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Article

Centromeric Cohesion Is Protected Twice at Meiosis, by SHUGOSHINs at Anaphase I and by PATRONUS at Interkinesis

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Summary

Background: At meiosis, two successive rounds of chromosome segregation lead to ploidy halving. This is achieved through a stepwise release of sister chromatid cohesion, along chromosome arms to allow homolog segregation at anaphase I and at centromeres to allow sister chromatid segregation at anaphase II. Cohesins, the protein complex that ensures cohesion, must then be protected at centromeres throughout meiosis, until the onset of anaphase II. Members of the Shugoshin protein family have been shown to protect centromeric cohesins at anaphase I, but much less is known about the protection of cohesion during interkinesis, the stage between meiosis I and meiosis II.

Results: Here, we (1) show that both *Arabidopsis SHUGOSHINs* paralogs are required for complete protection of centromeric cohesins during meiosis I, without apparent somatic function, and (2) identified PATRONUS (PANS1), a novel protein required for protection of meiotic centromeric cohesion. Although AtSGO1 and AtSGO2 protect centromeric cohesion during anaphase I, PANS1 is required at a later stage, during interkinesis. Additionally, we identified PANS2, a paralog of PANS1, whose mutation is synthetically lethal with *pans1* suggesting that *PANS* genes are also essential for mitosis. PANS1 interacts directly with the CDC27b and the CDC20.1 subunit of the Anaphase Promoting Complex (APC/C), in a manner suggesting that PANS1 could be both a regulator and a target of the APC/C.

Conclusions: This study reveals that centromeric cohesion is actively protected at two successive stages of meiosis, by SHUGOSHINs at anaphase I and by PATRONUS at interkinesis.

Introduction

Cohesion between sister chromatids ensures proper chromosome segregation during mitosis and meiosis. At metaphase, cohesion holds sister chromatids together and creates an opposing force to the spindle that pulls kinetochores to opposite poles of the cell. The release of cohesion at the onset of anaphase triggers chromosome segregation. Sister chromatid cohesion is ensured by a protein complex called the cohesin. This ring-shaped complex is thought to embrace the two chromatids and is composed of four conserved subunits [1]. One of these subunits, the Kleisin (Scc1/Rad21 and the meiosis specific variant Rec8), is the target of the Separase endopeptidase at the onset of anaphase [2, 3]. Cohesins are loaded on chromosomes at telophase or early G1, and cohesion is established during S phase [4]. In yeasts, mitotic cohesins are present along the chromosomes until their release at anaphase. In vertebrate mitotic cells, most cohesins are released from chromosomes during prophase [5]. This activity, called "prophase pathway," depends on cohesin phosphorylation by Polo and additional factors such as WAPL [6–8]. However, centromeric cohesins, which ensure sister chromatid cohesion at metaphase, are protected from prophase release. This protection involves Shugoshin1 (SGOL1, which stands for the Japanese "guardian spirit"), which recruits the protein phosphatase 2A (PP2A) to centromeres to prevent cohesin phosphorylation [9–11]. Thus, at metaphase of vertebrate mitosis, sister chromatids are connected by cohesins only at centromeres, giving rise to the emblematic X shape of chromosomes. At the onset of anaphase, cleavage of the Kleisin by the Separase allows the release of the remaining cohesins and sister chromatid separation.

Meiosis is a specialized cell division in which a single replication is followed by two rounds of chromosome segregation, leading to ploidy halving. The homologous chromosomes segregate at meiosis I, whereas the sister chromatids segregate at meiosis II [12]. Thus, a specific control of cohesion dynamics is required at meiosis. Indeed, sister chromatid cohesion along the arm in combination with crossovers (COs—exchanges of continuity between homologous chromatids) create a physical link between the homologous chromosomes at metaphase I. Arm cohesion is released at the onset of anaphase I to allow segregation of homologous chromosomes. However, centromeric cohesion must be protected during meiosis I and interkinesis (the period between meiosis I and meiosis II) to ensure cohesion between sisters at metaphase II. This cohesion between sisters is then essential for their proper alignment on the metaphase plate. At anaphase II, centromeric cohesion release allows segregation of sister chromatids. REC8 [13, 14] and SGO are key actors of the stepwise release of cohesion at meiosis [15–20]. During meiosis I, a SGO protein (SGO1 in yeasts, maize, and rice, and SGO2 in vertebrates) recruits PP2A at centromeres to dephosphorylate REC8, making it resistant to Separase cleavage [21, 22]. At the onset of anaphase II, REC8, which is then unprotected, will be cleaved by the Separase to liberate the sister chromatids. In absence of SGO activity, centromeric sister chromatid cohesion is prematurely released at anaphase I, and consequently sister chromatids fail to segregate properly at meiosis II [17]. Depending on the model species considered, there is one (*S. cerevisiae*, *D. melanogaster*) or two SGO paralogs (*S. pombe*; vertebrates, plants) in the genome [17]. In addition

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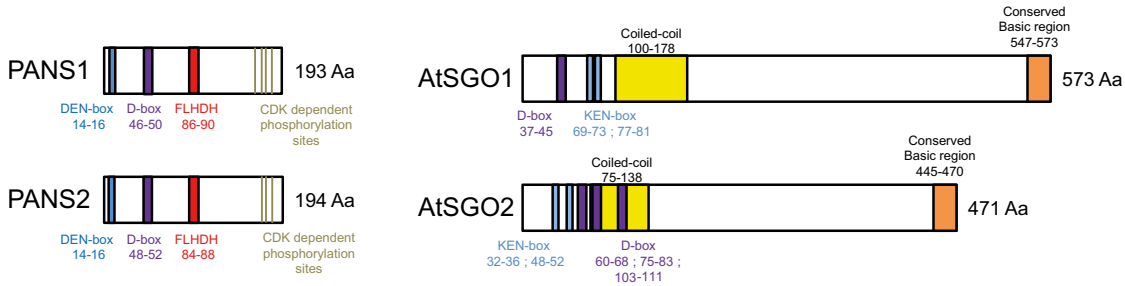

Figure 1. Schematic Representation of PANS1, PANS2, AtSGO1, and AtSGO2 Proteins

APC/C recognition motifs (DEN, KEN, and D boxes) are detected in each protein. PANS protein contains a conserved domain of unknown function (aa 81–97, around FLHDH) and a cluster of putative CDK-dependent phosphorylation sites. SGO proteins contain a predicted coiled-coil domain and a well conserved basic C-terminal domain. See also Figure S1.

to their function in protecting meiotic cohesion and to protect centromeric cohesion during mitotic prophase in vertebrates, SGO representatives have been shown to be involved in correct kinetochore orientation at mitosis and meiosis [23]. Consequently, mouse *sgo1* mutants are nonviable [19], and also *S. cerevisiae sgo1* and *S. pombe sgo2* have somatic defects [17]. Although the analysis of the SGO pathway shed light on the protection of centromeric cohesion at anaphase I, it remains to be clarified how this is achieved during interkinesis, a prominent stage in many species including animals and plants.

Here, we describe a novel protein, PANS1 (PATRONUS for protector in Latin), which is essential for the protection of centromeric sister chromatid cohesion at meiotic interkinesis. Additionally, we characterized the two *Arabidopsis thaliana SGOs* and showed that they are both required for full protection of centromeric cohesion during anaphase I. This shows that meiotic centromeric cohesion is actively protected not only at anaphase I by the conserved SGO pathway, but also at interkinesis thought a PATRONUS-dependent mechanism.

