The Kinesin AtPSS1 Promotes Synapsis and is Required for Proper Crossover Distribution in Meiosis

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Abstract

Meiotic crossovers (COs) shape genetic diversity by mixing homologous chromosomes at each generation. CO distribution is a highly regulated process. CO assurance forces the occurrence of at least one obligatory CO per chromosome pair, CO homeostasis smoothes out the number of COs when faced with variation in precursor number and CO interference keeps multiple COs away from each other along a chromosome. In several organisms, it has been shown that cytoskeleton forces are transduced to the meiotic nucleus via KASH- and SUN-domain proteins, to promote chromosome synapsis and recombination. Here we show that the Arabidopsis kinesin AtPSS1 plays a major role in chromosome synapsis and regulation of CO distribution. In Atpss1 meiotic cells, chromosome axes and DNA double strand breaks (DSBs) appear to form normally but only a variable portion of the genome synapses and is competent for CO formation. Some chromosomes fail to form the obligatory CO, while there is an increased CO density in competent regions. However, the total number of COs per cell is unaffected. We further show that the kinesin motor domain of AtPSS1 is required for its meiotic function, and that AtPSS1 interacts directly with WIP1 and WIP2, two KASH-domain proteins. Finally, meiocytes missing AtPSS1 and/or SUN proteins show similar meiotic defects suggesting that AtPSS1 and SUNs act in the same pathway. This suggests that forces produced by the AtPSS1 kinesin and transduced by WIPs/SUNs, are required to authorize complete synapsis and regulate maturation of recombination intermediates into COs. We suggest that a form of homeostasis applies, which maintains the total number of COs per cell even if only a part of the genome is competent for CO formation.

Introduction

During meiosis, chromosomes inherited from the mother and father are mixed in a process termed homologous recombination, to generate unique chromosomes that will be transmitted to the next generation. This genetic mixing has sustained the evolution of eukaryotes. There are typically one to four exchange points – crossovers (COs) – between homologous chromosomes at each meiosis. The distribution of these COs is under a series of constraints [1,2]. First, there is at least one CO per chromosome pair (obligatory CO or CO assurance). Indeed, beyond their genetic consequences, COs are also essential for holding homologous chromosomes together during meiosis I, ensuring their balanced distribution in daughter cells. Notably, a lack of or improper positioning of this obligatory CO causes aneuploidy in human oocytes [3]. Second, COs are subject to interference. This prevents the occurrence of COs next to each other, shaping an even distribution and limiting their number [4]. COs are also under homeostasis, meaning that their number tends to be stable even when faced with variation in precursor number [5–7]. Finally, looking at frequencies, COs are not homogenously distributed along the genome; hot and cold regions have been defined at the chromosome scale, and hotspots with a very high CO frequency have been observed at the kb scale [8,9].

COs are produced during meiotic prophase I concomitantly with and functionally connected to chromosome pairing and synapsis, which is the intimate association of homologous chromosomes lengthways with a protein structure, the synaptonemal complex (SC). Recombination is initiated at early prophase I by the formation of DNA double-strand breaks (DSBs) which largely outnumber the eventual CO number [10]. DSBs are subsequently resected to yield 3’ overhangs that invade the homologous chromosome, a step in which the recombinase DMC1 plays a prominent role [11]. In plants, as in mammals and budding yeast, these early steps of recombination also promote homologous chromosome synopsis. Indeed mutants affected in DSB formation or homologous template invasion (including Atdm1) fail in both synopsis and CO formation [12–

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repair. However, the prevalence of such sister-mediated repair in Arabidopsis thaliana reveals here that the AtPSS1 gene is required for proper localization of these homologous recombination events along the genome. We also show that AtPSS1, which belongs to a family of proteins able to move along the cytoskeleton, is likely part of a module that allows cytoplasmic forces to be transmitted through the nuclear envelope to promote chromosome movements during homologous recombination progression. 

