Involvement of the Cohesin Cofactor PDS5 (SPO76) During Meiosis and DNA Repair in Arabidopsis thaliana

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Maintenance and precise regulation of sister chromatid cohesion is essential for faithful chromosome segregation during mitosis and meiosis. Cohesin cofactors contribute to cohesin dynamics and interact with cohesin complexes during cell cycle. One of these, PDS5, also known as SPO76, is essential during mitosis and meiosis in several organisms and also plays a role in DNA repair. In yeast, the complex Wapl-Pds5 controls cohesion maintenance and colocalizes with cohesin complexes into chromosomes. In Arabidopsis, AtWAPL proteins are essential during meiosis, however, the role of AtPDS5 remains to be ascertained. Here we have isolated mutants for each of the five AtPDS5 genes (A–E) and obtained, after different crosses between them, double, triple, and even quadruple mutants (Atpds5a Atpds5b Atpds5c Atpds5e). Depletion of AtPDS5 proteins has a weak impact on meiosis, but leads to severe effects on development, fertility, somatic homologous recombination (HR) and DNA repair. Furthermore, this cohesin cofactor could be important for the function of the AtSMC5/AtSMC6 complex. Contrarily to its function in other species, our results suggest that AtPDS5 is dispensable during the meiotic division of Arabidopsis, although it plays an important role in DNA repair by HR.

Keywords: Arabidopsis thaliana, cohesin cofactor, DNA repair, homologous recombination, Meiosis, PDS5, SPO76

INTRODUCTION

Cohesin is a ring-shaped protein complex which holds sister chromatids together to prevent their separation prior to anaphase. Genes coding cohesin subunits are evolutionarily conserved as are the general mechanism of action of the corresponding proteins. The cohesin complex is formed by four core components, a heterodimer of Structural Maintenance of Chromosome proteins (SMC1 and SMC3) and two non-SMC proteins. The non-SMC proteins are SCC3 (SA1-SA2/STAG1-STAG2), and a member of the conserved α-kleisin family: SCC1 (Mcd1/RAD21, known as SYN proteins in Arabidopsis thaliana, see below) (Nasmyth and Haering, 2009). In germ cells, meiosis-specific cohesin subunits have been characterized: SMC1β and STAG3, encoded by SCC3 homologues,

Abbreviations: BAR, Bio-Analytic Resource for Plant Biology; CDDP, cisplatin; CL, cross-linking; CO, crossover; DMF, N,N-Dimethylformamide; DSB, Double-Strand Break; FISH, fluorescence in situ hybridization; GM, germination medium; HR, homologous recombination; MMC, mitomycin C; MMS, methyl methanesulphonate; NASC, Nottingham Arabidopsis Stock Centre; PDS5, Precocious Dissociation of Sisters 5; PMC, pollen mother cell; SC, synaptonemal complex; SIAS, sequences identities and similarities; SMC, structural maintenance of chromosome; WAPL, Wings Apart-Like; WT, wild-type.
and the α-kleisin REC8 (SYN1 in *A. thaliana*) (Suja and Barbero, 2009). In addition to RAD21 and REC8, a third α-kleisin gene, named RAD21L, specifically expressed in meiotic cells, has been identified in vertebrates (Gutiérrez-Caballero et al., 2011; Herrán et al., 2011; Ishiguro et al., 2011; Lee and Hirano, 2011). The cohesin protein complex is not only essential for sister chromatid cohesion, but it is also involved in chromosome condensation, gene expression, development, DNA repair and HR (Hirano, 2006; Dorsett, 2007; Onn et al., 2008; Barbero, 2009; Aragon et al., 2013).