Results

PATRONUS Encodes a Protein of Unknown Function with D and DEN Boxes

As a part of an expression profiling screen, 138 genes were selected as candidates for having a meiotic function because of their coregulation with known meiotic genes [24]. Among them, three were identified as being essential for normal meiosis: *PS1* (*PARALLE SPINDLES*) [24], *OSD1* (*OMISSION OF SECOND DIVISION*) [25], and finally *At3g14190* (*PANS1*). *PANS1* encodes a protein of 193 amino acids (Figure 1 and Figure S1 available online). The PANS1 transcript has been shown to be cell cycle regulated and to peak at late M/early G1 phase of mitosis [26, 27]. The *Arabidopsis* genome contains a *PANS1* paralog, encoding a protein of 194 amino acids that we named PANS2 (*At5g12360*). Both genes are expressed in somatic and reproductive tissues (Figure S1). PANS1 and PANS2 share 42% protein identity. Similarity searches identified PANS1/2 homologs in all Eudicots tested. Phylogeny analysis of the PANS1/2 homologs suggested a dynamic gain and loss of *PANS* gene copies in the evolution of Eudicots (Figure S1A). No sequence with overall similarity to PANS was detected outside of dicots, being notably absent in monocots, the other large group of flowering plants. However, PANS proteins do share limited similarity with RSS1 (RICE SALT SENSITIVE 1), a protein that regulates cell cycle under stress condition in rice [28]. The PANS/RSS1 sequence similarity is limited to the first third of the proteins, and it has been suggested that RSS1 has been specifically lost in Eudicots [28]. No protein sharing similarity to PANS has been found in eukaryotes other than land plants. Three domains appeared to be conserved in PANS1/2 homologs (Figure S1B). The first one is a DEN box (DEN aa 14–16 in PANS1) and the second a D box (destruction box, RxxLxxxN, aa 46–54). Both domains are domains of recognition by the anaphase promoting complex (APC/C), which trigger the destruction of the targeted protein by the proteasome. The third conserved domain (aa 81–97, spanning the motif FLHDH) appears to be specific to the PANS proteins and is thus of unknown function. In addition, a subgroup of PANS homologs contains an insertion of ~50 aa that contains an additional D box. Finally, a cluster of putative CDK phosphorylation sites ([S/T-P-x-[K/R]) is found close to the C-terminal end of the protein (Figures 1 and S1).

PATRONUS Is Required for the Protection of Centromeric Cohesion during Meiosis

Two mutant alleles, *pans1-1* (Salk_070337) and *pans1-2* (Salk_035661) [29], were identified as they provoke reduced fertility when homozygous (Figure 1). Homozygous *pans1-1* and *pans1-2* plants showed no obvious growth or developmental defects in standard growth conditions. Examination of pollen viability by Alexander staining [30] revealed a large proportion of nonviable pollen grains (Figures 2A and 2B), and 19% of the female gametophyte showed development arrest (n = 155). No defects were observed in the heterozygotes, showing that both mutations are recessive. Because gametophyte development defect could result from a meiotic defect, we investigated chromosome behavior during male meiosis in *pans1* mutants. The wild-type *Arabidopsis* meiotic stages are shown in Figure 3 (left column). At metaphase I five bivalents align, each composed of two homologous chromosomes connected by COs (Figure 3A). Cohesion release along chromosomes arms allows the balanced segregation of homologous chromosomes at anaphase I (Figures 3D and 3G). Chromosomes decondense at telophase I and form two nuclei (Figure 3J). The stage between the two meiotic division phases, during which two nuclei are visible, is called interkinesis (telophase I being thus the first substage of interkinesis). During interkinesis, the chromosomes show a typical X shape, reflecting the residual cohesion at centromeric regions (Figure 3J). Then, chromosomes condense again, and the two groups of five condensed chromosomes align on two metaphase II plates (Figure 3G). Centromeric cohesion release at anaphase II allows segregation of sister chromatids leading

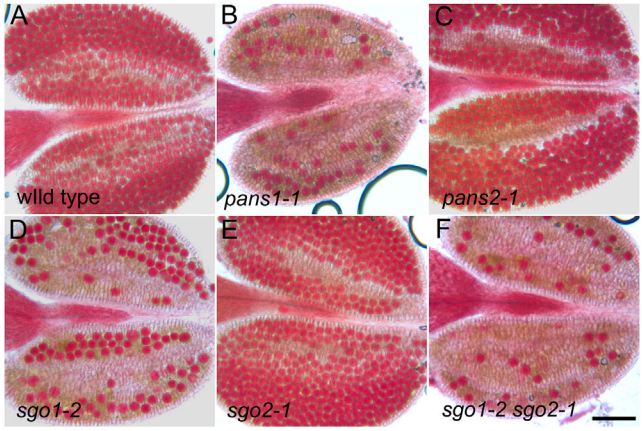

Figure 2. Pollen Grains Viability in Wild-Type and Mutants

Viable pollen grains are stained in red, whereas dead pollen grains appear deflated and gray/green. The scale bar represents 100 μM.
(A) Wild-type. All pollen grains are viable.
(B) *pans1-1*. A significant proportion of pollen grains are nonviable.
(C) *pans2-1*. Pollen grains are viable.
(D) *Atsgo1-2*. A significant proportion of pollen grains are nonviable.
(E) *Atsgo2-1*. Pollen grains are viable.
(F) *Atsgo1-2 Atsgo2-1*. Very few pollen grains are viable.

to the formation of four spores. Meiotic chromosomes in *pans1-1* and *pans1-2* mutants behaved similarly to the wild-type until meiosis II. Chiasmata formation, bivalent alignment at metaphase I (Figure 3B), and segregation of homologous chromosome at anaphase I (Figures 3E and 3H) were indistinguishable from wild-type. Consequently, five decondensed chromosomes were observed at each pole of meiocytes at telophase I, which were, like in wild-type, composed of two chromatids linked at their centromeres (Figure 3K). Meiosis II, however, differed markedly from wild-type in both *pans1* mutants. At metaphase II, we observed free single chromatids that did not align properly (Figure 3N). The phenotype, although drastic, was not fully penetrant in both alleles (see quantification on Figure 4). About 30% of the metaphase plates contained ten single chromatids, showing a complete loss of sister chromatid cohesion. About 60% showed one or two pairs of associated chromatids (and eight or six single chromatids, respectively). However, it should be noted that the residual cohesion may be slightly overestimated as two chromatids could associated by chance on the chromosome spread. The remaining ~10% metaphase plates showed wild-type-like configuration with the alignment of five pairs of chromatids. The single chromatids segregated erratically leading to the production of unbalanced tetrads. Complementation tests with *pans1-1* and *pans1-2* mutants showed that these mutations were allelic. Transformation of *pans1-1* with a genomic fragment containing the *PANS1* gene restored normal meiosis, confirming that the observed meiotic defect is due to the mutations in the *PANS1* gene. Thus, PANS1 (but not PANS2, see below) is required for the maintenance of centromeric cohesion until meiosis II.

Identification of *shugoshin* Mutants in *Arabidopsis thaliana*

The premature loss of sister chromatid cohesion in a *pans1* mutant is reminiscent of the phenotype of *sgo* mutants that has described in several eukaryotes, but not yet in *Arabidopsis*. Sequence similarity searches [17] detected two putative *SHUGOSHIN* genes in the *Arabidopsis* genome, *At3g10440* (At*SGO1*) and *At5g04320* (At*SGO2*) (Figures 1B and S1). *AtSGO1* and *AtSGO2* encode proteins that share 30% identity. In eukaryotes, which contain two *SGO* genes, only one is involved in protection of centromeric cohesion during meiosis [23]. To investigate which *AtSGO* gene(s) is involved in meiotic centromeric cohesion, we analyzed insertion mutants of both genes. Two mutants in *AtSGO1* were available in the T-DNA SK collection [31], *Atsgo1-1* (SK35523) and *Atsgo1-2* (SK2556) (Figure 1B). Because no insertion mutant in the *AtSGO2* coding region was available from public collections, we performed a PCR-based screen of the Koncz collection [32] and identified one insertion (line 34303, *Atsgo2-1*) (Figure 1B).

