}$*Δ* cells were homozygous for *URA3*–GFP. **d**, Wild-type, *Scbub1Δ* and *Scrts1*$^{B'}$*Δ* cells, taken from the same experiment as in **a**, were analysed for the presence of ScRec8-HA3 on chromosome spreads. Shown are typical examples of Rec8 staining in anaphase I nuclei, in which a single spindle pole body (marked by ScTub4 staining) was observed in each lobe of a bilobed chromatin mass. The percentage of such nuclei containing ScRec8-HA3 co-localizing with the spindle pole bodies (centromeric Rec8) was scored ($n = 15$). **e**, **f**, Homozygous diploid wild-type and mutant strains, carrying ScPds1-myc18 and heterozygous for *URA3*–GFP, were sporulated in liquid medium and samples taken for *in situ* immunofluorescence staining 6 h after resuspension of cells in sporulation medium. The ability of cells to undergo anaphase I was monitored in such cells containing low nuclear ScPds1-myc18 levels and a single bipolar spindle (**e**) ($n = 100$). In such strains that can efficiently undergo anaphase I, the segregation pattern of *URA3*–GFP was monitored (**f**) ($n = 100$).

vitally important fraction of PP2A-π to meiosis I centromeres and not *vice versa*. As predicted by this hypothesis, localization of SpSgo1 to metaphase I centromeres was unaffected by *Sppar1*$^{B'}\Delta$ or *Spppa2*$^{C}\Delta$ (Fig. 4a), although it was abolished by *Spbub1*Δ (ref. 8). To detect chromosomal SpPP2A-π, cells undergoing meiosis I were fixed with formaldehyde, and sheared DNA immunoprecipitated with epitope tags on SpPar1$^{B'}$, SpPpa2^{C}, SpSgo1 or SpRec8 was hybridized to a high-density oligonucleotide array covering chromosomes 2 and 3