### Results

**AtPSS1 is required for full synopsis and bivalent formation in meiosis**

A previous report showed that mutation of the rice class I kinesin I (named OsPSS1) leads to meiotic defects [38]. Reciprocal BLAST analysis and comprehensive sequence analysis of plant kinesins [39] unambiguously identified the product encoded by the *Arabidopsis* At3g63480 gene as the only putative orthologue of OsPSS1. The two proteins share high amino acid sequence identity (59%). We identified three T-DNA insertion lines from the public collections: Atpps1-1, Atpps1-2 and Atpps1-3. Insertion of the DNA in these loci was confirmed by sequencing the flanking sequences (Figure 1). Homozygous plants for all three lines have the same phenotype: normal vegetative growth but decreased fertility, as shown by reduced seed set (55 ± 6 seeds per silique for wild type versus 27 ± 5 for *Atpps1-1*) and reduced pollen viability (Alexander staining, Figure S1). Heterozygote plants for two *Atpps1* mutations had the same phenotype showing that the three mutants are allelic. Transformation of the *Atpps1-1* mutant with a 5 kb genomic region containing the *AtPSS1* coding and regulatory sequences restored pollen viability (7 independent transformants, Figure S1), confirming that the observed defects are due to disruption of the *AtPSS1* gene.

We used chromosome spreads to investigate male meiosis defects in the *Atpps1-1* mutant. Wild-type *Arabidopsis* meiosis was described in detail in [40], and the major stages are summarized in

### Author Summary

In species that reproduce sexually, diploid individuals have two copies of each chromosome, inherited from their father and mother. During a special cell division called meiosis, these two sets of chromosomes are mixed by homologous recombination to give genetically unique chromosomes that will be transmitted to the next generation. Homologous recombination processes are highly controlled in terms of number and localization of events within and among chromosomes. Disruption of this control (a lack of or improper positioning of homologous recombination events) can cause deleterious chromosome associations in the offspring. Using the model plant *Arabidopsis thaliana* we reveal here that the AtPSS1 gene is required for proper localization of these homologous recombination events along the genome. We also show that AtPSS1, which belongs to a family of proteins able to move along the cytoskeleton, is likely part of a module that allows cytoplasmic forces to be transmitted through the nuclear envelope to promote chromosome movements during homologous recombination progression.
Figure 2. At leptotene chromosomes appear as thin threads (Figure 2A), synapsis (the close association of two chromosomes via an SC) begins at zygotene and is complete by pachytene (Figure 2B). The SC is then depolymerized at diplotene and chromosomes condense so that the five bivalents are visible (pairs of homologues connected by COs) (Figure 2C). The bivalents align at metaphase I (Figure 2D), and chromosomes separate from their homologue at anaphase I leading to the formation of two pools of five chromosomes and two nuclei (Figure 2E). At the second meiotic division, the pairs of sister chromatids align on the two metaphase plates, and separate at anaphase II to generate four pools of five chromosomes, which gives rise to tetrads of four microspores (Figure 2F).

In Atpps1 mutants, leptotene and zygotene appeared similar to those in wild type (Compare figure 2A and 2G). Accordingly immunolocalization of two axial element proteins, ASY3 [41] and the homard domain containing protein ASY1 [42] did not reveal any difference between Atpps1 and wild type (Figure S2). However we were unable to find a typical pachytene stage among chromosome spreads of the Atpps1 mutant (n>300), as only partial synapsis was observed (Figure 2H). Synapsis was further examined by immunolocalization of REC8 and ZYP1 [43], which are chromosome axis and SC central element proteins, respectively (Figure 3). In flower buds whose size corresponds to late pachytene/diplotene stages, most wild-type cells showed almost complete synapsis, with the ZYP1 signal covering completely the REC8 signal (Figure 3A). In contrast, we were unable to find any meiocytes in which SC had undergone complete polymerization (n=102) in Atpps1. Atpps1 cells showed various levels of incomplete synapsis (Figure 3B), ranging from 4 to 91%, with less than half of the REC8 axis being covered with ZYP1 signal in most cells (distribution shown on Figure 3C). The observed partial ZYP1 loading could be the result of either delayed synapsis or failure in completing synapsis. However, the observation of diplotene stages on the same slides favors the hypothesis of incomplete synapsis (see also below).