AtSGO1 and *AtSGO2* Are Both Required for Full Protection of Centromeric Cohesion at Meiosis

Plants homozygous for *Atsgo1-1* or *Atsgo1-2* mutations did not show any growth or developmental defects in standard conditions but had reduced fertility. In both mutants, a large proportion of male gametophytes (pollen grains) (Figure 2D) and female gametophytes were unviable (*Atsgo1-1*, 55%, n = 269; *Atsgo1-2*, 58%, n = 271). In contrast to *Atsgo1* mutants, *Atsgo2-1* mutants did not show any defect in fertility, pollen (Figure 2E), or female gametophyte viability (97% viability, n = 169) and was neither affected in growth nor development. The double homozygous mutant *Atsgo1-2 Atsgo2-1* was, however, less fertile (shorter fruit) and showed a higher male (Figure 2F) and female gametophyte lethality (89%, n = 254) than *Atsgo1* single mutants, suggesting that *At*SGO1 and *At*SGO2 have partially redundant function in the sexual process. *Atsgo1-2 Atsgo2-1* double mutants did not show growth or developmental defects, suggesting that *At*SGO1 and *At*SGO2 have no critical role at mitosis. We thus investigated chromosome behavior during male meiosis in *Atsgo* single and double mutants. Consistent with its complete fertility, the *Atsgo2-1* mutant did not show any meiotic defects. In contrast, both *Atsgo1* mutant alleles exhibited a meiotic defect, which was amplified in the *Atsgo1-2 Atsgo2-1* double mutant (Figure 4). Meiotic chromosome behavior in these mutants was indistinguishable from wild-type during prophase, metaphase I, and early anaphase I (Figures 3C and 3F). In contrast, defects were visible at metaphase II, when a mixture of pairs of chromatids and single chromatids were observed (Figures 3I and 4). The defect had a variable intensity in *Atsgo1* single mutants, ranging from five pairs of chromatids normally aligned to ten single chromatids erratically distributed (Figure 4). In the *Atsgo1-2 Atsgo2-1* double mutant, cohesion was nearly completely lost at meiosis II, most metaphase II plates exhibiting ten single chromatids (Figures 3O and 4). Thus, *At*SGO1 and *At*SGO2 are both required for full protection of centromeric cohesion at meiosis, without obvious function at mitosis.

*At*SGOs and PANS1 Are Needed for Protection of Centromeric Cohesion at Distinct Stages

Plants lacking *PANS1* or *AtSGOs* exhibited similar defects at metaphase II. However, although no defect was detected in meiosis I in *pans1*, precocious split of chromatids were detected in *Atsgo1-2 Atsgo2-1* in the course of anaphase I (compare Figures 3H and 3I). Accordingly, free chromatids were observed at telophase I at each pole of the cell in *Atsgo1-2 Atsgo2-1* but not in *pans1* in which chromosome

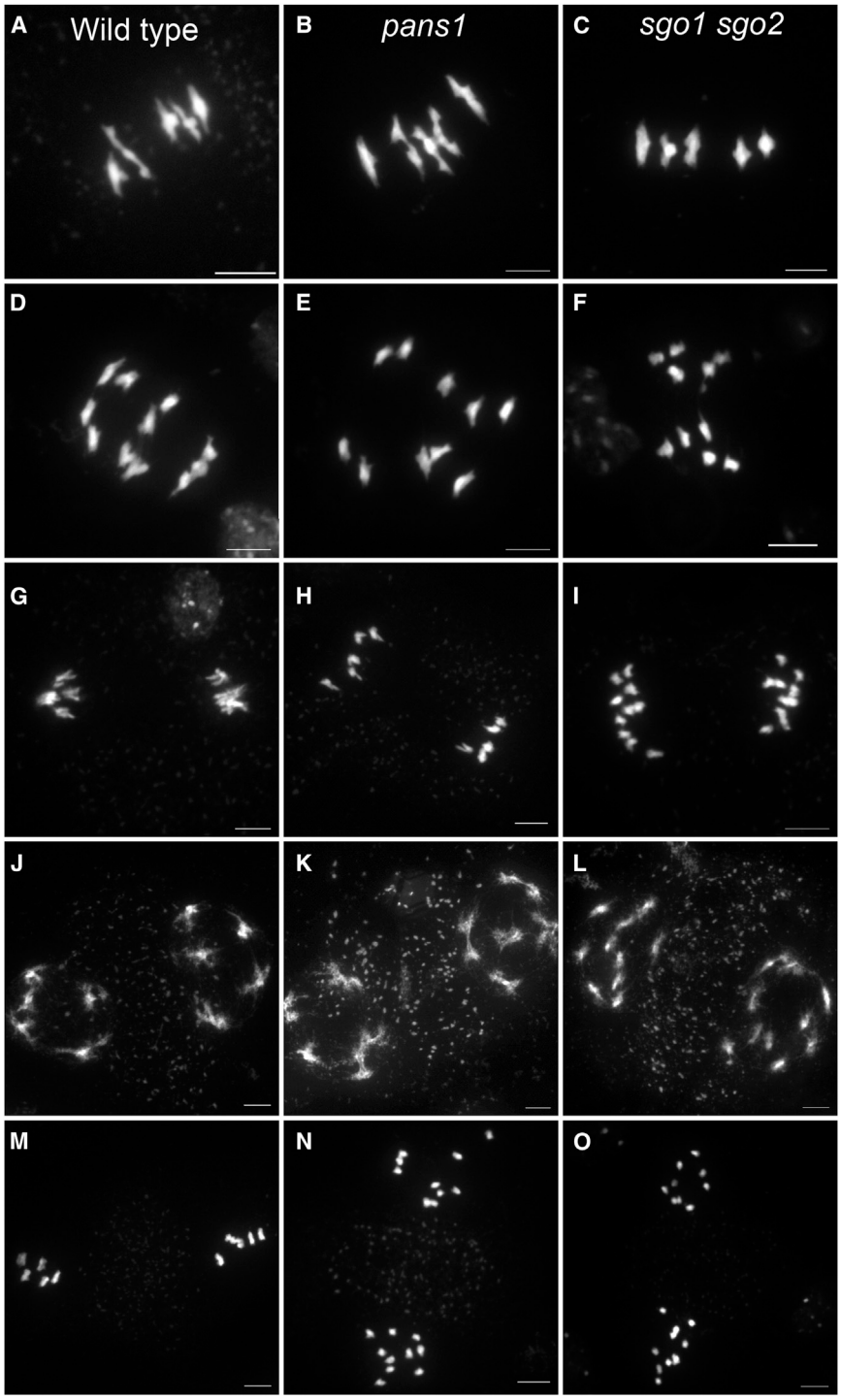

Figure 3. Male Meiosis in Wild-Type, *pans1-1*, and *Atsgo1-2 Atsgo2-1* Double Mutant

Chromosome spreads stained by DAPI. Metaphase I (A–C), early anaphase I (D–F), late anaphase I (G–I), interkinesis (J–L), and metaphase II (M–O) are shown. Scale bars represent 5 μm.

had the typical X shape reflecting sister chromatid cohesion around the centromere (compare Figures 3K and 3L, see quantification in Figure 4). Thus, like in other organisms, *Arabidopsis* SGOs appear to protect centromeric cohesion during anaphase I. The difference in timing of centromeric cohesion loss in *pans1-1* versus *Atsgo1-2 Atsgo2-1* strongly suggests that *At*SGOs and PANS1 are needed for protection of centromeric cohesion at distinct stages of meiosis, anaphase I, and interkinesis, respectively.

*At*REC8 Behavior in *pans1* and *Atsgo1 Atsgo2* Mutants

AtREC8 is a meiosis specific cohesin that is cleaved by the Separase at the anaphase onset [33–36]. We localized *At*REC8, in combination with the centromeric histone HTR12/CENH3 [37]. As previously shown, in wild-type meiocytes, *At*REC8 is present on chromosomes during prophase I [33, 35]. At metaphase I, REC8 was detected on the entire bivalent with the exception of the core centromeric region where HTR12 and REC8 signal did not overlap (Figure 5A). We were unable to detect a REC8 signal above the background during anaphase I and telophase I. However, at metaphase II, an unambiguous REC8 signal was detected between the two HTR12 signals that are oriented to opposite poles. This signal is much fainter than the metaphase I signal and presumably represents the pericentromeric cohesion that has been protected during meiosis I (Figure 5B). When sister chromatids segregated in anaphase II, no REC8 signal was detected on separating chromatids. We observed that REC8 and HTR12 signals in the single *pans1-1* mutant and the *Atsgo1-2 Atsgo2-1* double mutants were the same as in wild-type at prophase and metaphase I (Figures 5C and 5E), suggesting that cohesin loading was unaffected in both mutants. However, at metaphase II no signal was detected on the single chromatids (Figures 5D and 5F), showing that both SGOs and PANS1 are required to prevent precocious release of pericentromeric cohesins during meiosis.

