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ORIGINAL RESEARCH

Arabidopsis PTD Is Required for Type I Crossover Formation and Affects Recombination Frequency in Two Different Chromosomal Regions

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ABSTRACT

In eukaryotes, crossovers together with sister chromatid cohesion maintain physical association between homologous chromosomes, ensuring accurate chromosome segregation during meiosis I and resulting in exchange of genetic information between homologues. The *Arabidopsis PTD* (*Parting Dancers*) gene affects the level of meiotic crossover formation, but its functional relationships with other core meiotic genes, such as *AtSPO11-1*, *AtRAD51*, and *AtMSH4*, are unclear; whether *PTD* has other functions in meiosis is also unknown. To further analyze *PTD* function and to test for epistatic relationships, we compared the meiotic chromosome behaviors of *Atspo11-1 ptd* and *Atrad51 ptd* double mutants with the relevant single mutants. The results suggest that *PTD* functions downstream of *AtSPO11-1* and *AtRAD51* in the meiotic recombination pathway. Furthermore, we found that meiotic defects in *rck ptd* and *Atmsh4 ptd* double mutants showed similar meiotic phenotypes to those of the relevant single mutants, providing genetic evidences for roles of *PTD* and *RCK* in the type I crossovers pathway. Moreover, we employed a pollen tetrad-based fluorescence method and found that the meiotic crossover frequencies in two genetic intervals were significantly reduced from 6.63% and 22.26% in wild-type to 1.14% and 6.36%, respectively, in the *ptd-2* mutant. These results revealed new aspects of *PTD* function in meiotic crossover formation.

KEYWORDS: *PTD*; Meiosis; *MSH4*; Crossover; Recombination frequency

INTRODUCTION

Meiosis is a specialized cell division in eukaryotic sexual life cycles, producing haploid gametes by halving the parental diploid genome. The key events in meiosis are homologous chromosome pairing, synapsis, recombination, and segregation (Ma, 2005). Meiotic recombination increases genetic diversity by exchanging genetic materials between homologues (Felsenstein, 1974; Zickler and Kleckner, 1999), consequently increasing genetic variation among individuals of a population. Another important role for meiotic recombination is to ensure accurate homologue segregation during meiosis I. Proper segregation relies on the formation of special structures called chiasmata, which provide physical association between homologues. These connections are required for bivalent formation, eventually leading to accurate homologue segregation during anaphase I.

Molecular and genetic studies, mainly in yeast, have led to the development of the DNA double-strand break repair

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(DSBR) model of meiotic recombination (Szostak et al., 1983), as reviewed by Keeney (Keeney, 2001). This model is also supported by genetic studies in other fungi, animals and plants (Ma, 2005; Handel and Schimenti, 2010). According to the DSBR model, meiotic recombination is initiated by SPO11 which generates double-strand DNA breaks (DSBs) (Esposito and Esposito, 1969; Keeney, 2001; Lichten, 2001). Homologues of SPO11 have been identified in fungi, animals, plants and protists (Ma, 2005; Malik et al., 2007; Handel and Schimenti, 2010), suggesting that the initiation of meiotic recombination is evolutionarily conserved (Barlow and Hulten, 1998; Dernburg et al., 1998; McKim et al., 1998; Keeney et al., 1999; Romanienko and Camerini-Otero, 1999; Celerin et al., 2000; Grelon et al., 2001). In *Arabidopsis thaliana*, two *SPO11* homologues, *AtSPO11-1* and *AtSPO11-2*, are both required for meiotic recombination, with mutants in either gene displaying similar defects (Grelon et al., 2001; Stacey et al., 2006). Following DSBs formation, each end of the break is resected to form a single-stranded DNA (ssDNA) overhang. Afterward, the DSB repair protein RAD51 and its meiosis-specific homologue DMC1 bind to the ssDNA and facilitate its invasion into the intact duplex DNA of a homologous chromosome, forming an intermediate called a displacement loop (D-loop). The functions of RAD51 and DMC1 homologues are also highly conserved in many different organisms including plants (Hamant et al., 2006; Deyhle et al., 2007).