In addition to proteins mentioned above, several non-cohesin accessory proteins contribute to cohesion regulation, although they are not considered to be components of the canonical cohesin complex (Nasmyth, 2011). The adherin complex SCC2–SCC4 is involved in cohesin loading during early G1 in vertebrate cells and late G1 in yeast (Ciosk et al., 2000; Watrin et al., 2006; Hu et al., 2011). The establishment of cohesion also requires SMC3 acetylation by Eco1/Ctf7p in yeast and ESCO1 and ESCO2 in human cells (Skibbens et al., 1999; Tóth et al., 1999; Hou and Zou, 2005). In mammalian cells, SMC3 acetylation is necessary for the recruitment of Sororin to chromatin-bound cohesin complexes, a protein needed for maintaining cohesion during meiosis (Nasmyth, 2011). The adherin complex SCC2–SCC4 is involved in cohesin loading during early G1 in vertebrate cells and late G1 in yeast (Ciosk et al., 2000; Watrin et al., 2006; Hu et al., 2011). The establishment of cohesion also requires SMC3 acetylation by Eco1/Ctf7p in yeast and ESCO1 and ESCO2 in human cells (Skibbens et al., 1999; Tóth et al., 1999; Hou and Zou, 2005). In mammalian cells, SMC3 acetylation is necessary for the recruitment of Sororin to chromatin-bound cohesin complexes, a protein needed for maintaining cohesion during meiosis (Nasmyth, 2011). Sororin stabilizes cohesin on DNA by competing with the cohesin release factor WAPL (Gandhi et al., 2000; Kueng et al., 2006; Shintomi and Hirano, 2009). Sororin is a negative regulator of cohesion that interacts directly with the cohesin complex by binding α-kleisin (Neuwald and Hirano, 2000; Kueng et al., 2006; Shintomi and Hirano, 2009). Sororin also directly associates with PDS5 and thereby dissociates WAPL from PDS5, at least in vitro, implying that Sororin antagonizes WAPL by changing its interaction with PDS5 (Nishiyama et al., 2010). The interaction of PDS5 with either Sororin or WAPL could explain why this protein has both positive and negative effects on cohesion association.

Regarding the PDS5 function, there are also important differences among organisms. In *Saccharomyces cerevisiae*, Pds5p is essential for viability and is required to maintain sister chromatid cohesion and chromosome condensation (Hartman et al., 2000; Panizza et al., 2000; Stead et al., 2003; Tong and Skibbens, 2014). A recent study has determined that Pds5 in yeast maintains cohesion, at least in part, by antagonizing the polySUMO-dependent degradation of cohesin (D’Ambrosio and Lavoie, 2014). BIMD, encoded by the homolog of *PDS5* in *Aspergillus nidulans*, has also an important function in maintaining cohesion. Mutations in the *BIMD* gene result in mitotic arrest at anaphase and an increased sensitivity to DNA damaging agents (Denison et al., 1993). In contrast, *pds5*-null strains are viable in *Schizosaccharomyces pombe*, but a *pds5Δ* mutation confers cohesion defects after prolonged arrest in G2/M and increased chromosome loss rates (Tanaka et al., 2001; Wang et al., 2002). *Xenopus* eggs extracts depleted of both *PDSSA* and *PDSSB* (there are two *PDSS* genes in vertebrates) display an abnormal level of cohesin on chromosomes and altered centromeric cohesion (Sumara et al., 2000; Losada et al., 2005). Other studies have demonstrated that knockout mice for either *PDSSA* or *PDSSB* die at the perinatal age with several developmental anomalies that resemble those found in humans with Cornelia de Lange syndrome (Zhang et al., 2007, 2009). Recently, an analysis of primary mouse embryonic fibroblasts lacking *PDSSA*, *PDSSB*, or both has revealed that they contribute to telomere and arm cohesion. In addition, *PDSSB* is specifically required for centromeric cohesion (Carretero et al., 2013).

The meiotic function of PDS5 was initially described in *Sordaria macrospora*, where it was named SPO76 (Moreau et al., 1985; Huynh et al., 1986). In this species SPO76 is needed for normal meiotic chromosome morphogenesis. The spo76-1 mutant is defective in centromidal cohesion and chromosome compaction during prophase I, since chromatids are fully separated at diplotene. Meiotic recombination is also affected (van Heemst et al., 1999; Storlazzi et al., 2003). BIMD of *A. nidulans* is also required for cohesion and normal chromosome compactness during meiosis. However, in contrast to SPO76, it does not reveal defined axes during prophase I (van Heemst et al., 2001). In *Saccharomyces*, a meiosis-conditional *pds5* allele produces hypercondensed chromosomes and alterations in synopsis, DSB repair, and meiotic chromosome segregation (Jin et al., 2009). Cohesion defects were also observed during meiosis in *Caenorhabditis elegans* *elv-14/pds5* mutants. In this species, EVL-14 is not required for establishing sister chromatid cohesion but it is important for its maintenance (Wang et al., 2003).