At diakinesis and metaphase I, a mixture of univalents and bivalents (on average 3.1±1.2 bivalents and 1.9±1.2 univalent pairs) was observed in each Atpps1 allele (Figure 4), contrasting with wild type which always has five bivalents (Figure 2D, J and 5). FISH experiments using probes directed against 45S, 5S rDNA and the Fij2 BAC that allow the identification of the five Arabidopsis Col-0 chromosomes as described in [44], suggested that each chromosome is affected in bivalent formation. (The univalent frequency for chromosomes 1 to 5 were respectively 28%, 37%, 42%, 42% and 26%. N=43 Atpps1-1 cells). The presence of univalents resulted in missegregation of chromosomes in anaphase I and a subsequent aberrant number of daughter cells and/or unbalanced chromosome distribution (Figure 2K, L). Overall, our results showed that AtPSS1 is required for full synapsis and normal levels of bivalent formation at male meiosis. Observation of pistils [45], showed that 52% of the Atpps1 female gametophytes were defectsives (n=150). Further, univalents were detected at metaphase I of female meiosis (Figure S3), showing that AtPSS1 is essential for normal levels of bivalent formation in both male and female meiocytes.

The AtPSS1 mutation affects CO distribution but not frequency

The presence of bivalents in Atpps1-1 implies that CO formation is not completely impaired in this mutant. The nature of the COs produced in the absence of AtPSS1 was investigated by epistasis tests with zmm and mus81 mutants, which are defective in class I and class II CO formation, respectively. Mutation of a ZMM in Atpps1 reduced bivalent formation from 3.1±1.2 to 0±0,4, showing that most of the COs produced in the Atpps1 mutant are ZMM dependent. We then used MLH1 immunolocalization, a marker of class I COs, to explore CO distribution in Atpps1. The total number of MLH1 foci per cell during diplotene and diakinesis was similar in Atpps1 (11.9±2.7 and 10.2±2.3) and wild type (11.1±1.7and 10.5±1.3) (Figure 5). However, we found that the distribution of MLH1 foci among chromosomes was significantly affected in the Atpps1 mutant, as shown in figure 5. In wild type, 62% of the bivalents had exactly two MLH1 foci, 20% had three, 15% had one and less than 3% had four foci. In contrast, the number of MLH1 foci per chromosome was much more variable in Atpps1, with the appearance of classes not observed in wild type (Figure 5E). One quarter of the chromosome pairs appeared as univalents without MLH1 foci, fitting with the observed frequency of univalents at metaphase I, while bivalents with more than three foci were more frequent than in wild type (19.4% vs 2.7%). This suggests that CO distribution but not frequency is affected in Atpps1. Measurements of recombination rates in six genetic intervals using pollen tetrad analysis [46] showed that CO frequency is not reduced but even slightly higher in Atpps1 (Figure 6, Table S1a and Table S1b). CO interference, measured genetically, was significantly reduced compared to wild type, to a level no longer detected (Table S1b). While we cannot formally exclude that a low level of interference exists, this clearly establish that CO interference measured genetically is decreased in Atpps1. This further suggests that relative CO distribution is disturbed in Atpps1.

In Atpps1, synapsis and DSBs maturation into COs occur in the same regions

Overall, the above data showed that synapsis is incomplete and CO distribution among chromosomes is affected in Atpps1 mutants. As both synapsis and COs are promoted by DSB formation and repair, we carried out immunolocalization studies with DMC1, a protein which marks DSBs undergoing repair. In Atpps1, DMC1 foci decorated all chromosome axes and their total number was higher compared to wild type (+37%. 204±6 vs 279±8, T-test p=3.5.10^-10), suggesting that in the mutant DSB formation is enhanced or that DMC1 foci accumulate due to slower turnover (Figure 7). Thus in the mutant DSBs appear to occur on all chromosomes. We then examined whether the chromosome regions where COs occurred and that synapsed were the same. Because synapsis disappears before MLH1 foci numbers peak in Arabidopsis [47], we used HEI10/ZYP1 co-immunolocalization to explore this question (Figure 8 and S4). Indeed, HEI10 marks recombination progression from numerous faint foci at leptotene (Figure 8A) to about ten large foci labeling class I CO sites from late pachytene (Figure 8C) to diakinesis (Figure S4) [48]. At leptotene, Atpps1 and wild-type cells were indistinguishable with numerous small HEI10 foci (Figure 8A and 8D), further suggesting that early recombination events are unaffected in the mutant. At early wild type pachytene, numerous foci of variable
size are dispersed on the SC (Figure 8B). At the same stage in Atpps1, the synapsed regions were also decorated with numerous HEI10 foci, but the regions that failed to synapse were foci-free. At late pachytene, a small number of bright and homogeneous foci were observed in both wild type and the mutant (Figure 8C, F and G). Remarkably, while the total length of the SC in Atpps1 pachytene cells was on average one third that of wild type, confirming partial synapsis, the average number of HEI10 foci per cell was unaffected (Wild type: 10.3 ± 1.9, Atpps1: 11.2 ± 1.2, \( p = 0.19 \)) (Figure 8H). Accordingly, the number of HEI10 foci per 100 \( \mu m \) of SC was on average 3.1 ± 0.7 for wild type and 10.9 ± 4.8 for Atpps1 (these measurements were made on a cell per cell basis, because the entanglement of Arabidopsis pachytene chromosomes makes it difficult to unambiguously follow individual SCs). While the density of HEI10 foci was relatively stable in wild type (from 2 to 4.3 per 100 \( \mu m \)), it varied greatly in Atpps1 (from 4.3 to 23.6 per 100 \( \mu m \)) (Figure 8H). This is strikingly illustrated by the extreme case shown in figure 8G, where seven HEI10 foci can be seen on a single 30 \( \mu m \) SC stretch. At diplotene and diakinesis, the number of HEI10 foci per cell was similar and stable in the wild type and mutant. However, consistent with the MLH1 data, the distribution of HEI10 foci among chromosomes was significantly modified in Atpps1 (Figure S4), confirming that CO distribution but not number is affected. In summary, in Atpps1, COs and synapsis are jointly restricted to the same limited portion of the genome. Partial synapsis is accompanied by an increase in CO density per SC unit, resulting in –or caused by (see discussion)– an unaffected number of COs per cell.

