The *Arabidopsis AtRAD51* gene is dispensable for vegetative development but required for meiosis

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The maintenance of genome integrity and the generation of biological diversity are important biological processes, and both involve homologous recombination. In yeast and animals, homologous recombination requires the function of the RAD51 recombinase. In vertebrates, RAD51 seems to have acquired additional functions in the maintenance of genome integrity, and *rad51* mutations cause lethality, but it is not clear how widely these functions are conserved among eukaryotes. We report here a loss-of-function mutant in the *Arabidopsis* homolog of *RAD51*, *AtRAD51*. The *atrad51-1* mutant exhibits normal vegetative and flower development and has no detectable abnormality in mitosis. Therefore, AtRAD51 is not necessary under normal conditions for genome integrity. In contrast, *atrad51-1* is completely sterile and defective in male and female meioses. During mutant prophase I, chromosomes fail to synapse and become extensively fragmented. Chromosome fragmentation is suppressed by *atspo11-1*, indicating that AtRAD51 functions downstream of AtSPO11-1. Therefore, AtRAD51 likely plays a crucial role in the repair of DNA double-stranded breaks generated by AtSPO11-1. These results suggest that RAD51 function is essential for chromosome pairing and synapsis at early stages in meiosis in *Arabidopsis*. Furthermore, major aspects of meiotic recombination seem to be conserved between yeast and plants, especially the fact that chromosome pairing and synapsis depend on the function of SPO11 and RAD51.

Homologous recombination and DNA-damage repair are fundamental biological processes found in all life forms. Homologous recombination plays a major role in both maintaining genome stability (DNA-damage repair) and the generation of genetic variability. Defects in DNA-damage repair generally lead to genome instability and are increasingly found to be associated with cancer in mammals. The active surveillance mechanisms that can recognize and precisely repair DNA damage to prevent the accumulation of errors are meanwhile thought to be intimately involved in the prevention of cancer and the delaying of aging (1, 2). Genes playing critical roles in homologous recombination are important for these processes.

Homologous recombination has been intensively studied in budding yeast *Saccharomyces cerevisiae*, and a number of genes have been identified that function in this process. Some of these genes, including *RAD51*, were identified based on the hypersensitivity of their mutants to radiation (3). *RAD51* and another yeast gene, *DMC1*, share significant sequence homology with the bacterial *recA* gene (4). Similar to the bacterial RecA protein, the yeast RAD51 protein acts in homology searching, DNA pairing, and strand exchange (5), activities important for both DNA-damage repair and meiosis. *RAD51* homologs have been found in all eukaryotic organisms thus far and are well studied in the vertebrates human, mouse, and chicken. In contrast to yeast, the loss of *RAD51* function is lethal in both chicken DT40 and mouse cells (4). These *RAD51*-deficient cells arrest during the mitotic cell cycle, show signs of chromosome fragmentation, and undergo programmed cell death. Lethality of *rad51* mutant cells is delayed by a mutation in the p53 tumor suppressor gene. In addition, RAD51 and p53 proteins interact directly, and expression of a dominant negative *RAD51* gene that interferes with homologous recombination but not general DNA repair does not cause lethality in hamster cells (6). Furthermore, RAD51 and the cancer-susceptibility protein BRCA2 interact directly and colocalize to nuclear foci formed after DNA damage (7, 8), and defects in *BRCA2* affect homologous recombination and DNA-damage repair. These data suggest that the lethality caused by *rad51* defects in vertebrate cells is connected primarily to the effect of DNA damage on cell-cycle progression and not directly related to homologous recombination. However, it is unknown how conserved this feature is among higher eukaryotes.

Yeast *rad51* mutations also cause meiotic defects including an accumulation of meiosis-specific double-stranded breaks (DSBs) and reduced formation of physical recombinants (9). RAD51 also plays a role in meiosis of fission yeast, *Aspergillus nidulans*, *Caenorhabditis elegans*, and *Drosophila* (10–13). In addition, *RAD51* homologs are expressed at relatively high levels in the reproductive organs or meiotic cells of several organisms including chicken and mouse (14, 15). Furthermore, the RAD51 protein is localized to recombination foci along early meiotic chromosomes in yeast, maize, and mouse (16–19) and might be a component of early recombination nodules in lily (18). Detailed analysis of RAD51 foci localization during normal and mutant maize meiosis supports the idea that RAD51 is important for homologous chromosome pairing in addition to its role in recombination (20, 21). These findings suggest that *RAD51* operates in meiosis in a variety of organisms and plays important roles in multiple processes including homologous chromosome pairing and meiotic recombination.

Homologous recombination in meiosis is required for proper chromosome segregation and generation of genetic diversity (22). In budding yeast, *SPO11* plays a major role in the initiation of meiotic recombination (23) by catalyzing the formation of DSBs via a topoisomerase-like transesterase activity (24). This mechanism to initiate meiotic recombination seems to be conserved among eukaryotes because homologs of the *SPO11* gene have been identified in both animals and plants; additionally, *AtSPO11-1*, the *Arabidopsis SPO11* homolog, is also required for normal meiotic recombination and synapsis (25). However, little is known about the interplay between *RAD51* and *SPO11* in multicellular eukaryotes.