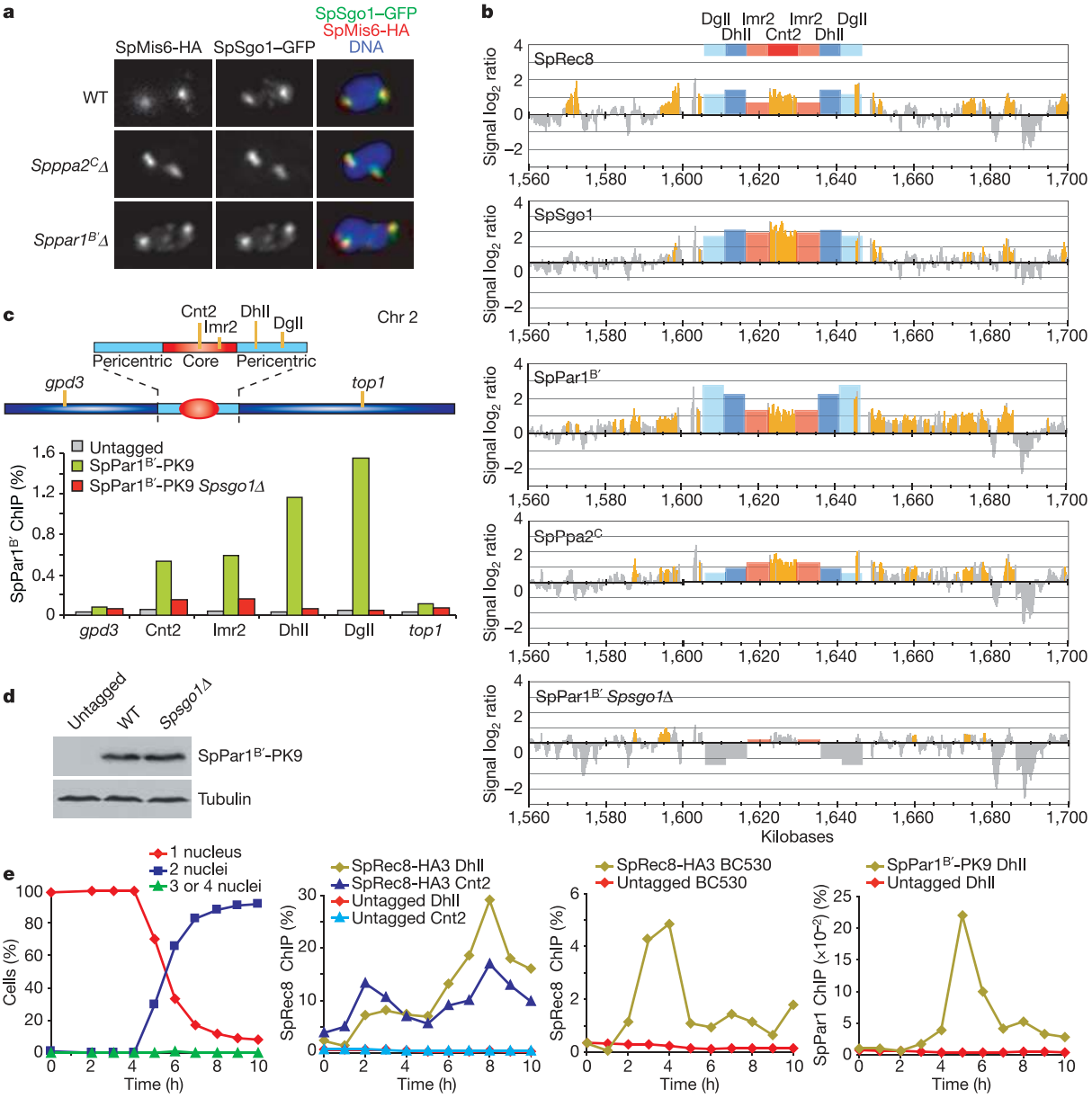


Figure 4 | PP2A-π localizes to *S. pombe* centromeres in an SpSgo1-dependent manner. **a**, SpSgo1 localizes to centromeres independently of PP2A-π. *h*^{90} wild-type, *Spppa2*$^{C}\Delta$ and *Sppar1*$^{B'}\Delta$ cells producing SpSgo1–GFP and the kinetochore protein SpMis6-HA3 were sporulated for 16 h in PMG-N media and samples taken. Co-localization of SpSgo1–GFP and SpMis6-HA3 was analysed by *in situ* immunofluorescence. **b**, *Sppat1-114* homozygous diploid cells carrying SpRec8-HA3, SpSgo1-PK9, SpPar1$^{B'}$-PK9, SpPpa2^{C}-HA3 and SpPar1$^{B'}$-PK9 *Spsgo1*Δ were harvested 4 h after induction of synchronous meiosis (that is, before anaphase I). The distribution of epitope-tagged proteins on chromosome 2 and most of chromosome 3 was analysed by ChIP and hybridization on a high-density oligonucleotide microarray. Only a 140-kb region surrounding the centromere of chromosome 2 (1,560–1,700 kb from the left telomere) is shown. The orange-shaded areas are regions containing significant enrichment of immunoprecipitated material, as detailed previously[27]. Grey-shaded areas represent signals that are not statistically significant. Signals for the DgII, DhII and Imr2 repetitive centromere regions (light blue, dark blue and light red boxes, respectively) were determined by ChIP experiments using quantitative real-time PCR (qRT–PCR) and calibrated to match the profile. For the complete microarray profiles showing entire chromosome 2 and most of chromosome 3 see Supplementary Figs S4 and S5. **c**, *Sppat1-114* homozygous diploid wild-type, SpPar1$^{B'}$-PK9 and SpPar1$^{B'}$-PK9 *Spsgo1*Δ cells were harvested 4 h after induction of synchronous meiosis. ChIP of various loci of chromosome 2, using qRT–PCR, was performed. The schematic drawing in the top panel illustrates the positions of amplified sequences. Both *gpd3* and *top1* are arm loci. **d**, Samples taken from the same cells as shown in **c** were analysed by western blotting for the expression levels of SpPar1$^{B'}$. Tubulin detection served as a loading control. **e**, *Spmes1-B44 Sppat1-114* homozygous diploid cells, producing the epitope-tagged proteins SpPar1$^{B'}$-PK9 and SpRec8-HA3, were induced to undergo meiosis I synchronously, eventually arresting before meiosis II. Chromatin binding of SpPar1$^{B'}$-PK9 and SpRec8-HA3 at various time points was analysed by ChIP and qRT–PCR. Amplified regions included the inner (Cnt2) and outer (DhII) centromeres, as well as a cohesion-associated chromosomal arm region (BC530).