In *A. thaliana*, single copy genes code for *AtSMC1*, *AtSMC3*, and *AtSCC3*. These proteins have been identified in both somatic and meiotic tissues (Liu et al., 2002; Chelysheva et al., 2005; Lam et al., 2005; Schubert, 2009). However, there are four SCC1 homologues: SYN1 (*DIF1*), SYN2 (*RAD21.1*), SYN3 (*RAD21.2*), and SYN4 (*RAD21.3*). Homozygous knockout mutants of any of these genes are viable, probably because of the functional redundancy of them. SYN1 is needed for meiotic cohesion, whereas SYN2 and SYN4 are mitotic α-kleisins, with SYN2 also playing a role in DNA repair (Bai et al., 1999; Bhatt et al., 1999; Dong et al., 2001; Cai et al., 2003; da Costa-Nunes et al., 2006; Jiang et al., 2007; Yuan et al., 2012). See also Schubert (2009) for a detailed description of SMC complexes in plants. Regarding non-cohesin accessory genes, as in yeast a Sororin ortholog has not been identified in *A. thaliana*. Mutations in *AtCTF7* cause embryo lethality, since *AtCTF7* is required for the establishment of sister chromatid cohesion and to avoid the premature dissociation of cohesin from chromosomes during meiosis (Jiang et al., 2010; Bolaños-Villegas et al., 2013; Singh et al., 2013). By contrast, plants without *AtWAPL* activity (there are two genes in *A. thaliana*) exhibit normal growth and development, but several defects during meiosis (De et al., 2014). Moreover, mutations in both *AtWAPL* genes suppress the lethality associated with inactivation of *AtCTF7*. The role of *AtPDS5* (there are five orthologs) and their possible involvement in *Arabidopsis* meiosis is still completely unknown. Here we report findings related to *AtPDS5* function, by analyzing the corresponding mutants. The results indicate that, contrary to *AtWAPL*, the absence of
AtPDS5 causes growth defects, hypersensitivity to DNA repair and a drastic reduction in HR, but only subtle meiotic alterations.

MATERIALS AND METHODS

Plant Material and Growth Conditions

*Arabidopsis thaliana* ecotype Columbia (0) was used for WT analysis. T-DNA lines corresponding to the five AtPDS5 genes were the following: AtPDS5A (SALK_114556), AtPDS5B (SALK_092843), AtPDS5C (SALK_013481), AtPDS5D (SALK_133849), and AtPDS5E (SAIL_287_D07). They were obtained from the Nottingham Arabidopsis Stock Centre (NASC1). Plants were grown on a soil mixture of vermiculite and commercial soil (3:1) with a light cycle of 16 h alternating with 8 h of darkness, at 20°C and 70% humidity.

In Silico Analysis

The program Clustal W2 was used for sequence alignment and to determine sequence identity between the proteins from different species (Larkin et al., 2007; McWilliam et al., 2013). The sequences were available in NCBI database. Scores for amino acid identity and similarity were retrieved from SIAS server2. The sequences were available in NCBIdatabase. Scores for amino acid identity and similarity were retrieved from SIAS server2. These sequences were available in NCBIdatabase. Scores for amino acid identity and similarity were retrieved from SIAS server2. These were obtained from the Nottingham Arabidopsis Stock Centre (NASC1). Plants were grown on a soil mixture of vermiculite and commercial soil (3:1) with a light cycle of 16 h alternating with 8 h of darkness, at 20°C and 70% humidity.

Interactions Viewer from the BAR3 was used to determine expression data with the experimental context variables anatomy and development (Hruz et al., 2008). The tool *Arabidopsis* Interactions Viewer from the BAR3 was used to determine AtPDS5 interacting proteins (Geisler-Lee et al., 2007).

Molecular Characterization of *AtPDS5* Mutants and AtPDS5 Expression Analyses

Genotyping of the different T-DNA lines and expression analyses were performed as previously described by Pradillo et al. (2012). Details of the primers used are given in Supplementary Tables S1 and S2. In the real time PCR expression was normalized against 18S rRNA and ACTIN2. 

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Cytological Procedures

Fixation, chromosome spread preparations of pollen mother cells (PMCs), fluorescence in situ hybridisation (FISH) and chiasma counts were carried out as described by Sánchez-Morán et al. (2001). Characteristics of 45S rDNA and 5S rDNA DNA probes are also provided in this reference.

Immunolocalization was performed by spreading as previously described (Armstrong et al., 2009). Primary antibodies used were: anti-ATZYP1 (rat; 1:500), anti-SYN1 (rabbit; 1:500), and anti-AtSMC3 (rabbit; 1:500) (Mercier et al., 2003; Higgins et al., 2005; Tjiang, 2010).

RESULTS

*Arabidopsis* Possesses Five *PDS5* Genes

According to previous results and database searches of putative *PDS5* genes in the *Arabidopsis* genome, five candidates were identified (Mercier et al., 2001; Figures 1A,C). The protein that displays the highest identity and similarity to *S. macrospora* sequences (SPO76, 15% identity and 27% similarity), *A. nidulans* (BIMD, 15% identity and 26% similarity) and *Mus musculus* (PDS5A, 22% identity and 30% similarity; and PDS5B, 22% identity and 32% similarity) is encoded by At5g47690. For this reason we designated it as AtPDS5A. The remaining proteins were named according to their identity (ranging from 31 to 23%) and similarity (ranging from 39 to 20%) respect to AtPDS5A: AtPDS5B (At1g77600), AtPDS5C (At4g31880),...
AtPDS5D (At1g80810) and AtPDS5E (At1g15940). AtPDS5D and AtPDS5E are the most similar to each other (53%). As well as PDS5 proteins from other organisms, according to UniProt database, all AtPDS5 proteins contain an Armadillo-type fold domain in the or near to the N-terminus (UniProt Consortium, 2015; Figure 1B). This domain presents an extensive solvent-accessible surface that promotes interactions with proteins and nucleic acids.