MUS81-dependant COs in Atpps1

The MUS81 pathway (Class II pathway) is minor in Arabidopsis wild type. Its disruption reduces CO frequency by \( \sim 10\% \), but does not affect bivalent formation \([49,50]\) (Figure 4). Mutation of MUS81 in the Atpps1 background did not further reduce bivalent frequency (Figure 4), which is consistent with the conclusion above that most COs are ZMM-dependent in Atpps1. At FANCM was previously shown to limit MUS81-dependant CO formation and bivalent formation is fully restored in zmm/Atfancm mutants due to a massive increase in class II COs \([22]\). Mutation of AtFANCM in Atpps1 did not increase the number of bivalents, suggesting that it did not restore CO formation in regions that are CO incompetent in the single Atpps1 mutant (but this does not exclude that there is an increase in CO frequency in regions that are CO competent) (Figure 4). However while bivalent formation was very low in Atpps1 Atzip4, bivalent formation was restored in the Atpps1 Atfancm Atzip4 triple mutant back to the level observed in the single Atpps1 mutant (Figure 4). Altogether, these results suggest that, in Atpps1, class II COs occur at a low frequency, and can be promoted by mutating AtFANCM but exclusively in regions that are already competent for class I CO formation.

A potential AtPSS1-SUNs-WIPs force transduction module

AtPSS1, which belongs to the kinesin family, appears to play a crucial role in meiosis. Kinesin proteins are characterized by their ability to walk on microtubules via a motor domain that uses ATP to
promote repetitive conformation changes [51]. We thus tested if the motor function of AtPSS1 is important for its function in meiosis. For this, we expressed an AtPSS1 protein modified in the conserved arginine (Arg-293\textsuperscript{HIs}) that was previously shown to abolish the microtubule-stimulated ATPase activity [38] in the Atpss1-1 mutant. When Atps1 plants were transformed with the control wild-type AtPSS1 gene, pollen viability and bivalent formation at metaphase I were fully restored (7 independent transformants). In contrast, transformation with AtPSS1-R293H, expressed behind the native AtPSS1 promoter, did not restore pollen viability and normal meiosis (4 independent transformants, see methods; Bivalent frequency: 4.2±1 \textit{p} = 0.035 (n = 35), 4.3±0.5 \textit{p} = 0.04 (n = 35), 3.5±0.6 \textit{p} = 0.21 (n = 4), 3.5±1.2 \textit{p} = 0.03 (n = 15), respectively), showing that the kinesin function of AtPSS1 is critical for its role in meiosis. In several model species, cytoskeleton-based forces were previously shown to be important for meiosis and to be transduced to the nucleus by KASH- and SUN-domain containing proteins [24,25].