(Fig. 4b; see also Supplementary Fig. S4). Association of proteins with the repetitive centromere regions, which are difficult to quantify by this method, was measured using a real-time polymerase chain reaction (PCR) assay. SpRec8 localized to inner (Cnt2 and Imr2) and outer (DhII and DgII) centromere regions, as well as to regions of convergent transcription along chromosome arms (Fig. 4b; see also Supplementary Figs S4 and S6). SpSgo1 localized to both inner and outer centromere regions, as did both SpPar1$^{B'}$ and SpPpa2^{C} (Fig. 4b; see also Supplementary Figs S4 and S6). SpPar1$^{B'}$ and SpPpa2^{C} might be more broadly distributed around centromeres than SpSgo1 (Fig. 4b).

To address whether SpPP2A-π persists at centromeres after meiosis I has been completed, we measured association of SpPar1$^{B'}$ and SpRec8 with chromosomes as *mes1-B44* mutants undergo meiosis I and arrest before the onset of meiosis II[24,25]. Whereas SpRec8 was absent from arm sequences after meiosis I was completed, it persisted or even increased at centromeres (Fig. 4e). SpPar1$^{B'}$ associated with centromeres shortly before the onset of meiosis I but disappeared when cells arrested at the onset of meiosis II. Crucially, the amount of SpPar1$^{B'}$ associated with inner and outer centromere regions (but not the level of total protein; Fig. 4d) was severely reduced in *Spsgo1Δ* cells during meiosis I (Fig. 4b, c). Chromatin immunoprecipitations (ChIPs) hybridized to microarrays suggested that centromeric SpPar1$^{B'}$ did not re-distribute to other chromosomal regions in *Spsgo1Δ* cells (Supplementary Fig. S5).