PANS1 and *PANS2* Are Synthetically Essential for Gametophyte Development

To investigate the function of PANS2, the single PANS1 paralog found in the *Arabidopsis* genome, we analyzed a T-DNA insertion *pans2-1* (Sail_305_G04) (Figure S1) [38]. Plants homozygous for the *pans2-1* mutation did not show any growth or developmental defects in standard growth conditions. Further, unlike *pans1* mutants, *pans2-1* presented no meiotic or pollen development defects (Figure 2C). With the intention of producing a double mutant, crosses between homozygous *pans1-1* and *pans2-1* plants were performed and the F2 progeny was genotyped. However, neither double homozygous *pans1-1*$^{-/-}$

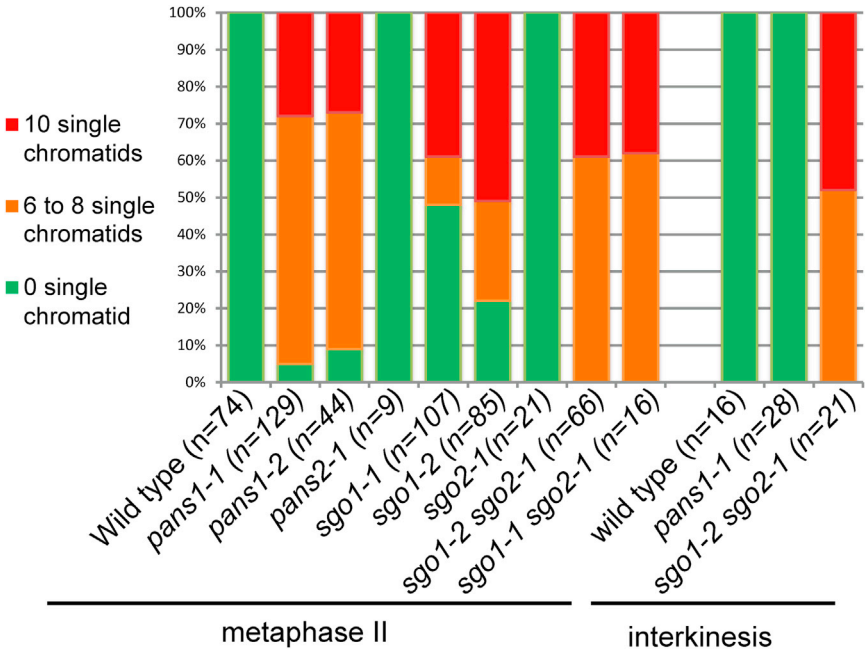

Figure 4. Quantification of Sister Chromatid Cohesion

Metaphase plates and interkinesis nuclei were sorted into three categories according to the number of single chromatids, from zero (five pairs of chromatids align on the metaphase plate, or connected by centromere at interkinesis) to ten. The proportion of each category is shown for wild-type and a series of mutant. The number of plates or nuclei observed is indicated in brackets.

pans2-1$^{-/-}$ plants (0/412 versus 26 expected) nor *pans1-1*$^{-/-}$ *pans2-1*$^{+/-}$ (0/412 versus 52 expected) were recovered. Very few *pans1-1*$^{+/-}$ *pans2-1*$^{-/-}$ were obtained (2/412 versus 52 expected). Similarly using the *pans1-2* allele and *pans2-1*, no plants double homozygote or homozygote for one mutation and heterozygote for the other one were obtained (0/47 versus 3 and 12 expected). These results strongly suggest transmission defects of gametes harboring the two mutations. We then performed reciprocal crosses with wild-type plants. When *pans1-1*$^{+/-}$ *pans2-1*$^{+/-}$ was used as female, only one double heterozygote was recovered (1/94, 23 expected). Similarly, when *pans1-1*$^{+/-}$ *pans2-1*$^{+/-}$ was used as male, only one double heterozygote plant out of 94 progeny was recovered (23 expected). This confirmed a strong defect in the simultaneous transmission of both *pans1-1* and *pans2-1* alleles through the male and the female gametes, showing that almost all of the double mutant gametophytes are defective. Consistently, 27% of the *pans1-1*$^{+/-}$ *pans2-1*$^{+/-}$ female gametophyte had development defects (n = 67) (Figure S2). However, no obvious defect was detected in pollen grains development (Figure S2), even if male genetic transmission is almost abolished. Altogether, these results suggest that PANS1 and PANS2 have a redundant essential role in gametophytic and likely somatic development, possibly at mitosis.

Although *pans1* single mutants showed no growth defects in greenhouse conditions, we detected a slight reduction of root growth in vitro. Further, *pan1-1* root growth was strongly affected by addition of NaCl in the medium (Figure S3). This confirms that *PANS1* also plays a role in somatic development. In contrast, the *pans2-1* mutant, the *Atsgo* single mutants, and the *Atsgo1 Atsgo2* double mutant were not affected (Figure S3), further supporting the absence of an essential function of AtSGOs in somatic development.

PATRONUS Interacts with APC/C Core Components

To identify proteins that interact with PANS1, tandem affinity purification (TAP) experiments were performed on cell culture [39–42] using PANS1 as baits. Transgenic cultures were generated expressing PANS1 fused at its N terminus with the GS TAPtag [40]. Copurified proteins were separated by polyacrylamide gel electrophoresis and sequenced through MALDI mass spectrometry (MS). The list of proteins detected by MS was cleaned up based on a list of nonspecific background proteins that was assembled by approximately 40 TAP experiments on wild-type cultures and cultures expressing TAPtagged mock proteins GUS, RFP, and GFP, a similar procedure we followed for TAP experiments on more than 100 cell-cycle-ted proteins produced in the same cell culture [42]. The final list of copurified proteins solely contained a small list of APC/C subunits: HOBBIT (*At*CDC27b), *At*APC6, *At*APC7, and *At*APC8 (Figure S4). This showed that PANS1 interacts with the APC/C, but, because TAP purifies protein complexes as a whole, these results do not permit determination of which APC/C subunit PANS1 directly interacts with. Therefore, we used yeast two-hybrid (Y2H) interaction matrix to test possible interactions with 14 different APC/C subunits [43]. Only the test with the APC/C component HOBBIT was positive (Figure 6). We further tested by one-to-one Y2H experiments the interaction of PANS1 with HOBBIT, AtAPC6, AtAPC7, the three CDH1 homologs (CCS52A1, CCS52A2, CCS52B), and CDC20.1 (which was missing in the matrix experiment). Only the PANS1-HOBBIT and the PANS1-CDC20.1 interaction tests were positive (Figure 6). Thus, PANS1 interacts directly with the APC/C through the HOBBIT (CDC27b) and CDC20.1 subunits.