Although *RAD51* function has been studied in organisms from fungi through animals, large gaps in our knowledge remain, especially for plants. Previously, an *Arabidopsis RAD51* homolog, *AtRAD51*, was described (26). To analyze the function of *AtRAD51*, we identified a knockout mutation in this gene, *atrad51-1*, and studied its role in development and meiosis.

Abbreviations: DSB, double-stranded break; T-DNA, portion of the tumor-inducing plasmid that is transferred to plant cells; SC, synaptonemal complex.

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Unexpectedly, the homozygous *atrad51-1* plants were fully viable and developed normally under standard conditions. However, mutant plants were completely male- and female-sterile, and cytological and genetic analyses indicated that *AtRAD51* is required for repair of meiotic DNA DSBs generated by *AtSPO11-1*. Our results demonstrate that *RAD51* function in a higher plant is significantly different from that in vertebrates but may be similar to that in yeast and invertebrate animals.

Materials and Methods

Plant Materials. Both wild-type and *atrad51-1* mutant plants are of the Columbia ecotype. Unless indicated otherwise, plants were grown under long-day conditions (16-h day/8-h night) at 22 ± 2°C.

Phenotypic Analysis. Tetrads were dissected and stained with 0.1% toluidine blue before being photographed. Viability of pollen grains was examined by staining with Alexander's solution (27). Mitosis was examined by using root tips of 4-day-old seedlings; spindle structure was examined by using antibodies against β-tubulin (Sigma). For mitotic index determination, cells that have a preprophase microtubule band and a perinuclear envelope were regarded as preprophase/prophase cells; cells with an established spindle structure and aligned condensed chromosomes but lack a preprophase microtubule band and a nuclear envelope were grouped as metaphase cells; anaphase cells were those having an anaphase spindle and newly separated chromosomes; and telophase cells were those with clustered and partially decondensed chromosomes (28). Meiotic chromosomal behavior in meiocytes was observed by preparing chromosome spreads (29) stained with 4′,6-diamidino-2-phenylindole (1 μg/ml). Female meiosis was analyzed essentially according to Armstrong *et al*. (30) by using floral buds at stages 10–11 (31).

RT-PCR. RNA was extracted from wild-type and *atrad51-1* leaves and young inflorescences and treated with RQ1 DNase (Promega). cDNA was synthesized by using Superscript II and random priming (Invitrogen) according to manufacturer instructions. Quantitative RT-PCR was performed by combining gene-specific primers atrad51-1-1 (5′-GGTTCCATCACGGAGTTATATGG-3′) and atrad51-2-1 (5′-AGCCATGATATTCCCACCAATC-3′) with plant-specific 18S rRNA primers (Quantum RNA 18S internal standards) (1:10 dilution; primer/competimer, 3:5) according to manufacturer instructions (Competimer primer kit, Ambion, Austin, TX).

Complementation with a *CaMV 35S*::*MYC*::*AtRAD51* Construct. The *AtRAD51* cDNA was cloned by PCR amplification of a wild-type young inflorescence cDNA sample with gene-specific primers oMC858 and oMC859 and inserted into a binary MYC vector (pROK2-MYC vector). An *Agrobacterium* C3581 strain containing that the confirmed construct was used to transform *AtRAD51/atrad51-1* plants. Transgenic plants that carried the *atrad51-1* allele but not the wild-type *AtRAD51* allele were identified, and the expression of the *MYC*::*AtRAD51* transgene was tested. Expression of the constitutive *APT1* gene was examined as a control (32) (for details, see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site).

Generation of the *atrad51-1 atspo11-1* Double Mutant. A cross was performed between *AtSPO11-1/atspo11-1* and *AtRAD51/atrad51-1* plants, and resulting F_1 double-heterozygous plants (*AtRAD51/atrad51-1*; *AtSPO11-1/atspo11-1*) were identified by using PCR with gene-specific primers. Progeny of the double-heterozygous plants were analyzed to identify double mutants by PCR (see *Supporting Materials and Methods* for details).

RNA *in Situ* Hybridization. Nonradioactive RNA *in situ* hybridization was performed essentially as described (33). The antisense and sense RNAs of *AtRAD51* were labeled with digoxigenin through *in vitro* transcription of linearized pTA-*AtRAD51*, which carries a fragment of the *AtRAD51* cDNA, amplified with gene-specific primers oMC1479 and oMC1480 (5′-CTGAATAATTTCCTGTCTCTGAGCATG-3′).

Transmission Electron Microscopy. For transmission electron microscopy, the fixation and infiltration procedures were performed according to ref. 34 with minor modifications. To determine appropriate meiotic stages, thick cross sections through the buds were cut, collected on glass slides, stained with 0.1% toluidine blue, and observed with a light microscope. Ultrathin sections were examined with a JEOL 1200 EXII transmission electron microscope, and at least 50 ultrathin sections were analyzed for each bud. Additional details can be found in *Supporting Materials and Methods*.