Characterization of Atpds5 Mutants

To unravel the function of the Arabidopsis PDS5 homologs, the NASC database was screened for lines containing a T-DNA insertion in the corresponding genes. Homozygous plants for the following lines were characterized: SALK_114556 (Atpds5a-1, T-DNA insertion located in the second exon), SALK_092843 (Atpds5b-1, T-DNA insertion located in the seventh exon), SALK_013481 (Atpds5c-1, T-DNA insertion located in the
double mutants were isolated in the F2 (Atpds5a Atpds5b seed set (and Atpds5b AtPDS5A and Atpds5e displayed normal fertility compared to WT plants, but Atpds5e proximity of AtPDS5 (obtain triple and quadruple mutant plants germinated normally without any obvious delay in growth during the first 2 weeks. Later, they displayed markedly smaller rosette sizes and were much shorter and less robust than WT throughout their life cycle (Figures 1D,E). Mutants also displayed early flowering and reduced fertility. The majority of triple and quadruple mutant plants bolted 20 days after sowing whereas WT plants did so 30 days after sowing. The reduction in fertility was not only due to a decrease in seed set (Table 1) but also to the presence of short siliques (Figure 1F, Supplementary Table S3). However, there were not differences between Atpds5a Atpds5b AtPDS5c and Atpds5e Atpds5b Atpds5c AtPDS5e neither with respect to average seed set nor silique length (p = 0.10 and p = 0.37, respectively).

Male Meiosis Displays Chromosome Bridges at Anaphase I in the Different Atpds5 Mutants
To determine whether meiosis defects could be responsible for the reduced fertility of Atpds5 mutants, we analyzed DAPI-stained chromosome spreads from PMCs. Analysis of PMCs in Atpds5 mutants revealed that meiosis proceeds without any important deviation from WT, even in the quadruple mutant (Supplementary Figures S3 and S4). We only detected, in contrast to the WT, the presence of some chromosome bridges at late anaphase I and telophase I in the single mutants, ranging from 20 to 25% (n = 40) (Figure 2). These bridges, originated probably as consequence of unresolved recombination intermediates, were also observed in the quadruple mutant with a similar frequency (22.5%; n = 40) and gave rise to fragments with a very low frequency (Figure 2).

Since sister chromatid cohesion is essential during meiotic recombination, we investigated whether the Atpds5 mutants are affected in CO formation. We used 5S and 45S rDNA as FISH probes to identify each chromosome and chromosome arm of the complement set (Sánchez-Morán et al., 2002; Supplementary Figure S5). We only detected a significant decrease in the mean cell chiasma frequency of Atpds5a Atpds5b AtPDS5c AtPDS5e with respect to WT (9.32 ± 0.25 vs. 10.20 ± 0.14; p = 0.004). This difference was due to a slight reduction in the number of chiasmata in chromosome 1, specifically in its long arm (1.16 ± 0.09 vs. 1.54 ± 0.06; p = 0.001).

AtSMC3 and SYN1 Localization Along Meiotic Chromosome Axes is Not Affected by the Absence of AtPDS5
To further investigate any effect on chromosome axis morphogenesis, chromosome spread preparations of Atpds5a Atpds5b AtPDS5c AtPDS5e PMCs were examined by using anti-AtSMC3 and anti-SYN1 antibodies in conjunction with an antibody against the synaptonemal cross filament protein, AtZYP1, to analyze the chronology of the early prophase I stages. AtZYP1 appears at early zygotene as short stretches which extend and produce a continuous signal between the synapsed homologous chromosomes at pachytene. AtSMC3 and SYN1 colocalize with chromosome axes during early prophase I. There were no obvious differences between WT and Atpds5a Atpds5b Atpds5c AtPDS5e (n = 20) (Figure 3). This suggests that

TABLE 1 | Comparison between Col and Atpds5 single, double, triple, and quadruple mutants respect to average seed set per silique.