PANS1 with Mutated Destruction Domains Interacts with CDC27b and Is Lethal In Vivo

PANS1 possess two putative APC/C targeting signals, a D box and a DEN box. For both the D box and DEN box, essential amino acid residues were substituted (RxxL → LxxV and DEN → AAA, PANS1ΔD and PANS1ΔDEN, respectively). Mutating either or both domains did not affect the Y2H PANS1- HOBBIT interaction. In contrast, the PANS1-CDC20.1 interaction was strongly affected by these mutations and almost abolished when both domains were mutated (Figure 6). To investigate whether these domains are needed for PANS1 function in vivo, we created versions of the genomic PANS1 gene with one or both mutations (PANS1ΔD, PANS1ΔDEN, and PANS1ΔDΔDEN) and tested their ability to complement the *pans1-1* mutant phenotype. The wild-type genomic and PANS1ΔDEN clones were equally able to restore the pollen viability and normal meiosis of the *pans1-1* mutant (number of independent transformants n = 11 and n = 8, respectively), showing that the DEN box is not essential for the PANS1 meiotic

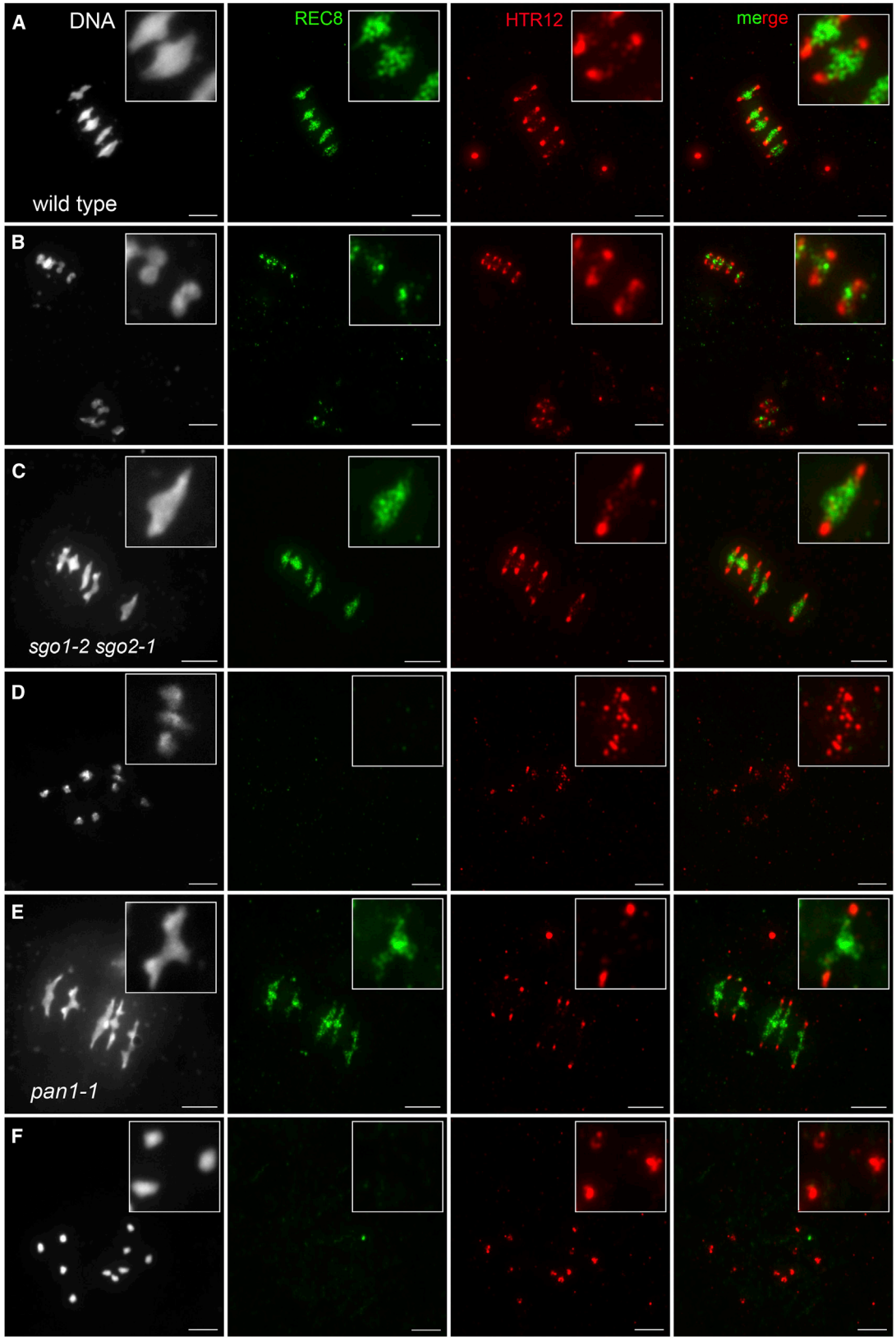

Figure 5. Immunolocalization of HTR12 and AtREC8

First column: DNA stained by DAPI (gray). Second column: localization of the cohesin AtREC8 (green). Third column: localization of the centromeric histone HTR12 (red). Fourth column: merge of AtREC8 and HTR12 localizations. Wild-type (A and B), *Atsgo1-2 Atsgo2-1* double mutant (C and D), and *pans1-1* mutant (E and F) are shown. Metaphase I (A, C, and E) and metaphase II (B, D, and F) are shown. Scale bars represent 5 μm. Magnifications are shown in the top-left corner for metaphase images.

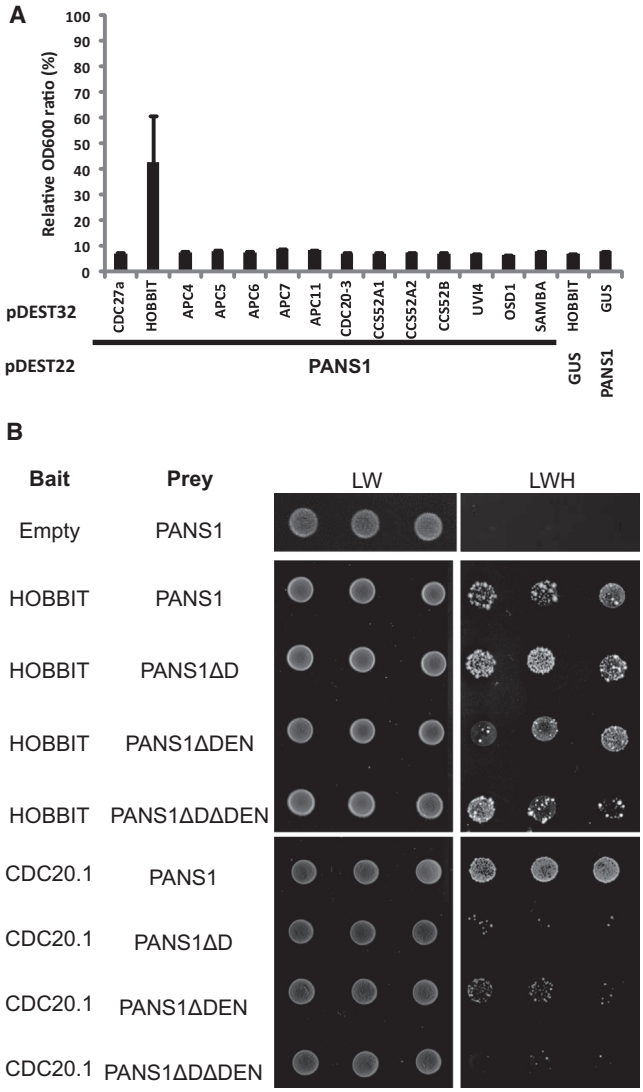

Figure 6. Y2H Interaction between Wild-Type and Mutant PANS1 Proteins with APC/C Subunits

(A) OD plot of Y2H interactions via mating testing PANS1 against various APC/C subunits [43]. The GUS protein was included as negative control.
(B) PANS1 interacts with HOBBIT/CDC27b independently of its D and DEN boxes. In contrast, PANS1 interacts with the APC/C activator CDC20.1 in a D- and DEN-box-dependent manner.
See also Figures S4 and S5.

function. When PANS1ΔD was expressed either in wild-type or the *pans1-1* mutant, a strong dominant defect in growth was observed (n = 10 and 3) (Figure S5). We were thus unable to test the capacity of this construct to complement the *pans1* meiotic phenotype. Further, although numerous transformed plants were obtained for each of the other constructions at each transformation experiment, no plant was obtained when PANS1ΔDΔDEN was used (four independent experiments). This strongly suggests that the ΔDΔDEN version of the PANS1 protein is lethal, impairing the recovery of transformed plants.

Discussion

At meiosis, centromeric cohesion must be protected through meiosis I, until anaphase onset of the second division, to ensure balanced segregation of sister chromatids. Here, we show in *Arabidopsis* that sister chromatid cohesion is protected twice at meiosis, during anaphase I by the two SHUGOSHIN proteins, and during interkinesis by PATRONUS, an APC/C interacting protein.