Results

Identification of an *AtRAD51* T-DNA Insertional Mutant. A T-DNA (portion of the tumor-inducing plasmid that is transferred to plant cells) insertion line containing an insert in the *AtRAD51* gene (line 134A01 [see Fig. 7*A*, which is published as supporting information on the PNAS web site]) was identified in the GABI-KAT population (35). This line contained two T-DNA inserts that segregated independently. From this line, plants that contained only the T-DNA insertion in *AtRAD51* were obtained by outcrossing, and this insertion was designated as the *atrad51-1* allele. The insert is a highly truncated copy of the pAC161 tagging vector (GenBank accession no. AJ537514) located between nucleotide positions 2830 and 2831 in the DNA sequence of the *AtRAD51* gene (26). Southern blotting and long-template PCR indicated that the *atrad51-1* insert did not cause any additional rearrangements of the *AtRAD51* sequence (data not shown). DNA sequencing of the PCR product showed that the insertion carried neither a complete resistance marker nor an intact right border. *atrad51-1* is likely a loss-of-function allele, because the *AtRAD51* mRNA was not detectable in plants homozygous for the T-DNA insertion (see Fig. 7*B*) by using quantitative RT-PCR that was sensitive enough to detect a <100-fold lower level of wild-type *AtRAD51* transcript.

AtRAD51 Function Is Dispensable for Vegetative Growth and Root Mitosis. Homozygous *atrad51-1* plants showed no apparent abnormal phenotype during the vegetative phase. *atrad51-1* homozygous plants were identified by PCR, grown under standard long- and short-day conditions, and indistinguishable from heterozygous and wild-type plants until they started to set seeds (see Fig. 8, which is published as supporting information on the PNAS web site). In particular, homozygous plants grew with a similar rate and developed a similar number of rosette and cauline leaves and a similar biomass as their wild-type neighboring plants (data not shown). Therefore, the loss of *AtRAD51* function did not cause detectable defects on vegetative development.

Loss of the *RAD51* function in mouse and chicken severely affects cell proliferation, and *RAD51*-defective cells have a severe defect in mitosis that is accompanied by chromosome fragmentation (reviewed in ref. 4). Because growth experiments might not detect minor defects in mitosis, a direct cytological analysis was performed. Mitosis in 4-day-old root tips of mutant plants (Fig. 1 *C*, *D*, *G*, *H*, *K*, *L*, *O*, and *P*) was indistinguishable from wild-type plants (Fig. 1 *A*, *B*, *E*, *F*, *I*, *J*, *M*, and *N*) in all parameters analyzed. No difference was observed in the congression of the chromosomes at metaphase and the segregation at anaphase that result in the formation of two daughter nuclei at the end of mitosis. Moreover, the typical ratio of representative mitotic stages was not altered in *atrad51* compared with

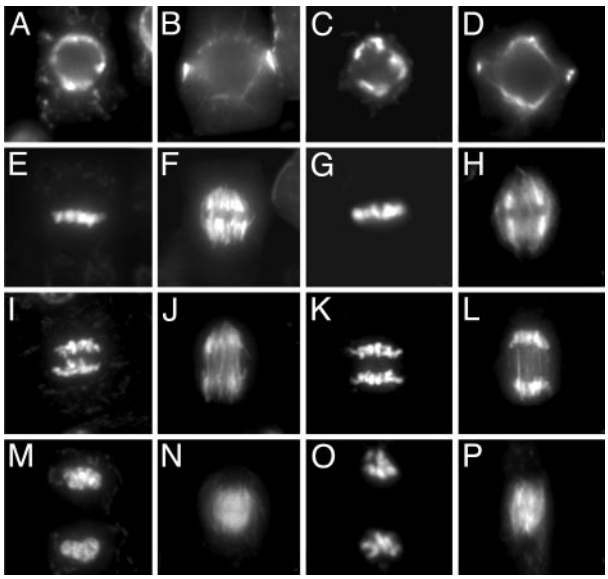

Fig. 1. Mitosis in root tips of wild-type (*A*, *B*, *E*, *F*, *I*, *J*, *M*, and *N*) and *atrad51-1* (*C*, *D*, *G*, *H*, *K*, *L*, *O*, and *P*) seedlings. Chromosomes (*Left*) and microtubule structures (*Right*) in the same cells were visualized with 4′,6-diamidino-2-phenylindole, and antibodies against β-tubulin were visualized at different stages during the mitotic cell cycle (preprophase, *A* and *C*; metaphase, *E* and *G*; anaphase, *I* and *K*; and telophase, *M* and *O*).