In Arabidopsis, two SUN proteins were recently shown to be redundant and important for meiosis (S.J.A. under review). As in Atps1, a mixture of bivalents and univalents are observed in Atsun1 Atsun2 double mutants. This defect is quantitatively identical in the Atps1, Atsun1 Atsun2 and the Atsun1 Atsun2 Atps1 triple mutants (Figure 4), suggesting that SUN proteins and AtPSS1 may act in the same pathway. WIP1-3 proteins were also recently identified as KASH containing proteins in Arabidopsis, and shown to interact with SUNs [52]. This raised the possibility that AtPSS1 could be involved in transmitting forces to the meiotic nucleus via a WIP-SUN module. Yeast two-hybrid experiments showed that AtPSS1 interacts directly with WIP1 and WIP2. The AtPSS1-WIP1 but not the AtPSS1-WIP2 interaction was confirmed by BiFC assays (Figure S5). The yeast two-hybrid also confirmed that WIPs interact with SUNs, as previously shown [52] (Figure 9).

Discussion

During meiotic prophase I, chromosome movements within the intact nucleus are prominent and have been shown to be involved in...
chromosome pairing, synapsis and recombination in a variety of species. Here we showed that AtPSS1, the Arabidopsis kinesin-1 like protein [39], is essential for full synapsis and is required for proper CO distribution. Furthermore, the bivalent shortage is identical when AtPSS1, SUNs or both, are knocked out suggesting that SUNs and AtPSS1 act in the same pathway to regulate CO formation. In addition, AtPSS1 interacts with the KASH-domain proteins WIP1 and WIP2 which themselves interact with SUN proteins [52]. Finally, we showed that the kinesin motor domain of AtPSS1 is required for its meiotic function. Kinesin is a motor protein which walks along microtubules with high processivity and for long distances (reviewed in [51]). We thus speculate that AtPSS1 moves...
along microtubules and generates forces that are transduced via a SUN-WIP module through the nuclear membrane to the chromosomes, promoting synapsis and regulating CO distribution (see below). The proteins that would connect SUNs to the chromosome telomeres remain to be identified. These results add to a growing amount of evidence showing that the transduction of cytoplasmic forces through the nuclear membrane is an important and conserved promoter of meiotic recombination. It should be noted here that the function we propose for AtPSS1 appears to be fulfilled by dynein in many organisms, and that dynein is absent from flowering plant genomes [53]. The rice PSS1 is also essential for normal meiosis [38]. Even though recombination and synapsis have not been extensively analyzed in the rice Osps1 mutants, univalent were observed at metaphase I, suggesting that the primarily defects may be similar to Atps1. This suggests that the meiotic function of AtPSS1 is conserved among flowering plants.

We showed that AtPSS1 is required for full synapsis and normal CO formation. In most species, the search for homologous sequences by recombinase-coated 3'-ssDNA promotes both CO formation and homologous synapsis. Indeed, in Arabidopsis both COs and synapsis are absent in mutants affecting DSB formation, but also homologous sequence invasion (RAD51, DMC1 and their co-factors) [12,14,19]. This appears to be a cooperative process as multiple repair events are required for initiation and progression of synapsis [54,55]. Atpss1 mutants have a novel defect: in each cell, COs and synapsis take place on only a subset of the genome (which varies from 10 to 90%). Initial DSB formation and processing do not appear to be involved in these defects, as DMC1 foci and early HEI10 are present on all chromosomes in the mutant. The number of DMC1 foci was higher in the mutant than wild type, possibly reflecting a delay in recombination progression. The increased number of DMC1 foci may also reflect an increase of the number of DSBs in response to the downstream defects [56]. However, we suggest that only a subset of these DSBs is efficiently matured into potential CO precursors and promoters of synapsis. This is supported by the observation that the segments of chromosomes which were seen to synapse were also the places where early HEI10 foci progressively matured into intermediate and then late CO-marking-foci. This model implies that chromosome movement involving AtPSS1 is required to efficiently mature DMC1-coated-DSBs into CO/synapsis precursors. This movement could be simply required for the homology searching DNA “tentacle” [57] to reach the homologous chromosome which can be at some distance in the nucleus [50]. Alternatively, the movement may be required to resolve the entanglement/clutter/interlocking which likely arises from multiple chromosome pairing attempts in the limited space of the nucleus [55]. The DSBs present on the portions of chromosomes which failed to reach homologues are likely repaired using the sister chromatids as template, thus failing to promote synapsis and homologous CO. Such sister-mediated repair occurs genome-wide in haploid Arabidopsis, where DMC1-coated resected DSBs are repaired on the sister, or in diploid mutants where DMC1 or one of its partners is absent [12–14,59].