We functionally characterized the two SGO paralogs in *A. thaliana*. The mutation of *AtSGO1* led to a partial loss of centromeric cohesion during meiosis I, leading to a mixture of single and cohesive chromatids at metaphase II. In contrast, mutation of *AtSGO2* alone appeared to have no effect on meiosis. Further, an *Atsgo1 Atsgo2* double mutant showed an almost complete loss of sister chromatid cohesion during anaphase I. At meiosis I, bivalents aligned normally, and the homologous chromosomes segregated evenly showing that the two *At*SGO genes are not required for the establishment of sister chromatid cohesion or the monopolar orientation of sister kinetochores that ensures cosegregation of sister chromatids at anaphase I. In wild-type, the AtREC8 cohesin is detectable on bivalents at metaphase I, and with a lower intensity on sister chromatid pairs at metaphase II. As shown for *sgo* mutants in several organisms, in the *Atsgo1 Atsgo2* double mutant, the REC8 signal is indistinguishable from wild-type at metaphase I but is no longer detectable on the single chromatids at metaphase II. Altogether, this shows that the two *At*SGO paralogs act redundantly to protect REC8 at centromeres during meiosis I. Thus, the *At*SGO genes appear to ensure the conserved role of REC8 protection during anaphase I, the difference with other organisms tested so far being that both genes are involved in this function. The two *AtSGO* genes appear to be issued from a recent duplication that is shared only with *Brassicales*, which may explain their partial functional redundancy. Interestingly, neither single nor *Atsgo1 Atsgo2* double mutants have detectable growth or developmental defects, suggesting that, in contrast to the situation in vertebrates and yeasts [10, 17, 44], none of the *SGOs* have an essential role at mitosis in *Arabidopsis*, though they are expressed in somatic tissues. In plants, only one of the two *SGO* genes has been characterized in rice and maize [15, 16]. In both species, this gene is involved only in meiosis ensuring the canonical role in protecting centromeric cohesion. It would be interesting to analyze the function of the other paralogs to test the putative meiotic function of the second gene and if the absence of the requirement for SGOs at mitosis is shared in the plant clade.

Further, using a genetic screen, we identified a novel actor essential for the persistence of cohesion until meiosis II and named it *PATRONUS* (*PANS1*) for "protector" in Latin. In *pans1* mutants, meiosis I occurred normally with bivalent alignment and regular homologous chromosome segregation. In contrast, single chromatids were observed at metaphase II, leading to random segregation. Also in *pans1,* the REC8 cohesin was normally present on metaphase I bivalents but was not detected on single chromatids at metaphase II. This defect is reminiscent of the *shugoshin* defect and shows that PANS1 is required for the protection of sister chromatid cohesion at meiosis, but not for cohesion establishment and monopolar orientation of kinetochores at meiosis I. However, the *pans1* defect differs from the *Atsgo1 Atsgo2* defect. Indeed, even if the defect is similar in both mutants at metaphase II, the single chromatids are visible as soon as late anaphase I/telophase I in *Atsgo1 Atsgo2*, although sister chromatids are still attached at the centromeres at interkinesis in *pans1* and wild-type. This shows that, although *At*SGOs protect sister chromatid cohesion during anaphase I, the persistence of this cohesion during

interkinesis depends upon PANS1. To our knowledge, this is the first demonstration of the existence of an active system of cohesion protection during meiotic interkinesis. Although the PANS protein may be not conserved, such a pathway may exist in other systems with a distinct interkinesis, such as male meiosis in mammals.

Beyond the crucial role of PANS1 at meiosis, the PANS protein family appears to have an important somatic function. Indeed, *pans1* mutant exhibited growth defects under stress conditions. In addition, the expression, under the endogenous *PANS1* promoter, of mutated versions of *PANS1* predicted to be resistant to degradation mediated by the APC/C led to growth defects or lethality. Finally, *PANS1* and its paralog *PANS2* are synthetically lethal. This strongly suggests that these genes play a redundant, essential role at mitosis. At least one *PANS* homolog is present in all dicot species. However, phylogenetic analysis strongly suggests that *PANS1* and *PANS2* separation of function appeared in the Brassicale clade (Figure S1). In monocots, no clear homolog is found, only a protein family that shows limited similarity. One of the two representatives of this family in rice, RSS1, has been shown to regulate the somatic cell cycle under stress conditions but was not reported as having a meiotic function [28].

TAP-TAG and Y2H experiments showed that PANS1 interacts with the APC/C through a direct interaction with the AtCDC27b/HOBBIT and CDC20.1 subunits. CDC20.1 is one of the APC/C activators that confers its target specificity [45, 46]. PANS1 possess two APC/C degradation motifs (D and DEN boxes) that are required for its Y2H interaction with CDC20.1. PANS1ΔDEN showed decreased interaction with CDC20.1 but appeared still fully functional in vivo. PANS1ΔD and PANS1ΔDΔDEN showed almost abolished interaction with CDC20.1 and provoked strong growth defects or lethality when expressed in vivo. This advocates that PANS1 is targeted by the APC/$C^{CDC20.1}$ through its DEN and D box domains and that a nondegradable version perturbs mitosis. However, PANS1 likely interacts with the APC/C beyond being an APC/C target. Indeed, TAP-TAG experiments using PANS1 as bait recovered APC/C subunits, although it is not the case when well-known APC/C targets such as cyclins are used as bait [42], likely because the interaction between APC/C and its targets is too transient. Further, PANS1 interacts with the APC/C subunit HOBBIT/CDC27b in Y2H experiments, independently of its D and DEN boxes. Altogether, this suggests that PANS1 may be both a regulator of the APC/C through its interaction with AtCDC27b, independently of it DEN and D boxes, and a target of the APC/$C^{CDC20.1}$ for degradation, through these boxes. At least three hypotheses can be proposed for how an APC/C regulator could be involved in cohesion protection during meiotic interkinesis. First, the Separase that cleaves cohesins along chromosomes may be prevented by PANS1 from cleaving centromeric cohesion at interkinesis. One possibility would be that PANS1 protects SGO from APC/C destruction, because SGO proteins have predicted APC/C degradation motifs. Second, PANS may protect centromeric cohesion from Separase independently of SGO, if SGO no longer protects centromeric cohesion during interkinesis. SGO cytological dynamics has not been established so far in *Arabidopsis*, but, in *S. pombe*, rice and maize SGO is no longer detectable after anaphase I [15–18]. Under this second hypothesis, PANS1 may prevent Separase activity either by stimulating its targeting by APC/C or conversely by preventing the destruction by APC/C of a Separase inhibitor (i.e., the Securin, which has not been identified yet in plants). Third, PANS1 may inhibit the other known pathway of cohesin release that is normally activated at the end of mitosis (telophase/G1 of the next round of mitosis). This pathway allows dynamic renewal of cohesins at G1, through cohesin loading catalyzed by SCC2/SCC4 and cohesin release by catalyzed by WAPL [47]. Such WAPL activity must be prevented at meiotic interkinesis, which somehow resembles mitotic telophase/G1. This could be the function of PANS1 to inactivate WAPL activity via APC/C regulation. *Arabidopsis* possesses two WAPL homologs that have not yet been characterized. It would be of particular interest to address the functional relationship of PANS1 with the WAPL genes on one hand and with the Separase on the other, to understand the molecular bases of this novel mechanism of cohesion protection involving PANS1.