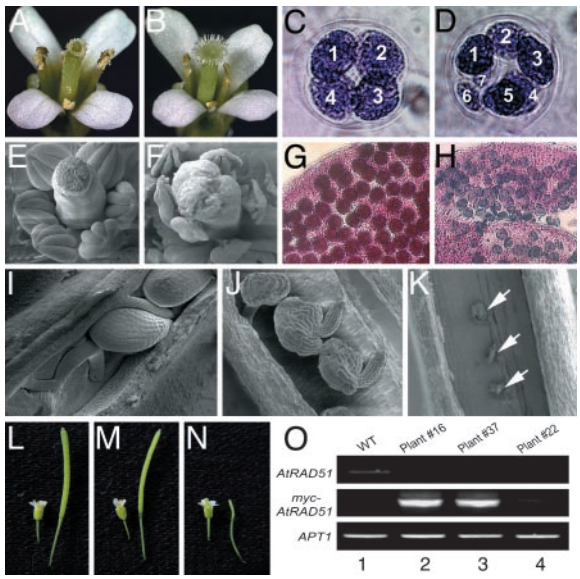

Fig. 2. Phenotypes of wild-type (*A*, *C*, *E*, *G*, *I*, and *L*), *atrad51-1* (*B*, *D*, *F*, *H*, *J*, *K*, and *N*), and *atrad51-1*/*atrad51-1 35S*::*MYC*::*AtRAD51* transgenic plants (*M*). Shown are opened flowers (*A* and *B*), stamens and carpels dissected from unopened flowers (*E* and *F*), tetrad (*C* and *D*) with numbered microspores, pollen grain (*G* and *H*), ovule (*I–K*; arrows point at aborted ovules), and young siliques (*L* and *N*). Two independent *35S*::*MYC*::*AtRAD51* transgenic lines (plants 16 and 37) produced siliques (*M*) that were similar in size to the wild-type ones (*L*). RT-PCR analysis of these two lines and another sterile transgenic line was performed and shown in *O*. Lanes: 1, wild type; 2, T1 transgenic plant 16; 3, T1 transgenic plant 37; 4, T1 transgenic plant 22. *APT1* expression was determined as a positive control.

wild type [wild type: 56.9% preprophase/prophase, 12.6% metaphase, 6.5% anaphase, and 24.0% telophase cells (n = 462); *atrad51-1*: 58.0% prophase/preprophase, 11.7% metaphase, 7.7% anaphase, and 22.6% telophase cells (n = 350)].

The *atrad51-1* Mutant Is Male- and Female-Sterile. In contrast to the normal vegetative development, the *atrad51-1* mutant plants were sterile (Fig. 2*B*). The mutant pollen grains were nonviable (as shown by staining with Alexander's solution; Fig. 2*H*), variable in size, and usually smaller than wild-type ones (data not shown). Although wild-type meioses (>99%, 230 meioses from four plants) produced four spores (Fig. 2*C*), mutant meioses produced multiple spores ranging in number from five to nine, and most tetrads contained six or seven spores (Fig. 2*D*). Although *atrad51-1* flowers have normal carpels (Fig. 2 *B* and *F*) and produce wild-type-like ovules (Fig. 2*J*), the ovules did not develop further after meiosis and subsequently degenerated (Fig. 2*K*), preventing further seed development (Fig. 2*N*). Female sterility is confirmed by the complete failure to obtain seed set after the mutant stigma was pollinated with wild-type pollen grains (10 stigma from four mutant plants).

The sterility phenotype is genetically linked to the *atrad51-1* mutation, because in a population of 149 plants derived from a heterozygote, only the 38 homozygous *atrad51-1* plants were sterile. In addition, the defect can be complemented by the introduction of a *CaMV 35S*::*MYC*::*AtRAD51* gene, which completely restored fertility in homozygous mutants (Fig. 2*M*). Fertility was correlated with high-level expression of the introduced transgene (plants 16 and 37), whereas lines with low-level expression remained sterile (e.g., plant 22; Fig. 2*O*). Expression of the endogenous *AtRAD51* gene was not detectable in any of these plants (Fig. 2*O*), consistent with their *atrad51* mutant background. Complementation in the next generation strictly cosegregated with the transgene (data not shown). Therefore, the loss of *AtRAD51* function is the cause of the sterility phenotype.

The *AtRAD51* Gene Is Highly Expressed in Meiotic Cells. *AtRAD51* was shown previously by Northern blotting to be expressed widely at low levels, and the highest expression was in the floral buds (26). We examined the *AtRAD51* expression in detail in reproductive organs by using RNA *in situ* hybridization. *AtRAD51* expression was first detected in flower primordia at a rather low level (Fig. 3*A*). An increased level was observed in young anthers before meiosis (Fig. 3*B*), and its expression became even stronger in flowers at the time of male meiosis, particularly in the meiocytes (Fig. 3 *C* and *D*). *AtRAD51* was expressed also in the female meiotic cells (Fig. 3*E*). However, *AtRAD51* expression could not be detected in developing male gametophytes (Fig. 3*F*) or in carpels (Fig. 3*G*) after meiosis. The expression of *AtRAD51* in both male and female meiocytes is consistent with an important function in meiosis.