The distributions of PP2A-π, ScSgo1, ScBub1 and ScRec8 on

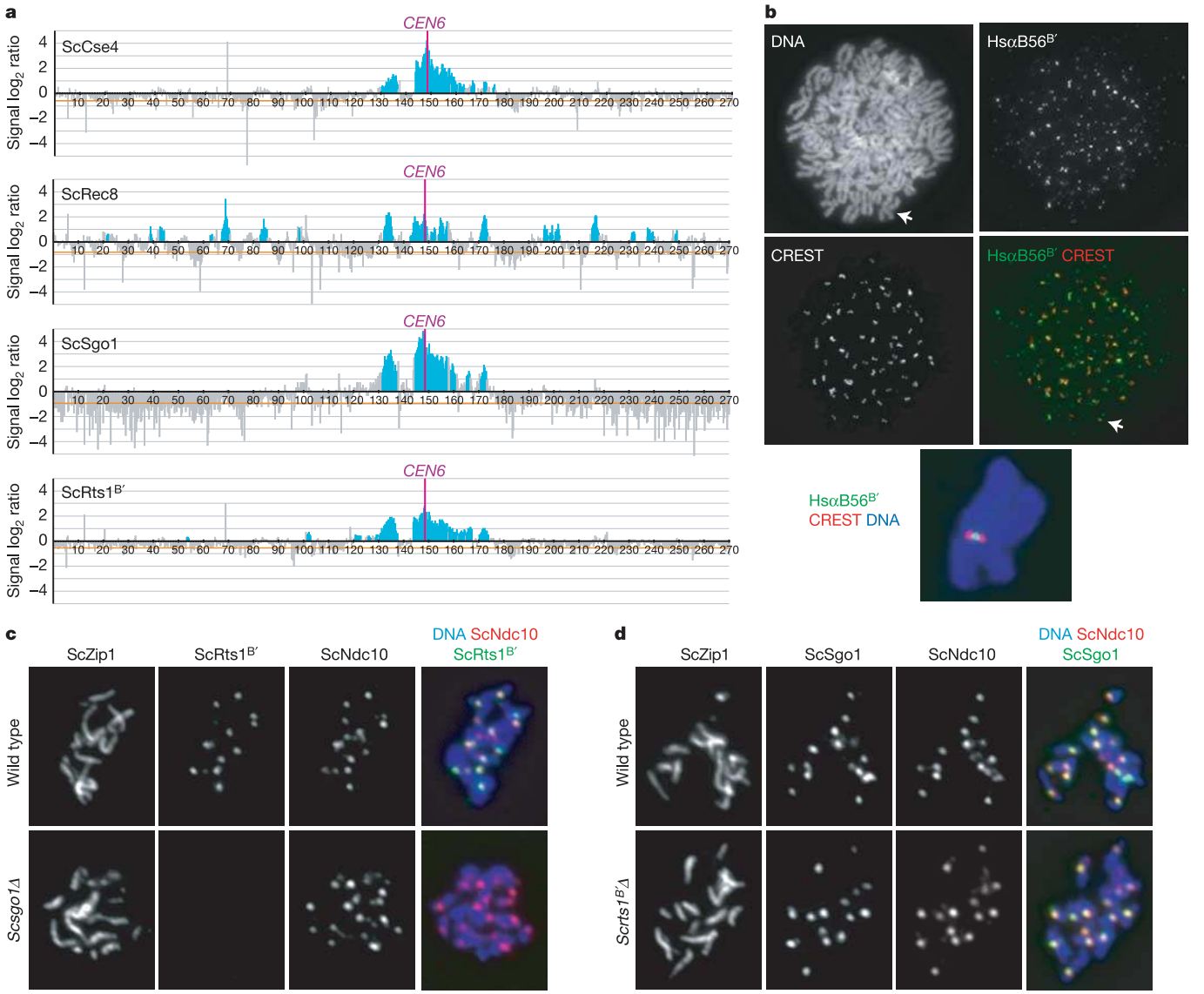


Figure 5 | PP2A-π is recruited to the centromeres in *S. cerevisiae* and mammalian cells. **a**, Meiotic cells producing ScCse4-HA6, ScRec8-HA3, ScSgo1-PK6 and ScRts1$^{B'}$-PK3 were harvested 4 h after shifting cells to sporulation medium (that is, before the first meiotic division). Benomyl (30 μg ml^{-1}) was added 1 h after transfer to ensure that cells did not progress past meiosis I. The distribution of the tagged proteins on chromosome VI was analysed by ChIP and hybridization on a high-density oligonucleotide microarray. The blue-shaded areas are regions of the chromosome that contain significant enrichment of immunoprecipitated material, as detailed previously[27]. Grey-shaded areas represent signals that are not statistically significant. The orange horizontal line indicates the average signal ratio of loci not enriched in the immunoprecipitated fraction. **b**, Distribution of HsαB56$^{B'}$ on HeLa chromosomes. Shown is a typical chromosome spread of a mitotic cell immunostained to detect endogenous HsαB56$^{B'}$ and the centromere marker CREST. The arrow points to a single chromosome pair, of which an enlargement is shown in the lowest panel. **c**, **d**, Meiotic diploid wild-type, *Scsgo1Δ* and *Scrts1*$^{B'}$*Δ* cells were harvested 5 h after transfer to sporulation medium and the localization of ScRts1$^{B'}$-myc9 (**c**) and ScSgo1-myc9 (**d**) on late pachytene chromosome spreads was analysed. All spreads were co-stained for ScNdc10-HA3 (used as a marker for centromeres) and the synaptonemal complex protein ScZip1.