After ssDNA invasion, there are at least two repair pathways for meiotic crossovers, interference-sensitive (type I) and interference-insensitive (type II) pathways (Zalevsky et al., 1999; Bishop and Zickler, 2004). The interference-sensitive pathway, which experiences inhibition of additional crossovers near existing ones, relies on proteins like the MSH4−MSH5 heterodimer and a DNA helicase called MER3 in yeast. On the other hand, the interference-insensitive pathway generates randomly positioned crossovers, and depends on the MUS81/MMS4/EME1 endonuclease activities (Hollingsworth and Brill, 2004; Berchowitz et al., 2007). In *Arabidopsis*, the interference-sensitive pathway produces approximately 80% of meiotic crossovers (Copenhaver et al., 2002). The roles of *MSH4* and *MER3* and their homologues during meiotic crossovers formation have been well studied in yeast (Nakagawa and Ogawa, 1999; Hoffmann and Borts, 2004; Mazina et al., 2004). The *Arabidopsis* homologue of *MER3* has been identified and functionally characterized independently by two groups, and named as *RCK* (*ROCK-N-ROLLERS*) and *AtMER3*; although slightly weaker than the *Atmsh4* mutant, the *rck Atmer3* mutant phenotypes suggested that it plays a role in the type I crossover pathway (Chen et al., 2005; Mercier et al., 2005).

Another important *Arabidopsis* gene for meiotic crossovers formation is *PTD* (*Parting Dancers*), which was identified as a plant specific gene (Wijeratne et al., 2006). Light and transmission electron microscopy (TEM) analyses showed that the number of chiasmata is reduced in the *ptd* male meiocytes, statistically consistent with a role of PTD in the same crossover pathway as that requires AtMSH4 and RCK/MER3 (Wijeratne et al., 2006). However, the *ptd* mutant phenotypes seemed less severe than that of the *Atmsh4* mutant and the previous results did not exclude possible additional functions of *PTD*, which might not be detectable in a single mutant. Such a possible hidden function could sometimes be revealed by double mutant analysis. Also, genetic evidence for interaction between *PTD* and other meiotic genes is not available. To further investigate *PTD* function and to test for genetic interactions with other genes, we generated double mutants between *ptd* and mutants of other meiotic recombination genes in *Arabidopsis*, including *Atspo11-1 ptd-2*, *Atrad51 ptd-1*, *rck-4 ptd-1* and *Atmsh4 ptd-2* double mutants by genetic crosses and examined their meiotic phenotypes in comparison to the relevant single mutants. Our genetic analyses indicated that PTD acts downstream of AtSPO11 and AtRAD51, and works in the MSH4−MSH5-dependent crossover pathway. Furthermore, although the previous study revealed that crossover formation was greatly reduced at the whole genome level in *ptd* mutants compared to wild-type, the cytological analyses that were used could not examine the meiotic crossover frequency in different genomic regions. Therefore, we examined the crossover frequency in two intervals on chromosome 1 and 3 in *ptd-2* mutants by employing the pollen tetrad-based fluorescence method (Francis et al., 2006). Our data show that crossover frequencies are reduced in *ptd-2* mutants in the two intervals tested.

RESULTS

PTD functions downstream of *AtSPO11* during meiotic recombination

Genetically testing whether two genes are involved in the same biological pathway can be achieved by comparing the phenotypes of the two corresponding single mutants with that of the double mutant. If they function in the same pathway, the double mutant phenotype is expected to be similar to the single mutants (when they are similar), or to that of the more severe single mutant (when they are different). Conversely, if the two genes are in different pathways, the double mutant is expected to show a more severe phenotype than either of the two single mutants. Previously, two independent T-DNA insertion mutants for *PTD* were identified. One is *ptd-1* (SALK_127447), in which the T-DNA was inserted in the first exon, 40 bp downstream of the beginning of the protein-coding region; the other is *ptd-2* (SAIL_567_D09), carrying an insertion in the fifth exon (430 bp downstream of the start codon) of a total of nine exons (Wijeratne et al., 2006). If a truncated protein is produced in the *ptd-2* mutant, it lacks the conserved C-terminal portion of the PTD protein. Both *ptd-1* and *ptd-2* displayed similar fertility defects, and RT-PCR results showed that transcripts containing the full length CDS (coding domain sequence) were not detectable in either mutants (Wijeratne et al., 2006). Therefore, both alleles were used for further studies here.

To test whether *PTD* and *AtSPO11-1* are involved in the same pathway during meiotic recombination, we obtained