Male Meiosis Is Disrupted in *atrad51-1* Mutant Plants. Because the *atrad51-1* mutant tetrads indicated that male meiosis is defective,

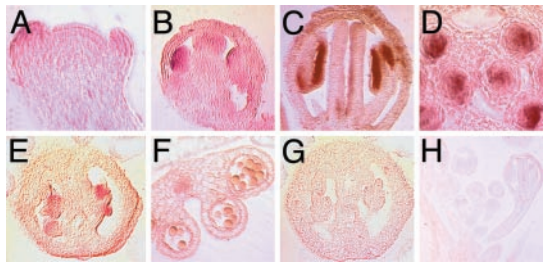

Fig. 3. *AtRAD51* gene expression pattern in floral organs. *AtRAD51* expression was detected in floral primordia (*A*) and at moderate levels in very young flower buds (*B*). In later anthers, the expression is strong in meiocytes (*C*) and restricted in the anther locules (*D*). Expression of *AtRAD51* in carpels is restricted to ovules (*E*). There was no detectable expression in pollen grains (*F*) or gynoecium after meiosis (*G*) or when a sense probe was used (*H*).

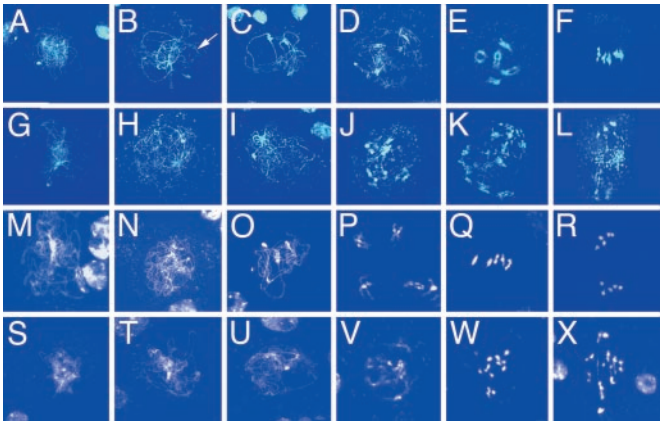

Fig. 4. Male and female meiosis in wild-type (male, *A–F*; female, *M–R*) and *atrad51-1* (male, *G–L*; female, *S–X*) plants. For male meiosis, stages included for wild-type (*A–F*) and *atrad51* (*G–L*) meiosis are leptotene (*A* and *G*), zygotene (*B* and *H*), pachytene (*C* and *I*), diplotene (*D* and *J*), diakinesis (*E* and *K*), and metaphase I (*F* and *L*). For female meiosis, stages included for wild-type (*M–R*) and *atrad51-1* (*S–X*) meiosis are leptotene (*M* and *S*), zygotene (*N* and *T*), pachytene (*O* and *U*), diakinesis (*P* and *V*), metaphase I (*Q* and *W*), and anaphase I (*R* and *X*). The arrow in *B* indicates a pairing fork.

we compared male meiosis in wild-type and *atrad51-1* mutant plants. Wild-type and mutant male meiocytes were similar at early prophase I (leptotene; Fig. 4 *A* and *G*). However, at zygotene, homologous chromosome pairing can be seen as pairing forks in wild type (Fig. 4*B*) but not in *atrad51-1* (Fig. 4*H*). In wild type, meiosis proceeds by completing synapsis of homologous chromosomes at pachytene (Fig. 4*C*) and further condensation at diplotene (Fig. 4*D*), leading to the formation of five highly condensed bivalents at diakinesis (Fig. 4*E*). These bivalents further condense and align at the division plane at metaphase I (Fig. 4*F*). In *atrad51-1*, no typical fully synapsed chromosomes were observed among >1,000 meiocytes. Additional analysis with transmission electron microscopy verified that the vast majority of *atrad51-1* meiocytes did not have any synaptonemal complexes (SCs) (Fig. 5*C*), although an occasional SC was observed (Fig. 5*E*). At the stages corresponding to zygotene through late pachytene, axial elements remained unpaired in the mutant cells when chromosomes appeared much more condensed (Fig. 5*D*), whereas in wild-type zygotene cells, most axial elements were associated into SCs (Fig. 5*A*). Extension of SCs was increased during progression of chromosome condensation, and by late pachytene no unpaired axial elements were found (Fig. 5*B*).

In addition to the absence of SCs, mutant meiocytes contain multiple brightly stained spots, ranging from 20 to 50 per meiocyte from diakinesis through the end of meiosis I (Fig. 4 *J–L*). These observations indicate that severe chromosome fragmentation had occurred at an early stage. From anaphase I through the end of meiosis II, the *atrad51-1* meiocytes are abnormal (see Fig. 9, which is published as supporting information on the PNAS web site), largely as a consequence of earlier mutant defects. Chromosome fragmentation together with the absence of normal chromosomal pairing and synapsis indicates an essential role of *AtRAD51* in *Arabidopsis* meiosis.