Figure 6. Genetic recombination in wild type and Atpss1-1. Genetic distances in six intervals using tetrad analysis with fluorescent-tagged lines (FTL), were calculated with the Perkins equation [67] and are given in centiMorgans. l1b and l1c are adjacent intervals on chromosome 1 and so on for the other pairs of intervals as described in (Table S1). doi:10.1371/journal.pgen.1004674.g006

Figure 7. DMC1 immunolocalization. Immunolocalization of DMC1 at early zygotene is shown (A) in wild type and (B) in Atpss1-1. Cells were prepared according to Armstrong et al. [42] Scale bar = 10 μm. (C) Scatter plot of DMC1 foci number per cell. doi:10.1371/journal.pgen.1004674.g007
One intriguing feature of the *AtPSS1* mutant is that CO frequency per cell is not reduced, but instead the subparts of the chromosomes that do synapse and recombine make a similar total number of COs per cell as in wild type. This is strikingly shown in figure 8G, where a single SC stretch was formed in a cell on which seven class I COs occurred, while CO number rarely exceeds four on an entire wild-type chromosome. The smaller size of the competent regions appears to be compensated by an increased CO density, which implies that interference is no longer acting or that the distance at which interference spreads is reduced. Unfortunately, the difficulty in following individual SCs prevented us from cytologically measuring CO interference. The stable number of COs per cell in *AtPSS1* could reflect a form of CO homeostasis, which is defined as the tendency to preserve CO number despite a variation in DSB number through a modulation of the probability for DSBs to become COs [5]. We suggest that such homeostasis applies in the *AtPSS1* mutant, and that the decrease in the number of CO-competent DSB is compensated for by an increased probability of the eligible DSB becoming a CO. It is possible that the total number of COs per cell is defined, and then ~10 COs per cell occurs on licensed regions. However, the mechanism that would count the number of COs per cell remains elusive. Alternatively, we suggest that a feedback loop could sense some unachieved event (e.g. the presence of chromosomes lacking COs, or incomplete synopsis), and then increase the propensity of precursors to be designated for CO. This feed-back loop would therefore modulate the parameters of interference (possibly by a progressive increase in CO-promoting mechanical stress or progressive increase in the sensitivity of precursors to this stress [60,61]). Finally, *AtPSS1* could have a dual function, on one hand promoting synopsis and recombination intermediate maturation, and on the other preventing an excess of COs on selected regions, both via chromosome movement [24].

**Materials and Methods**

**Plant material**

Col-0 lines were obtained from the collection of T-DNA mutants from the Salk Institute Genomic Analysis Laboratory (Columbia accession) [SIGnAL, http://signal.salk.edu/cgi-bin/tdnaexpress] and provided by NASC [http://nasc.nott.ac.uk/]. Mutant alleles used in this study were: *Atmsh5-2* (SALK_026553) [62]; *Atzip4-2* (SALK_068052) [63]; *Atmus81-2* (SALK_107515) [49,50], *Aflancm-1* [22]; *Atsun1* (SAIL_84_G10); *Atsun2* [24].
Yeast two-hybrid and BIFC assays

The AtPSS1, AtWIP1, AtWIP2, AtWIP3, AtSUN1 and AtSUN2 open reading frames were amplified from Arabidopsis cDNA clones (Columbia ecotype) using specific primers flanked by the AttB1 and AttB2 sites (Table S2), cloned into Gateway vector pDONR207 using BP recombination (Invitrogen), and sequenced. Expression vectors were obtained after LR recombination (Invitrogen) between these entry vectors and destination vectors (pGADT7-GW and pGBK7-3-GW for Y2H, and pBiFP vectors for BIFC). Yeast two-hybrid interactions were tested using AtPSS1, AtWIP1, AtWIP2, AtWIP3, AtSUN1 and AtSUN2 as bait (pGADT7-GW) or as prey (pGBK7-3-GW) by matching with the AH109 and Y187 yeast strains. For fluorescence complementation tests, transient expression of all eight compatible combinations between protein pairs (i.e., providing both parts of the YFP) was assayed. Each expression vector was introduced into Agrobacterium tumefaciens strain C58C1(pMP90) by electroporation. Agrobacterium bacterial cultures were incubated overnight at 28°C with agitation. Each culture was pelleted, washed, and resuspended in infiltration buffer (13 g/L borturage N2 medium [Duchefa Biochemie] and 40 g/L sucrose, pH 5.7) to an OD600 of 0.5. The inoculum was delivered to the lamina tissue of N. benthamiana leaves by gentle pressure infiltration through the lower epidermis. To enhance transient expression of BIFC fusion proteins, the P19 viral suppressor of gene silencing was coexpressed [66]. YFP fluorescence was detected three days after infiltration. Tissue was mounted in low-melting-point agarose (0.4% in water) and viewed directly using an inverted Zeiss Observer Z1 spectral confocal laser microscope LSM 710 using a C-Apochromat ×63/1.20 W Corr objective (Carl Zeiss). Fluorescence was recorded after an excitation at 514 nm (Argon laser) and using a selective band of 514 to 568 nm.