meiotic *S. cerevisiae* chromosomes were analysed by hybridizing chromatin immunoprecipitates to chromosome VI oligonucleotide microarrays[26,27] (Fig. 5a; see also Supplementary Fig. S7a). The ScSgo1, ScBub1, ScRts1$^{B'}$ and ScPph22^{C} distributions were similar to those of two kinetochore proteins, CENP-A (ScCse4) and ScNdc10. All six proteins localized to a large (~45-kilobase (kb)) region surrounding the 125-base-pair *CEN6* sequence that confers centromere function. This result was surprising because previous studies concluded that ScCse4 and ScNdc10 are restricted to a single centromeric nucleosome[28,29]. The extended 'centromeric' region marked by these kinetochore proteins contained several prominent and closely spaced peaks of ScRec8, which was also detected at regions of convergent transcription along chromosome arms (Fig. 5a; see also refs 27, 30). We suggest that the fraction of ScRec8 that co-localizes with ScPP2A-π within the 45-kb centromere region is resistant to separase cleavage at the onset of anaphase I, whereas the fraction of ScRec8 along chromosome arms that does not co-localize with ScPP2A-π is susceptible. ScCse4 and ScNdc10, as well as ScPP2A-π, ScSgo1 and ScBub1, were found in a narrower window (~20 kb) around *CEN6* in mitotic cells (Supplementary Fig. S7b).

We also observed ScPP2A-π at centromeres on chromosome spreads of late pachytene cells (Fig. 5c). The concentration of ScRts1$^{B'}$ at centromeres depended on ScSgo1 and ScBub1, whereas that of ScSgo1 depended on ScBub1 but not ScRts1$^{B'}$ (Fig. 5c, d; see