<table>
<thead>
<tr>
<th></th>
<th>Seed set per silique</th>
<th>p-value</th>
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<tr>
<td>Col</td>
<td>51.53 ± 3.31</td>
<td>–</td>
</tr>
<tr>
<td>Atpds5a</td>
<td>54.33 ± 4.48</td>
<td>NS</td>
</tr>
<tr>
<td>Atpds5b</td>
<td>47.80 ± 3.73</td>
<td>**</td>
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<tr>
<td>Atpds5c</td>
<td>44.53 ± 5.50</td>
<td>***</td>
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<tr>
<td>Atpds5d</td>
<td>50.33 ± 4.47</td>
<td>NS</td>
</tr>
<tr>
<td>Atpds5e</td>
<td>47.67 ± 6.91</td>
<td>NS</td>
</tr>
<tr>
<td>Atpds5a Atpds5b</td>
<td>37.00 ± 3.59</td>
<td>***</td>
</tr>
<tr>
<td>Atpds5a Atpds5b Atpds5c</td>
<td>30.27 ± 4.73</td>
<td>***</td>
</tr>
<tr>
<td>Atpds5a Atpds5b Atpds5c Atpds5e</td>
<td>27.33 ± 4.84</td>
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Significance testing was carried out using the Student’s t-test. NS, not significant; **p < 0.01; ***p < 0.001.
FIGURE 2 | Loss of AtPDS5 function generates chromosome bridges at anaphase I and telophase I. Arrows indicate chromosome bridges, which probably arise because of the existence of unresolved recombination intermediates, and a fragment.

both proteins, AtSMC3 and SYN1, load normally on mutant chromosomes. Hence, AtPDS5 seems to be dispensable for meiotic cohesin complex formation.

AtPDS5 Proteins Play a Role During DNA Repair

Many proteins involved in sister chromatid cohesion are important for the maintenance of genome integrity and repair of DNA damage during the cell cycle (da Costa-Nunes et al., 2006; Bolaños-Villegas et al., 2013). To assess whether AtPDS5 plays a similar role, we tested Atpds5 mutants for hypersensitivity to γ-irradiation, a DSB-inducing agent, and the CL agents MMC, which mainly produces inter-strand CLs (Rink et al., 1996), and CDDP, which preferentially forms intra-strand CLs (Eastman, 1985; Boulikas and Vougiouka, 2003). Although both types of CL agents induce partially different types of DNA damage, both are expected to create DSBs during DNA synthesis, which are mostly repaired by HR. A hypersensitive response to γ-rays was consistently observed in the double, triple and quadruple mutants when compared with WT (Figure 4). Triple and quadruple mutants also showed hypersensitivity to MMC (Supplementary Figure S6). Finally, only the quadruple mutant revealed higher sensitivity than WT to high CDDP doses (Supplementary Figure S7). The global assessment of these results indicates that the quadruple mutant is more sensitive to DNA damage than the triple mutant, which in turn is more sensitive than the double mutant. Therefore, AtPDS5 genes are involved in DNA damage response and the function of the different AtPDS5 genes in DSB repair seems to be non-redundant.

Alterations in the Expression of Several Genes Denote the Function of AtPDS5 Genes in DNA Damage Response

Exposure of plants to DSB-inducing agents increases transcript levels of genes involved in DNA repair (Culligan et al., 2006). For this reason, we tested by qPCR the expression levels of the different AtPDS5 genes after γ-irradiation treatment. The results obtained revealed increased levels in the expression of these genes at 300 and 500 Gy in leaf tissue (Figure 5A). The most noteworthy increase was observed in AtPDS5E at 500 Gy, the expression of this gene was 10-fold higher than in the untreated control. However, no significant change was observed for the expression of AtPDS5 genes (except a slight increase for AtPDS5E) using samples from buds, containing meiocytes (Supplementary Figure S8A). We also investigated whether the loss of AtPDS5 genes alters the expression of genes required for DNA repair, which includes the kinases AtATM and AtATR, AtRAD50, the ubiquitin ligase AtBRCA1, the recombinase AtRAD51 and the SMC genes AtSMC6A and AtSMC6B (Gallego et al., 2001; Daoudal-Cotterell et al., 2002; Lafarge and Montané, 2003; Li et al., 2004; Culligan and Britt, 2008; Watanabe et al., 2009; Amiard et al., 2010; Pradillo et al., 2012). Small and unremarkable changes (down and up) were observed for AtATR, AtRAD50, AtBRCA1, and AtRAD51 expression in leaf samples. AtATM exhibited a reduced expression in both the triple and the quadruple mutants and all these genes were down-regulated in bud samples (Supplementary Figure S9). However, the most significant result was observed for AtSMC6A and AtSMC6B since the transcripts of these genes in the triple and quadruple mutants were halved and reduced by more than half, respectively.
FIGURE 3 | Meiotic chromosome axes are normal in *Atpds5a Atpds5b Atpds5c Atpds5e* as revealed by immunolocalization of AtSMC3 and SYN1 at pachytene meiocytes.