Experimental Procedures

Growth Conditions and Genotyping

Arabidopsis plants were cultivated in greenhouse as previously described [48]. The *pans1-1* (Salk_070337), *pans1-2* (Salk_035661), *pans2-1* (Sail_305_G04), *Atsgo1-1* (SK35523), *Atsgo1-2* (SK2556), and *Atsgo2-1* (line 34303) were genotyped by PCR with two primer pairs. The first pair is specific to the wild-type allele, and the second pair is specific to the left border of the inserted sequence as follows: *pans1-1 and pans1-2*, N570337U (5′-CCGTTAAACACTCTAAGCGC-3′) and N570337L (5′-ATGC TTCTCCTTGATGCTGG-3′), N570337L and LBSalk2; *pans2-1*, N814152 U (5′-GAGGTAGAGCTCCTGGCAAC-3′) and N814152 L (5′-ACCCCCTAGAAA CAATGCTG-3′), N814152 L and Lb3Sail; *Atsgo1-1*, SK35523U (5′-ATT CAAATTTCGATCCTGTTA-3′) and SK35523L (5′-ACCGCCAAGATACGAGT GTT-3′), SK35523U and pSKTail1; *Atsgo1-2*, SK2556U(5′-GAGGTCGGCAA TAAGGTTAG- 3′) and SK2556L (5′-CCCGATAGACTGTTCACTTG-3′), SK2556U and pSKTail1; *Atsgo2-1*, SGO2U (5′-TAAGTTATTCCGTTGGCC TAC-3′) and SGO2L (5′-GATTGCCTTCGTGTACATTGC-3′), SGO2U and GABI (5′-ATATTGACCATCATACTCATTGC-3′).

Directed Mutagenesis Constructs, Plant Transformation, and Plasmid Constructs

PANS1 genomic fragment was amplified by PCR using PANS1U (5′-CAC CATCAACTTCCGGTTGG-3′) and PANS1L (5′-GCAGCAAGTTATGAAAA GGTGG-3′). The amplification covered 964 nucleotides before the ATG and 477 after the stop codon. The PCR product was cloned, by Gateway (Invitrogen) into the pDONR207 (Invitrogen) to create pENTR-PANS1, on which directed mutagenesis was performed using the Stratagene Quickchange Site-Directed Mutagenesis Kit. The primers used to create the PANS1ΔDEN and PANS1ΔD mutations were 5′-GATCTTCCCAGCCGCG GCCGCTCCAATTCATC-3′ and 5′-CCTGGAGGAGCTCTTAAGGCTGTGAAT GATATTAC-3′, respectively. For plant transformation, LR reaction was performed with the binary vector pGWB1 [49]. The resulting binary vectors, pPANS1, pPANS1ΔDEN, pPANS1ΔD, and pPANS1 ΔDENΔD, were transformed using the *Agrobacterium*-mediated floral dip method [50] on plant population segregating for the *pans1-1* mutation. For Y2H experiments with mutant alleles, the same primers were used and mutagenesis was performed on the pENTR-PANS1 vector. Partial REC8 cDNA was amplified by 5′-GCTGATGCCGAGAATATCACAC-3′ and 5′-AGAACTGTCCTTTTCAAA GAG-3′ and cloned into a pDEST17 (Invitrogen) for recombinant protein production.

Cytology

Chromosomes spreads, alexander staining, and immunolocalization were performed according to published methods [30, 51, 52]. The anti-HTR12 antibody was described by [37] and was used at 1:400. The anti-REC8 antibody was raised against a partial recombinant protein of 174 aa and produced as described in [48]. It was used at a dilution of 1:250. The absence of signal in *Atrec8* mutant (N836037) confirmed its specificity. Observations were made using a ZEISS AxioObserver microscope.

TAP-TAG/Y2H

PANS1 cDNA was amplified by PCR using 5′-ATGGCGAACATGAA CGCTCT-3′ and 5′-TCAGAAGAGGTCGTCAGAGTC-3′. PCR product was cloned by Gateway (Invitrogen) into the pDONR221 vector (Invitrogen) to

create pENTR-cPANS1. LR reactions were done into the pDEST32 (bait) and pDEST22 (prey) vector (Invitrogen). Cloning of transgene encoding tag fusion under control of the constitutive cauliflower tobacco mosaic virus 35S promoter and transformation of *Arabidopsis* cell suspension culture was carried out as previously described [39]. Tandem affinity purification of protein complexes was done using the GS tag [40], followed by the GS purification protocol as described in [41]. For the protocols of proteolysis and peptide isolation, acquisition of mass spectra by a 4800 Proteomics Analyzer (Applied Biosystems) and MS-based protein homology identification based on the TAIR genomic database, we refer to [42]. Y2H interaction matrix was performed as described in [43]. Y2H interaction testing using PANS1 mutant allele as prey (pDEST22) with APC/C subunits as bait (pDEST32) was performed by mating, as described previously [43] into the yeast strain AH109 and Y187 (Clontech).

Supplemental Information

Supplemental Information includes five figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.08.036.

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References

1. Nasmyth, K., and Haering, C.H. (2009). Cohesin: its roles and mechanisms. Annu. Rev. Genet. *43*, 525–558.
2. Uhlmann, F., Wernic, D., Poupart, M.A., Koonin, E.V., and Nasmyth, K. (2000). Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell *103*, 375–386.
3. Hauf, S., Waizenegger, I.C., and Peters, J.M. (2001). Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. Science *293*, 1320–1323.
4. Peters, J.-M., Tedeschi, A., and Schmitz, J. (2008). The cohesin complex and its roles in chromosome biology. Genes Dev. *22*, 3089–3114.
5. Waizenegger, I.C., Hauf, S., Meinke, A., and Peters, J.M. (2000). Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell *103*, 399–410.
6. Hauf, S., Roitinger, E., Koch, B., Dittrich, C.M., Mechtler, K., and Peters, J.-M. (2005). Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS Biol. *3*, e69.
7. Kueng, S., Hegemann, B., Peters, B.H., Lipp, J.J., Schleiffer, A., Mechtler, K., and Peters, J.-M. (2006). Wapl controls the dynamic association of cohesin with chromatin. Cell *127*, 955–967.
8. Sumara, I., Vorlaufer, E., Stukenberg, P.T., Kelm, O., Redemann, N., Nigg, E.A., and Peters, J.M. (2002). The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. Mol. Cell *9*, 515–525.
9. Kitajima, T.S., Hauf, S., Ohsugi, M., Yamamoto, T., and Watanabe, Y. (2005). Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. Curr. Biol. *15*, 353–359.
10. McGuinness, B.E., Hirota, T., Kudo, N.R., Peters, J.-M., and Nasmyth, K. (2005). Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. PLoS Biol. *3*, e86.
11. Tang, Z., Shu, H., Qi, W., Mahmood, N.A., Mumby, M.C., and Yu, H. (2006). PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. Dev. Cell *10*, 575–585.
12. Zickler, D., and Kleckner, N. (1999). Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. *33*, 603–754.
13. Watanabe, Y., and Nurse, P. (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature *400*, 461–464.
14. Klein, F., Mahr, P., Galova, M., Buonomo, S.B., Michaelis, C., Nairz, K., and Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell *98*, 91–103.
15. Hamant, O., Golubovskaya, I.N., Meeley, R., Fiume, E., Timofejeva, L., Schleiffer, A., Nasmyth, K., and Cande, W.Z. (2005). A REC8-dependent plant Shugoshin is required for maintenance of centromeric cohesion during meiosis and has no mitotic functions. Curr. Biol. *15*, 948–954.
16. Wang, M., Tang, D., Wang, K., Shen, Y., Qin, B., Miao, C., Li, M., and Cheng, Z. (2011). OsSGO1 maintains synaptonemal complex stabilization in addition to protecting centromeric cohesion during rice meiosis. Plant J. *67*, 583–594.
17. Kitajima, T.S., Kawashima, S.A., and Watanabe, Y. (2004). The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature *427*, 510–517.
18. Rabitsch, K.P., Gregan, J., Schleiffer, A., Javerzat, J.-P., Eisenhaber, F., and Nasmyth, K. (2004). Two fission yeast homologs of Drosophila Mei-S332 are required for chromosome segregation during meiosis I and II. Curr. Biol. *14*, 287–301.
19. Lee, J., Kitajima, T.S., Tanno, Y., Yoshida, K., Morita, T., Miyano, T., Miyake, M., and Watanabe, Y. (2008). Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. Nat. Cell Biol. *10*, 42–52.
20. Llano, E., Gómez, R., Gutiérrez-Caballero, C., Herrán, Y., Sánchez-Martín, M., Vázquez-Quiñones, L., Hernández, T., de Alava, E., Cuadrado, A., Barbero, J.L., et al. (2008). Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. Genes Dev. *22*, 2400–2413.
21. Ishiguro, T., Tanaka, K., Sakuno, T., and Watanabe, Y. (2010). Shugoshin-PP2A counteracts casein-kinase-1-dependent cleavage of Rec8 by separase. Nat. Cell Biol. *12*, 500–506.
22. Katis, V.L., Lipp, J.J., Imre, R., Bogdanova, A., Okaz, E., Habermann, B., Mechtler, K., Nasmyth, K., and Zachariae, W. (2010). Rec8 phosphorylation by casein kinase 1 and Cdc7-Dbf4 kinase regulates cohesin cleavage by separase during meiosis. Dev. Cell *18*, 397–409.
23. Gutiérrez-Caballero, C., Cebollero, L.R., and Pendás, A.M. (2012). Shugoshins: from protectors of cohesion to versatile adaptors at the centromere. Trends Genet. *28*, 1–10.
24. d'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., Simon, M., Jenczewski, E., and Mercier, R. (2008). Mutations in AtPS1 (Arabidopsis thaliana parallel spindle 1) lead to the production of diploid pollen grains. PLoS Genet. *4*, e1000274.
25. d'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., and Mercier, R. (2009). Turning meiosis into mitosis. PLoS Biol. *7*, e1000124.
26. Menges, M., Hennig, L., Gruissem, W., and Murray, J.A. (2003). Genome-wide gene expression in an Arabidopsis cell suspension. Plant Mol. Biol. *53*, 423–442.
27. Gauthier, N.P., Jensen, L.J., Wernersson, R., Brunak, S., and Jensen, T.S. (2010). Cyclebase.org: version 2.0, an updated comprehensive, multi-species repository of cell cycle experiments and derived analysis results. Nucleic Acids Res. *38*(Database issue), D699–D702.
28. Ogawa, D., Abe, K., Miyao, A., Kojima, M., Sakakibara, H., Mizutani, M., Morita, H., Toda, Y., Hobo, T., Sato, Y., et al. (2011). RSS1 regulates the cell cycle and maintains meristematic activity under stress conditions in rice. Nat. Commun. *2*, 278.
29. Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., Stevenson, D.K., Zimmerman, J., Barajas, P., Cheuk, R., et al. (2003). Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science *301*, 653–657.
30. Alexander, M.P. (1969). Differential staining of aborted and nonaborted pollen. Stain Technol. *44*, 117–122.
31. Robinson, S.J., Tang, L.H., Mooney, B.A., McKay, S.J., Clarke, W.E., Links, M.G., Karcz, S., Regan, S., Wu, Y.-Y., Gruber, M.Y., et al. (2009). An archived activation tagged population of Arabidopsis thaliana to facilitate forward genetics approaches. BMC Plant Biol. *9*, 101.
32. Ríos, G., Lossow, A., Hertel, B., Breuer, F., Schaefer, S., Broich, M., Kleinow, T., Jásik, J., Winter, J., Ferrando, A., et al. (2002). Rapid identification of Arabidopsis insertion mutants by non-radioactive detection of T-DNA tagged genes. Plant J. *32*, 243–253.
33. Cai, X., Dong, F., Edelmann, R.E., and Makaroff, C.A. (2003). The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm

cohesion and homologous chromosome pairing. J. Cell Sci. *116*, 2999–3007.

34. Bhatt, A.M., Lister, C., Page, T., Fransz, P., Findlay, K., Jones, G.H., Dickinson, H.G., and Dean, C. (1999). The DIF1 gene of Arabidopsis is required for meiotic chromosome segregation and belongs to the REC8/RAD21 cohesin gene family. Plant J. *19*, 463–472.
35. Chelysheva, L., Diallo, S., Vezon, D., Gendrot, G., Vrielynck, N., Belcram, K., Rocques, N., Márquez-Lema, A., Bhatt, A.M., Horlow, C., et al. (2005). AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. J. Cell Sci. *118*, 4621–4632.
36. Liu, Z., and Makaroff, C.A. (2006). Arabidopsis separase AESP is essential for embryo development and the release of cohesin during meiosis. Plant Cell *18*, 1213–1225.
37. Talbert, P.B., Masuelli, R., Tyagi, A.P., Comai, L., and Henikoff, S. (2002). Centromeric localization and adaptive evolution of an arabidopsis histone H3 variant. Plant Cell *14*, 1053–1066.
38. Sessions, A., Burke, E., Presting, G., Aux, G., McElver, J., Patton, D., Dietrich, B., Ho, P., Bacwaden, J., Ko, C., et al. (2002). A high-throughput Arabidopsis reverse genetics system. Plant Cell *14*, 2985–2994.
39. Van Leene, J., Stals, H., Eeckhout, D., Persiau, G., Van De Slijke, E., Van Isterdael, G., De Clercq, A., Bonnet, E., Laukens, K., Remmerie, N., et al. (2007). A tandem affinity purification-based technology platform to study the cell cycle interactome in Arabidopsis thaliana. Mol. Cell. Proteomics *6*, 1226–1238.
40. Van Leene, J., Witters, E., Inzé, D., and De Jaeger, G. (2008). Boosting tandem affinity purification of plant protein complexes. Trends Plant Sci. *13*, 517–520.
41. Van Leene, J., Eeckhout, D., Persiau, G., Van De Slijke, E., Geerinck, J., Van Isterdael, G., Witters, E., and De Jaeger, G. (2011). Isolation of transcription factor complexes from Arabidopsis cell suspension cultures by tandem affinity purification. Methods Mol. Biol. *754*, 195–218.
42. Van Leene, J., Hollunder, J., Eeckhout, D., Persiau, G., Van De Slijke, E., Stals, H., Van Isterdael, G., Verkest, A., Neirynck, S., Buffel, Y., et al. (2010). Targeted interactomics reveals a complex core cell cycle machinery in Arabidopsis thaliana. Mol. Syst. Biol. *6*, 397.
43. Heyman, J., Van den Daele, H., De Wit, K., Boudolf, V., Berckmans, B., Verkest, A., Alvim Kamei, C.L., De Jaeger, G., Koncz, C., and De Veylder, L. (2011). Arabidopsis ULTRAVIOLET-B-INSENSITIVE4 maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. Plant Cell *23*, 4394–4410.
44. Salic, A., Waters, J.C., and Mitchison, T.J. (2004). Vertebrate shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis. Cell *118*, 567–578.
45. Pesin, J.A., and Orr-Weaver, T.L. (2008). Regulation of APC/C activators in mitosis and meiosis. Annu. Rev. Cell Dev. Biol. *24*, 475–499.
46. Kevei, Z., Baloban, M., Da Ines, O., Tiricz, H., Kroll, A., Regulski, K., Mergaert, P., and Kondorosi, E. (2011). Conserved CDC20 cell cycle functions are carried out by two of the five isoforms in Arabidopsis thaliana. PLoS ONE *6*, e20618.
47. Nasmyth, K. (2011). Cohesin: a catenase with separate entry and exit gates? Nat. Cell Biol. *13*, 1170–1177.
48. Vignard, J., Siwiec, T., Chelysheva, L., Vrielynck, N., Gonord, F., Armstrong, S.J., Schlögelhofer, P., and Mercier, R. (2007). The interplay of RecA-related proteins and the MND1-HOP2 complex during meiosis in Arabidopsis thaliana. PLoS Genet. *3*, 1894–1906.
49. Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., Toyooka, K., Matsuoka, K., Jinbo, T., and Kimura, T. (2007). Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. J. Biosci. Bioeng. *104*, 34–41.
50. Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. *16*, 735–743.
51. Azumi, Y., Liu, D., Zhao, D., Li, W., Wang, G., Hu, Y., and Ma, H. (2002). Homolog interaction during meiotic prophase I in Arabidopsis requires the SOLO DANCERS gene encoding a novel cyclin-like protein. EMBO J. *21*, 3081–3095.
52. Chelysheva, L., Grandont, L., Vrielynck, N., le Guin, S., Mercier, R., and Grelon, M. (2010). An easy protocol for studying chromatin and recombination protein dynamics during Arabidopsis thaliana meiosis: immunodetection of cohesins, histones and MLH1. Cytogenet. Genome Res. *129*, 143–153.