Female Meiosis in *atrad51-1* Is Defective Also. The defects observed in mutant female meiocytes were similar to those in male meiosis (Fig. 4). In *atrad51-1*, homologous chromosome paring at zygotene was absent (Fig. 4*T*). In addition, we could not find fully synapsed chromosomes, and thus no typical pachytene stage was observed (Fig. 4*U*). Mutant female meiocytes contained >10 brightly stained chromosomal structures, indicating that chromosome fragmentation also occurred in female meiocytes (Fig. 4 *V–X*).

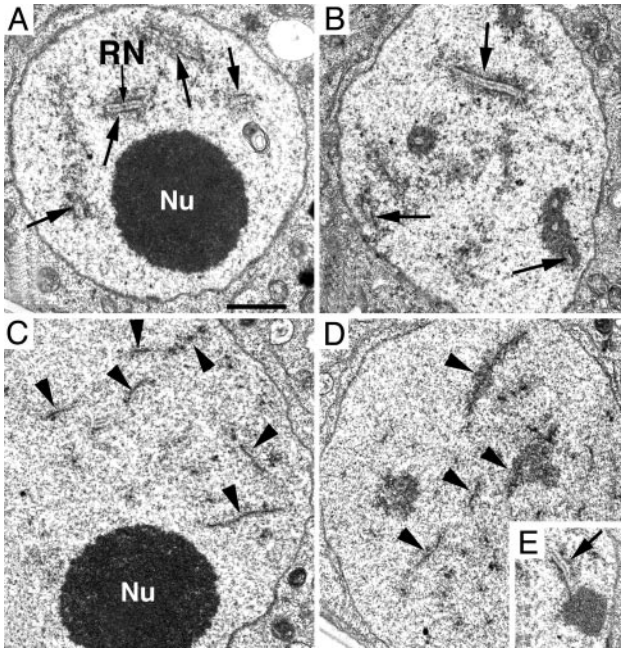

Fig. 5. Transmission electron micrographs of male meiocyte nuclei in wild-type (*A* and *B*) and *atrad51-1* (*C–E*) plants. SCs (arrows) were observed at zygotene (*A*) and pachytene (*B*) stages in wild type. In *atrad51-1*, the axial elements (arrowheads) remained unpaired in nuclei corresponding to zygotene (*C*) or pachytene (*D*) stages; an occasional SC was observed (*E*). Nu, nucleolus; RN, recombination nodule. (Scale bar, 100 nm.)

DSB Formation by *SPO11* Is a Prerequisite for Chromosome Fragmentation. To investigate the relationship of SPO11-induced DSBs and chromosome fragmentation in *atrad51-1* cells, we analyzed *atspo11-1 atrad51-1* double mutants (Fig. 6). The chromosomal behavior of double-mutant plants (*atrad51-1/atrad51-1*; *atspo11-1/atspo11-1*) was similar (Fig. 6 *G–L*) to that of the *atspo11-1* single mutant (Fig. 6 *A–F*). In particular, chromosome fragmentation was absent, and no meiocytes with >10 brightly stained dots were observed (Fig. 6 *H* and *I*). In addition, the absence of typical zygotene chromosome structures characteristic for *atspo11-1* male meiosis was observed also in the *atrad51-1 atspo11-1* double mutant (Fig. 6 *A* and *G*). Moreover, similar to those seen in the *atspo11-1* mutant (Fig. 6 *A–D*), multiple unpaired chromosomes (6–10 per meiocyte) with only occasional chromosomal pairs were seen in the *atrad51-1 atspo11-1* double mutant (Fig. 6 *H–J* and data not shown). Consistently, both *atspo11-1* and the double mutant had 20 sister chromatids

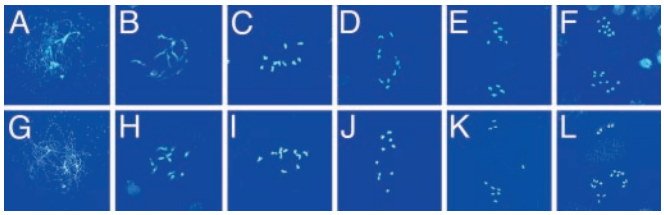

Fig. 6. Male meiosis in *atspo11-1* (*A–F*) and *atspo11-1 atrad51-1* (*G–L*). Stages included are zygotene (*A* and *G*), diakinesis (*B* and *H*), metaphase I (*C* and *I*), anaphase I (*D* and *J*), metaphase II (*E* and *K*), and anaphase II (*F* and *L*). The arrows in *D* and *I* indicate the presence of bivalents. See Fig. 4 for a comparison of the wild-type and *atrad51-1* meioses.

at anaphase II (Fig. 6 *F* and *L*). The epistatic analysis showed that *AtRAD51* functions downstream of *AtSPO11-1* and suggests that a major function of *AtRAD51* is to repair DSBs generated by *AtSPO11-1*. The *atrad51-1 spo11-1* double mutant can also produce a small number of seeds (data not shown), similar to the *spo11-1* single mutant, suggesting that segregation of the univalents can yield a low percentage of viable pollen.