*Pradillo et al.* AtPDS5 involvement during homologous recombination

(Figure 5B). This reduction was also observed in bud samples (Supplementary Figure S8B).

**The Loss of AtPDS5 Genes Affects Homologous Recombination in Somatic Cells**

Taking into account the DNA repair defects and the alterations in the expression of several genes involved in DNA damage response observed, we decided to analyze a possible effect on somatic HR. As the T-DNA in *Atpds5e* contains a GUS gene that might interfere with the reporter system that is based on the restoration of this gene by HR, we concentrated our effort on the triple mutant *Atpds5a Atpds5b Atpds5c*. We crossed it with the reporter line IC9 (Molinier et al., 2004). After isolation of the *Atpds5a Atpds5b Atpds5c* IC9 line, we determined the HR frequency with and without induction of DSBs by bleomycin. In untreated plants, the WT IC9 control showed about 0.8
recombination events per plant, while the HR frequency in the Atpds5a Atpds5b Atpds5c IC9 line was reduced by 50% to about 0.4 (Figure 6A; p < 0.001, n = 4, 50 plantlets each). Treatment with the DSB-inducing agent bleomycin induced the overall number of recombination events in both lines by a factor of 27. However, the Atpds5a Atpds5b Atpds5c IC9 line still displayed a HR frequency that was only about 50% of the WT IC9 control line (Figure 6B; p < 0.001, n = 4, 50 plantlets each). Thus, HR efficiency is indeed reduced in the mutant background.

DISCUSSION

Cohesin cofactors are essential proteins during cohesin dynamics, although they are not components of the cohesin complexes. In this study we describe the role of one of these cofactors, PDS5, in A. thaliana, focusing on meiosis and DNA repair. This cofactor, together with WAPL, has been shown to play a role in the removal of cohesin from chromosomes (Sutani et al., 2009), although it is also required for the maintenance of cohesion by promoting a stable
also been reported in other species. BIMD (A. nidulans), Pds5 (S. cerevisiae) and PDS5B (M. musculus) are also associated with meiotic chromosomes and required for normal chromosome compactness, playing an important role during meiosis (van Heemst et al., 2001; Jin et al., 2009; Fukuda and Hoog, 2010). Indeed, the absence of Pds5 in yeast produces SC formation between sister chromatids and not between homologs. This inter-sister SC formation requires the meiosis-specific cohesion subunit Rec8. Mice defective for REC8 also present inter-sister SC formation (Xu et al., 2005). Taken together, these data reveal that PDS5 family proteins are functional during meiosis, although with particular features in different species.

**Loss of AtPDS5 Proteins Does Not Affect Meiotic Chromosome Structure and Disturbs Slightly Meiotic Progression**

In contrast to the species mentioned above, A. thaliana contains five AtPDS5 genes. In this study we investigated their putative role during meiosis. The results obtained revealed that mutations in ATPDS5B and ATPDS5C produce a reduction in fertility. Curiously, and according to Genevestigator database, the expression of these genes is slightly higher in the inflorescence. Fertility defects are increased progressively when two, three or four AtPDS5 proteins are absent (Table 1, Figure 1). However, despite reduction in the average seed set, an exhaustive cytological examination of the meiotic process in PMsCs of these mutants has not revealed apparent defects during this division (Supplementary Figures S3 and S4). Cohesion between sister chromatids appears mainly intact (Figure 3), as well as in yeast, in which Pds5 deletion does not affect chromosomal localization of Rec8 (Jin et al., 2009). Only AtPds5a AtPds5b AtPds5c AtPds5e showed a significant reduction in the mean cell chiasma frequency with respect to WT, because of a decrease in chromosome I. Therefore, AtPDS5 proteins are not essential for chiasma formation. More noteworthy is the presence of chromatin bridges at anaphase I in all single mutants, which probably arise as a consequence of the existence of unresolved recombination intermediates (Figure 2). The frequency of these bridges is not increased in the quadruple mutant, suggesting all AtPDS5 proteins participate in release of sister chromatid cohesion during the first meiotic division.

On the other hand, according to the Bio-Analytic Resource for Plant Biology (BAR), the meiosis-specific cohesin SYN1 and AtSCC2, required for meiotic sister chromatid cohesion (Sebastian et al., 2009), are possible interactors of AtPDS5A. Moreover, topoisomerase AtTOPII and AtSUMO1 may interact with AtPDS5A and AtPDS5B. Interestingly, Topoisomerase II and SUMOylation of this protein have been revealed to be necessary for stress-relief along axis chromosomes during meiotic recombination in yeast (Zhang et al., 2014). Further analyses will be needed to determine a possible relationship between the function of AtTOPII and the presence of these chromatin bridges.