Supporting Information

Figure S1 Pollen grain viability is affected in Atpps1. (A) Wild type. All the pollen grains appear viable. (B) Atpps1-1. A significant proportion of the pollen grains are dead (~30%). (C) Transformation of the Atpps1-1 mutant with a 5 kb genomic region containing the AtPSS1 gene restored pollen viability. Scale bar = 50 μm (TIF).

Figure S2 Immunolocalization of ASY1 and ASY3 at leptotene in wild type and Atpps1-1. Cells were prepared according to Armstrong et al. [42]. Scale bar = 10 μm. (TIF)

Figure S3 Female meiosis is affected in Atpps1. (A) Wild-type ovule containing a meiocyte at metaphase I. Five bivalent are aligned on the metaphase plate. (B) An Atpps1 ovule at the same stage. Two bivalents are aligned
on the metaphase plate and six univalents are scattered in the meioocyte. b = bivalent, u = univalent. Scale bar = 10 μm. (TIF)

Figure S4 Immunolocalization of HEI10 at diakinesis. Immunolocalization of HEI10 at diakinesis is shown (A) in wild type and (B) in Atpss1-1. (C, D) Scatter plot of HEI10 foci number per cell at diplotene and diakinesis. (E) Distribution of chromosomes according to their HEI10 foci number at diakinesis. Cells were prepared according to Chehlysheva et al. [47]. Scale bar = 10 μm. (TIF)

Figure S5 AtPSS1 and AtWIP1 interact in BiFC. Nicotiana benthamiana cells were infiltrated with different combinations of split YFP fusions with AtPSS1 and AtWIP1. (A) Co-expression of benthamiana cells were infiltrated with different combinations of split YFP fusions with AtPSS1 and AtWIP1. (A) Co-expression of BiFC constructs YFP5-AtWIP1 and YFP2-AtPSS1 gave a clear cytoplasmic YFP fluorescence signal, revealing interaction between AtWIP1 and AtPSS1. (B, C) Negative controls correspond to co-expression of YFP C-AtPSS1 with the unrelated YFP N-AtWIP1 and AtPSS1 with the unrelated YFP C-DEFICIENS protein. (D) Positive control corresponds to co-expression of YFP2-GLOBOSA with the YFP2-DEFICIENS protein. Scale bar = 50 μm. (TIF)

Table S1 Tetrad analysis. (A) Tetrad raw data set. The FTL system relies on transgenic markers conferring cyan, yellow or red fluorescence of pollen grains within tetrads. Drawings above each column represent the different distribution possibilities of markers among the four chromatids and the corresponding distribution of colors in the tetrad, according to the nomenclature of Berchowitz and Copenhaver [46]. For each pair of intervals (e.g. I1b and I1c are two adjacent intervals on chromosome 1) and each genotype the observed number of each type of tetrad is given. (B) Interference analysis. Interval-interaction interference was measured by comparing the genetic size of an interval (d, Perkins equation, cm) when a crossover occurs in an adjacent interval to the genetic size of the same interval when no crossover occurs in the adjacent interval. The ratio of these two distances, called the interference ratio (IR), gives a measurement of the strength of interference between two intervals [46] (e.g. IR^{IR} = d(EB2)/d(EB1) with CO in I2a)/ (d(EB2) without CO in I2a)). The more this interference ratio is inferior to 1, the stronger interference is. Using the raw data from Table S1A, calculations and statistical analyses have been performed according to Berchowitz and Copenhaver [46] and Suhlab Lab Online tools (http://www.molbio.uoregon.edu/~suhlab/). For the three pairs of interval tested, genetic CO interference was detected in wild type (IR<1). In Atps1, the IRs were not different from 1 and were statistically different from the wild-type IRs, showing that genetic CO interference is reduced or abolished in Atps1.