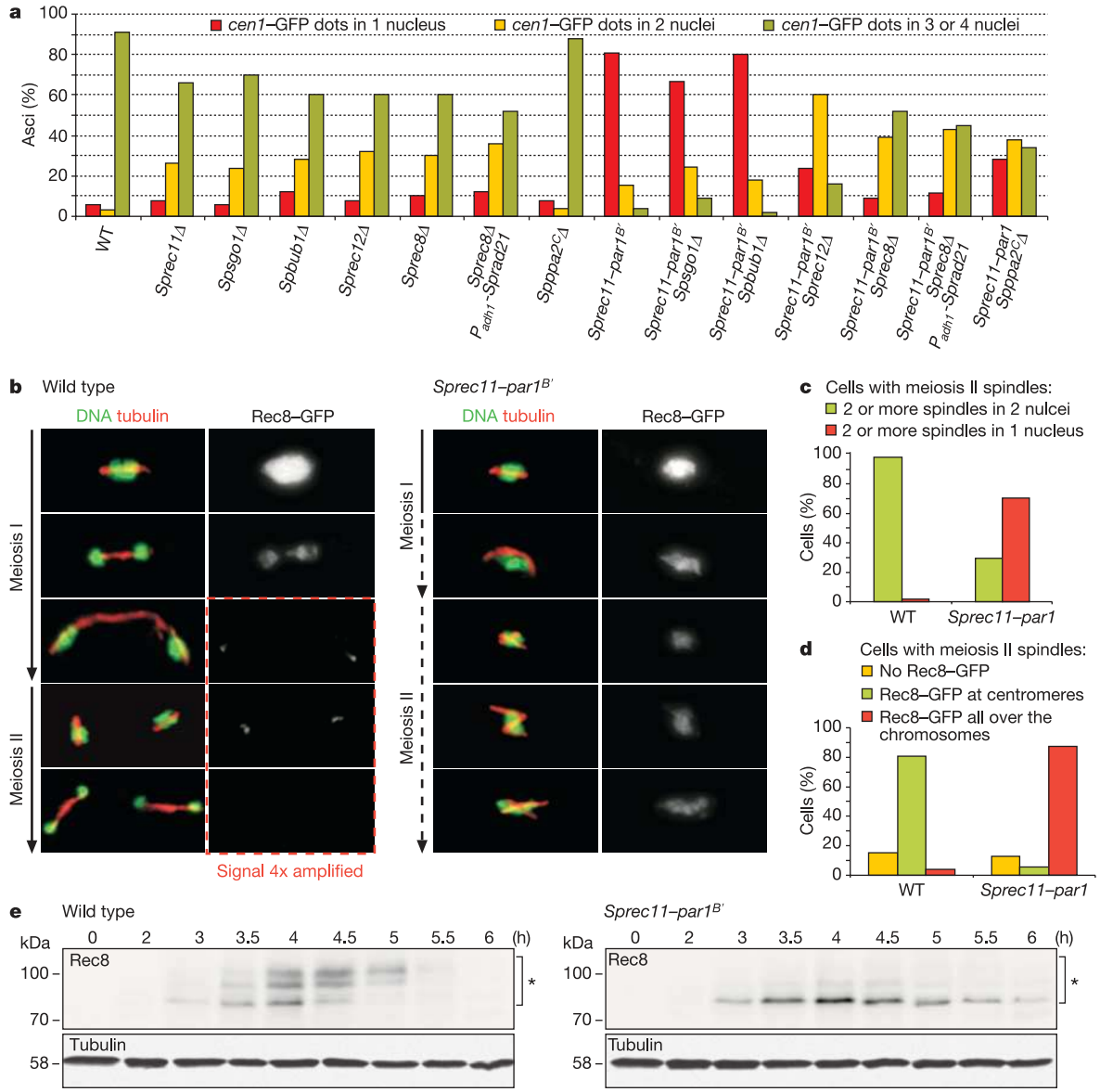


Figure 6 | Artificial recruitment of PP2A-π to chromosome arms in *S. pombe* prevents removal of arm cohesin, blocks nuclear divisions and causes dephosphorylation of SpRec8 during meiosis. **a**, h^{90} cells, expressing the SpRec11-HA3–Par1$^{B'}$ fusion protein from the endogenous *Sprec11* locus, together with control strains as shown, were sporulated for 40 h on PMG-N plates. Nuclear division was monitored by scoring the segregation of homozygous *lys1*–GFP into separate nuclei ($n = 100$). This was done to avoid scoring of nuclear fragmentation as divisions, as they frequently occur upon blocked chromosome segregation[6]. **b**, h^{90} cells, expressing either SpRec8–GFP or both SpRec8–GFP and the SpRec11-HA3–Par1$^{B'}$ fusion protein, were sporulated for 20 h in PMG-N medium and samples were taken. Cells were stained for the presence of SpRec8–GFP and tubulin. Representative cells in various meiotic stages are shown. **c**, Cells from **b** that contained two or more spindles were scored for the presence of a divided nucleus ($n = 100$). **d**, Cells from **b** that contained two or more spindles were scored for the presence and localization of SpRec8–GFP ($n = 100$). **e**, *Sppat1-114* homozygous diploid cells producing either SpCut2-myc13 or both SpCut2-myc13 and the SpRec11-HA3–Par1$^{B'}$ fusion protein were sporulated synchronously with samples taken at the indicated time points after induction of meiosis. Samples were analysed by western blotting for the presence of SpRec8 and tubulin. The asterisk denotes the migration of SpRec8 including the various slower-migrating phosphorylated forms of the protein.

also Supplementary Fig. S8a, b). ScBub1's association with centromeres was independent of both ScSgo1 and ScRts1$^{B'}$ (Supplementary Fig. S8c). These data suggest that ScBub1 recruits ScSgo1 to centromeres, which in turn recruits PP2A-π. Interestingly, both ScSgo1 and ScRts1$^{B'}$ were found at low levels along the length of most chromosomes in *Scbub1Δ* mutants and in 'kinase dead' *Scbub1(K733R)* mutants (Supplementary Fig. S8a, b). Thus, the kinase activity of ScBub1 seems to be required to restrict the chromatin association of both ScSgo1 and PP2A-π to centromeric regions.