Unlike AtPDS5, other cohesin cofactors have been shown to play an essential role during Arabidopsis meiosis. Atctf7 mutants, putative defective in AtSMC3 acetylation required for cohesion establishment, present defects in chromosome condensation and
sister chromatid cohesion during male meiosis in addition to chromosome fragmentation. Furthermore, the localization of the cohesin complex subunits AtSMC3, SYN1 and AtISCC3 is diffuse and irregular during prophase I in some meiocytes of these mutants (Bolaños-Villegas et al., 2013; Singh et al., 2013). On the other hand, inactivation of the two AtWAPL genes also produces meiotic defects consisting of incomplete synopsis at pachytene, chromosome bridges at anaphase I and uneven nuclei at second meiotic division (De et al., 2014). In yeast, Ctf7 acetylation of Smc3 is critical for the establishment of cohesion by counteracting the Walp(Wpl1)-Pds5 complex (Rolef Ben-Shahar et al., 2008; Ünal et al., 2008; Rowland et al., 2009; Sutani et al., 2009), although recently it has been proposed that actually Walp counteracts sister chromatid cohesion after it has been established. In addition, Wapl seems to be non-essential during meiotic chromosome segregation (Lopez-Serra et al., 2013). Thus, the function and interplay between AtWAPL and AtPDS5 seems to be different in A. thaliana, at least during meiosis. Obviously, a different result in complete absence of AtPDS5 function cannot be ruled out since we have only studied a situation in which four of the five genes are inactivated. However, the fact that a mutation in one AtPDS5 gene is not compensated by overexpression of the others genes (Supplementary Figures S1 and S2) and the apparently absence of possible interactions identified between both cofactors (according to the BAR), suggest that AtWAPL and AtPDS5 play a different role in cohesion dynamics during A. thaliana meiosis, since AtWAPL is essential and AtPDS5 seems to have no (or little) impact on this division.

**AtPDS5 Proteins are Involved in Homologous Recombination During DNA Repair**

Cohesins are essential proteins in the repair of DSBs. They facilitate DNA repair by holding sister chromatids together at the DSBs. Furthermore, apart from their genome-wide cohesion function, they have a direct role in DNA damage recognition and repair (Kim et al., 2002). The increase in the expression levels of the different AtPDS5 genes we have found after γ-irradiation treatment may indicate their possible role in DSB repair (Figure 5A). Thus, the α-kleisin SYN2 (also known as AtRAD21.1) is also overexpressed after γ-irradiation (da Costa-Nunes et al., 2006). This protein has a specific function in DNA repair in Arabidopsis somatic cells and, unlike other cohesin complex subunits, its absence does not affect sister chromatid cohesion (Schubert et al., 2009). A similar function has also been recently described for SYN4 (AtRAD21.3), which has synergistic and non-redundant effect on the SYN2 function (da Costa-Nunes et al., 2014). We confirmed that the increase in AtPDS5 transcripts is due to a specific role in DSB repair and not a consequence of a general deregulation produced by DNA damage by means of analyzing hypersensitivity to different DNA damage agents. We proved the quadruple mutant Atpds5aAtpds5bAtpds5cAtpds5e is hypersensitive to γ-rays, MMC and CDDP. We did not detect hypersensitivity to CDDP in the triple mutant Atpds5aAtpds5bAtpds5c, whereas the double mutant Atpds5aAtpds5b was only hypersensitive to γ-rays (Figure 4, Supplementary Figures S6 and S7). These findings suggest that AtPDS5 proteins share overlapping functions in DNA repair. Finally, we obtained more evidence for a specific role of AtPDS5 genes in HR by analyzing blue sectors resulting from HR events affecting the GUS reporter gene. Results obtained in the HR assay reveal a strong reduction in the basic level of HR in somatic cells of the triple mutant Atpds5aAtpds5bAtpds5c respect to the WT. This mutant also exhibits a decrease in HR induction upon bleomycin treatment (Figure 6). Especially the reduced HR frequency after bleomycin treatment indicates a function of AtPDS5 genes in DSB repair by HR. This might be a direct role in the regulation of cohesins at the site of a DSB. However, the same phenotype might also be explained by indirect effects of the Atpds5aAtpds5bAtpds5c mutations, e.g., the strong down-regulation of the expression of genes required for HR such as AtSMC6 (for a detailed discussion see below).