Discussion

A T-DNA insertion was identified that caused a loss-of-function mutation in the *Arabidopsis AtRAD51* gene. Phenotypic characterization showed that *RAD51* is dispensable for normal vegetative development in *Arabidopsis* but has an essential function in male and female meiosis. The phenotype of this T-DNA-generated mutant is different from a report that used RNA interference to suppress RAD51 function in meiosis, and no effect on fertility was observed (36). These data imply that RNA-interference-generated data need to be treated with caution and support our conclusion that the T-DNA insertion is likely to have caused loss of RAD51 function.

***RAD51* Is Not Necessary for Vegetative Development in Plants.** *rad51* mutant yeast cells are hypersensitive to ionizing radiation and DNA-damaging chemicals (9). Even more severely, *rad51* mutations in vertebrates cause a severe defect in cell proliferation that eventually causes cell death. In the absence of data from a larger variety of organisms, different scenarios were discussed to explain this difference in lethality between yeast and vertebrates. One scenario was the need for a more efficient recombination apparatus in vertebrates, because such cells have a more complex genome and a different chromatin structure. Our data show that *RAD51* is normally not required for vegetative and flower development in *Arabidopsis*. This fact indicates that an organism with high genome complexity and chromatin structure can survive without *RAD51* function, and thus genome complexity is not likely to be the cause of lethality in vertebrates.

Another possibility is that *RAD51* in higher organisms has acquired an additional function that links quality control in genome maintenance to cell-cycle progression (4, 37). Such a link might exist in human cells, in which RAD51 has been shown to interact with tumor suppressor genes p53, *BRCA1*, and *BRCA2* (4). However, it is still unclear whether the relationship of RAD51 to cell-cycle control or recombination functions more downstream are the primary cause of lethality in vertebrates. To resolve this question, additional data from other complex, multicellular organisms such as *Arabidopsis* are needed. *Arabidopsis* differs in major aspects in the signal transduction chain linking genome integrity to cell-cycle progression. The genome contains no homolog for p53, and the BRCA1 (38) and the BRCA2 (36) homologs that exist in *Arabidopsis* may differ from vertebrate BRCA proteins. Homology of AtBRCA1 to the vertebrate proteins is rather low, and AtRAD51 differs in amino acid sequence from vertebrate proteins in one of the interaction domains with BRCA2 that is considered important for regulation (data not shown). Therefore, no effect on normal mitotic growth together with major differences in the signal transduction pathway may indicate that plants differ from animals in the link of RAD51 to cell-cycle control and apoptosis and thus mitotic progression and DNA-damage control. *RAD51* knockouts in other organisms, including multicellular animals *C. elegans* (39) and *Drosophila* (12), also do not cause lethality. Therefore, viability is the more common phenotype, clearly indicating that the link of RAD51 to cell-cycle control is the cause of lethality in vertebrates. In addition, these data suggest that the additional functions of RAD51 have mostly evolved in vertebrates, possibly because they need a more stringent control of DNA-damage repair.

A number of proteins with limited sequence homology to RAD51 are found in all eukaryotic genomes (reviewed in ref. 4). Although their function in homologous recombination is less well understood, they seem to be nonredundant with RAD51 but have partially overlapping functions in vertebrates. Recently, such a paralog, *AtXRCC3*, has been characterized in *Arabidopsis* (40). In contrast to vertebrates, loss of *AtXRCC3* function also does not impair viability in *Arabidopsis*. Therefore, it is unlikely that paralogs can substitute for RAD51 function in vegetative development.

***AtRAD51* Is Required for Normal Meiosis.** The fact that the *atrad51-1* mutant is viable and healthy is in sharp contrast to its severe meiotic defects. Light-microscopic analysis of both male and female meioses showed that meiosis in *atrad51-1* proceeded normally through leptotene. However, in contrast to wild type, *atrad51-1* chromosomes were not paired at zygotene and did not form SCs. Transmission electron microscopy strongly supported the conclusion that *atrad51-1* is defective in synapsis. In addition, chromosomes become fragmented at the onset of diakinesis, and fragmentation remains visible through the rest of meiosis. The extensive chromosome fragmentation is likely to be the cause of abnormal segregation of chromosomes and, consistently, of complete sterility. The failure of *atrad51-1* meiocytes in chromosome pairing and synapsis strongly suggests that RAD51 is important for interhomolog recognition in *Arabidopsis* meiosis. In maize and lily, RAD51 is localized to numerous loci at early zygotene and associated with pairing, then subsequently found at greatly reduced foci during pachytene (18, 19). Furthermore, RAD51 foci are greatly reduced in many maize mutants that are defective in pairing (20). Our analysis of *atrad51-1* strongly supports the conclusion drawn from these results that RAD51-mediated homology search is critical for pairing and synapsis in plants. This situation may be different from animals, because a knockout of *RAD51* in *C. elegans* resulted in abnormal chromosomal morphology and univalent formation at diakinesis but did not affect meiotic homology recognition and synapsis (39). In addition, the *atrad51-1* defects in pairing and recombination do not seem to activate a checkpoint that could prevent progression through meiosis. This phenotype is in contrast to *Drosophila melanogaster*, in which a meiotic checkpoint is directly involved in the generation of the meiotic phenotype (9, 12), and *Tetrahymena thermophila*, in which *rad51*-null individuals resulted in an arrest during meiosis I (41). These data suggest that *RAD51* deficiencies affect meiosis differently in different organisms and that meiosis in *Arabidopsis* is more similar to budding yeast than to animals. Our results indicate a direct involvement of RAD51 in chromosome pairing and synapsis in *Arabidopsis*; however, further experiments are needed to show that this role for RAD51 also applies to other organisms.