The distribution of mammalian PP2A-π was also analysed on chromosome spreads. The endogenous α-isoform of the human B′ subunit (HsαB56$^{B'}$) co-localized with centromeres marked by CREST in HeLa cells (Fig. 5b). Its depletion by RNA interference (RNAi) greatly reduced the protein level of HsαB56$^{B'}$ and the number of HsαB56$^{B'}$ foci co-localizing with CREST (Supplementary Fig. S9a, b), showing that the antibody used for detecting HsαB56$^{B'}$ was specific to this isoform. In addition, overexpressed epitope-tagged versions of α-, β-, γ-, δ- and ε-isoforms of HsB56$^{B'}$ were also observed to co-localize with CREST in chromosome spreads from stably transfected NIH3T3 cells (Supplementary Fig. S9c).

Is PP2A-π sufficient to confer protection of Rec8?

If the sole role of Bub1 and Sgo1, with regards to their function in protecting centromeric cohesion, is to recruit PP2A-π to centromeres, and the role of PP2A-π is to remove phosphate groups from proteins whose phosphorylation is required for Rec8's cleavage, then the artificial recruitment of PP2A-π to chromosome arms should inhibit Rec8 cleavage at this location and thereby hinder the first meiotic division. To test this, we fused SpPar1$^{B'}$ to the C terminus of the meiosis-specific Scc3-like cohesin subunit SpRec11, which is essential for sister chromatid cohesion along chromosome arms[31]. Upon transferring *h*^{90} cells to sporulation conditions, SpRec11–Par1$^{B'}$ accumulated on chromosomes and co-localized with SpRec8 during meiosis I (Supplementary Fig. S10a). Most cells failed to undergo the first meiotic division but nevertheless produced asci containing either one or more unequally sized spores (Fig. 6a). Their lack of chromosome segregation was not caused by a lack of meiotic progression because cells formed first meiosis I and subsequently meiosis II bipolar spindles. Consequently, mononucleate cells with multiple spindles spanning four spindle pole bodies could be seen with high frequency (Fig. 6b, c). *Sprec11–par1*$^{B'}$ cells with meiosis II spindles contained SpRec8 throughout the chromosomes (and not merely at centromeres, as occurs in wild type) (Fig. 6b, d). Elimination of crossing over by deleting *Sprec12* permitted most *Sprec11–par1*$^{B'}$ cells to undergo one, but not a second, division (Fig. 6a), suggesting that their meiosis I defect may be in resolving chiasmata. Crucially, deleting *Sprec8* enabled *Sprec11–par1*$^{B'}$ cells to undergo two divisions, even when they expressed high levels of SpRec8's counterpart SpRad21. Because the lack of meiotic divisions caused by SpRec11–Par1$^{B'}$ was largely, but not completely, suppressed by deleting *Sppa2*^{C} (Fig. 6a), we suggest that the phenotype is caused by the abnormal recruitment to chromosome arms of SpPP2A-π's catalytic activity. We propose that this blocks chiasmata resolution by blocking SpRec8 (but presumably not SpRad21) cleavage. Notably, SpPP2A-π recruited directly to chromosome arms in this fashion blocked meiosis I independently of both SpSgo1 and SpBub1 (Fig. 6a). This suggests that neither of these proteins is essential for the catalytic activity of PP2A-π in preventing Rec8 cleavage once PP2A-π has been recruited to chromosomes.

To investigate whether SpPP2A-π causes dephosphorylation of Rec8, we compared its electrophoretic mobility in *Sprec11* and *Sprec11–par1*$^{B'}$ cells as they undergo synchronous meiosis triggered by SpPat1 inactivation. Under these conditions, *Sprec11–par1*$^{B'}$ had a less severe but still significant effect on meiosis I (data not shown). It did not, however, affect pre-meiotic DNA replication or delay securin degradation (Supplementary Fig. S10b, c). Importantly, fusion of SpRec11 to SpPar1$^{B'}$ largely eliminated the formation of slow-migrating forms of SpRec8 (Fig. 6e). These forms normally appear shortly before the degradation of SpRec8 and have previously been shown to be caused by phosphorylation[32].