The role of PDS5 in DNA repair has previously been described. spo76-1 and bimD6 mutants are sensitive to DNA-damaging agents (Moreau et al., 1985; Denison et al., 1993). In addition, the frequency of spontaneous mitotic interhomolog recombination is strongly reduced in bimD6 (van Heemst et al., 2001). In S. cerevisiae, Pds5 is also involved in DNA repair and mutations in the gene produce accumulation in DNA breaks (Hartman et al., 2000; Ren et al., 2005). S. pombe pds5 mutants are hypersensitive to both the alkylating agent methyl methanesulphonate (MMS) and bleomycin (Wang et al., 2002).

Contrary to the situation observed in meiosis, Atpds5 mutants seem to be more similar to other A. thaliana mutants defective for cohesin related proteins involved in DNA repair. AtCTF7 is also required to DNA repair as revealed by comet assay after a bleomycin treatment and, as Atpds5aAtpds5bAtpds5cAtpds5e quadruple mutant, Atctf7 also displays developmental defects (Bolaños-Villegas et al., 2013; Singh et al., 2013). However, results related to transcription expression levels of DNA repair genes are different since AtATM, AtBRCA1 and AtRAD51 are overexpressed in Atctf7 with respect to WT (Bolaños-Villegas et al., 2013), whereas they do not in the quadruple Atpds5 mutant or even show an underexpression as AtATM. Regarding AtWAPL, double mutants Atwapl1 Atwapl2 do not display a dwarf phenotype. Nevertheless, the presence of chromosome bridges and chromosome fragments could indicate a role in DNA repair (De et al., 2014). Indeed, wpl yeast mutants are hypersensitive to DNA damaging agents (Game et al., 2003).

**AtPDS5 and the AtSMC5/AtSMC6 Complex**

The architecture of the Smc5–Smc6 complex resembles that of the other SMC complexes. However, unlike cohesin, this complex is primarily required for DNA repair and mutations do not lead to premature chromatid separation (Torres-Rosell et al., 2005; Lindroos et al., 2006). Also, meiotic chromosome segregation and recombination are disturbed when the Smc5–Smc6 complex is dysfunctional in both fission and budding yeast (Pebernard et al., 2004; Farmer et al., 2011; Copsey et al., 2013). At present, no proper meiotic function has been described in A. thaliana for AtSMC5, AtSMC6A or AtSMC6B
(there are two AtSMC6 paralogs; Schubert, 2009). However, the complex is required for efficient repair by HR after DNA damage. Atsmc5 homozygous mutants are lethal. mim mutants (defective for AtSMC6B) are sensitive to UV-C, X-rays, MMS and MMC (Mengiste et al., 1999). Furthermore, AtMSC6A and AtSMC6B are both necessary for the establishment of DSB-induced cohesion between sister chromatids to facilitate repair by HR. Indeed, recombination events, detected by scoring GUS-stained blue sectors, are drastically reduced in the single Atsmc6 mutants, which are also defective in HR induction after bleomycin and MMC treatment (Watanabe et al., 2009). Therefore, the similarity between phenotypes corresponding to Atsmc6 and Atpds5 mutants could be related to the reduced expression of AtSMC6 genes in the latter one (Figure 5B).

We do not know whether the down-regulation of AtSMC6 genes is a direct consequence of AtPDS5 failure, but the results suggest that the function of AtPDS5 might be related to the AtSMC5/AtSMC6 complex. In agreement with qPCR results discussed above, the expression pattern of AtSMC6B is different between Atctf7 and Atpds5a Atpds5b Atpds5c Atpds5e, since this gene is overexpressed in Atctf7 (Bolaños-Villegas et al., 2013).

In summary, the results presented here indicate that the AtPDS5 proteins are mainly involved in DNA repair, playing an important role during HR, and their function being very similar to that of the AtSMC5/AtSMC6 complex. Although we cannot rule out the possibility that a residual amount similar to that of the AtSMC5/AtSMC6 complex. Although we cannot rule out the possibility that a residual amount similar to that of the AtSMC5/AtSMC6 complex. Although we cannot rule out the possibility that a residual amount similar to that of the AtSMC5/AtSMC6 complex. Although we cannot rule out the possibility that a residual amount similar to that of the AtSMC5/AtSMC6 complex. Although we cannot rule out the possibility that a residual amount similar to that of the AtSMC5/AtSMC6 complex. Although we cannot rule out the possibility that a residual amount similar to that of the AtSMC5/AtSMC6 complex.

ACKNOWLEDGMENTS
We thank Eugenio Sánchez-Morán for useful discussions and Prof. Chris Franklin for providing the antibodies used in this study. Both researchers work at University of Birmingham (UK). We also thank Sabrina Wagner (Karlsruhe Institut für Technologie, KIT, Germany) and Bianca Martín (Universidad Complutense, Madrid, Spain) for technical assistance.

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2015.01034

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