Table S2 PCR Primers used in this study. (DOCX)
47. Chelysheva L, Grandont L, Vrielynck N, le Guin S, Mercier R, et al. (2010) An
    46. Berchowitz LE, Copenhaver GP (2008) fluorescent Arabidopsis tetrads: a visual
    45. Motamayor JC, Vezon D, Bajon C, Sauvanet A, Grandjean O, et al. (2000)
    44. Chelysheva L, Vezon D, Belcram K, Gendrot G, Grelon M (2008) The
    43. Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FCH (2005)
    42. Zhou S, Wang Y, Li W, Zhao Z, Ren Y, et al. (2011) Pollen semi-sterility1
    41. Roberts NY, Osman K, Armstrong SJ (2009) Telomere distribution and
    40. Ross KJ, Fransz P, Jones GH (1996) A light microscopic atlas of meiosis in
    39. Richardson DN, Simmons MP, Reddy ASN (2006) Comprehensive comparative
    38. Zhou S, Wang Y, Li W, Zhao Z, Ren Y, et al. (2011) Pollen semi-sterility1
    37. Roberts NY, Osman K, Armstrong SJ (2009) Telomere distribution and
    36. Zickler D, Kleckner NE (1998) The leptotene-zygotene transition of meiosis.
    35. Koszul R, Kim KP, Prentiss M, Kleckner NE, Kameoka S (2008) Meiotic
    34. Ding X, Xu R, Yu J, Xu T, Zhuang Y, et al. (2007) SUN1 is required for
    33. Chikashige Y, Haraguchi T, Hiraoka Y (2007) Another way to move
    32. Scherthan H (2001) A bouquet makes ends meet. Nat Rev Mol Cell Biol 2: 621–
    31. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    30. Scherthan H, Weich S, Schwegler H, Heyting C, Ha¨rle M, et al. (1996)
    29. Shibuya H, Ishiguro K-I, Watanabe Y (2014) The TRF1-binding protein
    28. Sato A, Isaac B, Phillips CM, Rillo R, Carlton PM, et al. (2009) Cytoskeletal
    27. Chelysheva Y, Tsutsumi C, Yamane M, Okamatsu K, Haraguchi T, et al. (2006)
    26. Chikashige Y, Ding DQ, Funahachi H, Haraguchi T, Mashiko S, et al. (1994)
    25. Koszul R, Kim KP, Prentiss M, Kleckner NE, Kameoka S (2008) Meiotic
    24. Ding X, Xu R, Yu J, Xu T, Zhuang Y, et al. (2007) SUN1 is required for
    23. Chelysheva Y, Haraguchi T, Hiraoka Y (2007) Another way to move
    22. Scherthan H (2001) A bouquet makes ends meet. Nat Rev Mol Cell Biol 2: 621–
    21. Scherthan H, Weich S, Schwegler H, Heyting C, Ha¨rle M, et al. (1996)
    20. Sato A, Isaac B, Phillips CM, Rillo R, Carlton PM, et al. (2009) Cytoskeletal
    19. Zickler D, Kleckner NE (1998) The leptotene-zygotene transition of meiosis.
    18. Zhou X, Graumann K, Evans DE, Meier I (2012) Novel plant SUN-KASH
    17. Perkins DD (1949) Biochemical Mutants in the Smut Fungus Ustilago Maydis.
    16. Chelysheva L, Gendrot G, Vezon D, Doutriaux M-P, Mercier R, et al. (2007)
    15. Zickler D, Kleckner NE, HC (2003) Localization and roles of Ski1p protein in
    14. Thacker D, Mohihullah N, Zhu X, Kenney S (2014) Homologue engagement
    13. Shibuya H, Ishiguro K-I, Watanabe Y (2014) The TRF1-binding protein
    12. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    11. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    10. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    9. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    8. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    7. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    6. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    5. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    4. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    3. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    2. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)
    1. Scherthan H, Weich S, Schweger H, Hryting C, Harle M, et al. (1996)