Discussion

The preservation of centromeric sister chromatid cohesion during meiosis I is a crucial aspect of meiosis. Defects in this process might contribute to the precocious separation of sister chromatids observed in human oocytes[33–35] that might in turn cause fetal aneuploidy and thereby Down's syndrome. Our work suggests that Bub1 and Sgo1 perform this task in both fission and budding yeast by recruiting to meiosis I centromeres a specific form of PP2A (PP2A-π). Because transient treatment of mouse oocytes with okadaic acid, a potent inhibitor of PP2A, also causes the precocious separation of sister centromeres[36], we suggest that this mechanism might be conserved between fungi and mammals. Bub1, Sgo1 and PP2A-π co-localize with kinetochore-specific nucleosomes in *S. cerevisiae* in a surprisingly large interval surrounding the core centromere-determining sequence *CEN6*, which suggests that the centromeres in this organism might be much larger than previously suspected.

We suggest that PP2A-π protects centromeric cohesin from separase activity by dephosphorylating proteins whose phosphorylation is required for efficient Rec8 cleavage, such as separase or Rec8 itself. The finding that neither ScScc1 nor SpRad21 can be protected from separase activity by Sgo1 proteins[3,37] suggests that Rec8 might be the key target of PP2A-π. Consistent with this hypothesis is the observation that SpPP2A-π can prevent Rec8 removal from chromosomes and causes its dephosphorylation when artificially recruited to chromosome arms. Phosphorylation of Rec8, possibly by Polo-like kinases[38–40], might promote cleavage whereas dephosphorylation by PP2A-π might hinder it. Our finding that Sgo1 is associated with PP2A-π in mitotic HeLa cells raises the possibility that Sgo1 protects centromeric cohesin from the prophase pathway during mitosis by a similar mechanism. In this case, the target of PP2A-π is likely to be cohesin's Scc3/SA2 subunit[13,14], the phosphorylation of which causes cohesin to dissociate from chromosomes in the absence of α-kleisin cleavage. It is also possible that PP2A-π hinders removal of cohesin from meiotic centromeres by a prophase pathway[41].

METHODS

Yeast genetics and molecular biology. Deletion or replacement of *S. pombe* and *S. cerevisiae* genes was performed as detailed in Supplementary Information. Genotypes of strains used are listed in Supplementary Table S2. Induction of meiosis in diploid *S. pombe pat1-114* cells was performed as described[7]. Sporulation of diploid *S. cerevisiae* in liquid culture was performed essentially as previously published[5].

Mammalian cell lines and RNAi. Mouse Sgo1–TAP was expressed in HeLa cells from a bacterial artificial chromosome (RP24-185F17) integrated into the genome of HeLa cells[22]. Mouse NIH3T3 cell lines, stably expressing HA4-tagged human α-, β-, γ-, δ- and ε-isoforms of B56, were obtained by transfection[42]. RNAi was performed by standard methods using oligonucleotides 5′-GUUCUU AUUCCUAUGCAUA-3′ or 5′-CAGCUUGCCUCUCAAUUCA-3′. See Supplementary Information for further details.

TAP and mass spectrometry. TAP-tagged proteins were purified from yeast and mammalian cultures essentially as previously described[43], with minor modifications. Protein samples were trypsin digested, and the resulting peptides separated via nano-capillary liquid chromatography and identified by online tandem mass spectrometry. See Supplementary Information for details.

Immunostaining of whole cells and chromosome spreads. *S. pombe* and *S. cerevisiae* cells were fixed and stained for immunofluorescence microscopy essentially as described[7,10]. *S. cerevisiae*, HeLa and NIH3T3 chromosome spreads were performed and immunostained as described previously[10,44].

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) and hybridization to Affymetrix high-density oligonucleotide arrays of *S. cerevisiae* chromosome VI and *S. pombe* chromosomes 2 and 3 were performed essentially as previously described[26,27]. ChIP on repetitive *S. pombe* centromere sequences by quantitative real-time PCR was performed essentially as described previously[45]. See Supplementary Information for details.

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