***AtRAD51-1* Likely Acts Downstream of *AtSPO11-1*.** In yeast, meiosis is initiated by a DSB introduced by SPO11, and RAD51 is involved in the repair of such DSBs (9, 12). Chromosome fragmentation caused by the *atrad51-1* mutation is suppressed by an *atspo11-1* mutation, implying that RAD51 also functions in meiotic DSB repair in *Arabidopsis*. Furthermore, RAD51 acts downstream of SPO11 in the *Arabidopsis* meiotic recombination pathway. Therefore, major aspects of meiotic recombination are conserved between yeast and plants, not only the function of key players but also the molecular connections between them.

A variety of mutants affecting meiosis have been reported in *Arabidopsis*. Mutants defective in synapsis include *asy1*, *syn1*, *solo dancers*, *atspo11-1*, and *dmc1* (25, 42–44). *SYN1* is especially interesting, because the mutation also to leads chromosome fragmentation in meiosis. SYN1 encodes a cohesin that possibly is required for the establishment of the chromosomal structure during prophase I. Therefore the *syn1* phenotype suggests that a close interaction between homologous chromosomes is a prerequisite for meiotic DSB repair, in which RAD51 is involved. The yeast DMC1 protein, a RAD51 paralog, has roles

both overlapping with and distinct from those of RAD51 in meiotic recombination and synapsis. The yeast *dmc1* mutant exhibited extensive meiotic chromosome fragmentation, and DMC1 may also function at a later stage than does RAD51 (45); however, *dmc1* knockouts in animals and plants lack chromosome fragmentation, although they were sterile and defective in meiotic chromosome synapsis, indicating that these proteins have separated functions in meiosis (46, 47). In contrast to yeast in which both RAD51 and DMC1 are critical for the repair of SPO11-dependent DSBs, RAD51 alone may have this function in multicellular organisms, whereas the plant and animal DMC1 is not required and may be specific for late functions in meiotic recombination and synapsis.

Defects in *AtXRCC3* also lead to chromosome fragmentation during meiosis I in *Arabidopsis* (40), suggesting that AtXRCC3 is involved in repair of meiotic DSBs as well. Apparently, however, RAD51 paralogs cannot fully substitute for RAD51 in meiosis. Therefore, *AtRAD51* and its paralogs are likely to play distinct roles in plant meiosis, and possibly both are required for a certain process (*AtRAD51* and *AtXRCC3*) or act in succession (*AtRAD51* and *AtDMC1*). Knockout phenotypes of other recombination genes including *AtRAD50* are similar to *atrad51-1* in *Arabidopsis*. In contrast to animals, knockouts of *AtXRCC3* and the *AtRAD50* show no phenotype in vegetative development; however, meiosis is severely affected (40, 48). These results suggest that homologous recombination or major genes operating in this pathway have evolved in plants to function mainly in meiosis rather than DNA-damage repair.

Plant genomes are constantly challenged by genotoxic stresses such as oxidative damage or stalled replication forks that arise with cell division. The absence of a phenotype in the vegetative phase of *Arabidopsis atrad51-1*, *atxrcc3*, and *atrad50* mutants suggests that plants might use pathways other than homologous recombination for DNA-damage repair as suggested by the high efficiency of nonhomologous DNA integration routinely observed in flowering plants (49). However, it is hardly conceivable that such imprecise repair mechanisms are used exclusively for DNA-damage repair in plants. Therefore, other homologous recombination mechanisms might also function in somatic cells or in mitosis, or yet-uncharacterized protein(s) might substitute for RAD51 function. Furthermore, the finding that *AtRAD51* is important for chromosome pairing and synapsis during meiosis supports the idea that *AtRAD51*-dependent homology search is important for pairing and synapsis, in addition to its role in meiotic recombination (20, 21, 50). Additional investigations are needed to understand the function of AtRAD51 and also its relationship between its paralogs, such as AtXRCC3 and AtDMC1, in pairing, synapsis, and recombination.

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