The *AtRAD51C* Gene Is Required for Normal Meiotic Chromosome Synapsis and Double-Stranded Break Repair in Arabidopsis[1]

Wuxing Li, Xiaohui Yang, Zhenguo Lin, Ljudmilla Timofejeva, Rong Xiao, Christopher A. Makaroff, and Hong Ma*

Department of Biology and the Huck Institutes of the Life Sciences (W.L., Z.L., L.T., R.X., H.M.) and Intercollege Graduate Program in Plant Physiology (W.L., R.X., H.M.), the Pennsylvania State University, University Park, Pennsylvania 16802; Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio 45056 (X.Y., C.A.M.); and Department of Gene Technology, Tallinn University of Technology, Tallinn 12618, Estonia (L.T.)

Meiotic prophase I is a complex process involving homologous chromosome (homolog) pairing, synapsis, and recombination. The budding yeast (*Saccharomyces cerevisiae*) *RAD51* gene is known to be important for recombination and DNA repair in the mitotic cell cycle. In addition, *RAD51* is required for meiosis and its Arabidopsis (*Arabidopsis thaliana*) ortholog is important for normal meiotic homolog pairing, synapsis, and repair of double-stranded breaks. In vertebrate cell cultures, the *RAD51* paralog *RAD51C* is also important for mitotic homologous recombination and maintenance of genome integrity. However, the function of *RAD51C* in meiosis is not well understood. Here we describe the identification and analysis of a mutation in the Arabidopsis *RAD51C* ortholog, *AtRAD51C*. Although the *atrad51c-1* mutant has normal vegetative and flower development and has no detectable abnormality in mitosis, it is completely male and female sterile. During early meiosis, homologous chromosomes in *atrad51c-1* fail to undergo synapsis and become severely fragmented. In addition, analysis of the *atrad51c-1 atspo11-1* double mutant showed that fragmentation was nearly completely suppressed by the *atspo11-1* mutation, indicating that the fragmentation largely represents a defect in processing double-stranded breaks generated by AtSPO11-1. Fluorescence in situ hybridization experiments suggest that homolog juxtaposition might also be abnormal in *atrad51c-1* meiocytes. These results demonstrate that *AtRAD51C* is essential for normal meiosis and is probably required for homologous synapsis.

Meiosis is essential for eukaryotic sexual reproduction, allowing the production of haploid gametes. In addition, meiotic recombination during the early stages of meiosis allows the exchange of genetic information, serving as an important source of genetic diversity. The success of meiosis depends on a complex and prolonged prophase I that involves homologous chromosome (homolog) pairing, synapsis, and recombination (Zickler and Kleckner, 1999; Page and Hawley, 2003; Schwarzacher, 2003). After pairing, the homologs continue to associate and this interaction has been referred to as homolog juxtaposition (Zickler and Kleckner, 1999). Recombination results in crossover events that correspond to cytologically observed chiasmata, which, in combination with sister chromatid cohesion, maintain the association between homologs in the form of bivalents, ensuring proper segregation of homologs at anaphase I. Synapsis, the formation of synaptonemal complexes (SCs) between closely associated chromosomes, has also been implicated to play important roles in meiotic prophase I, although its relationship with recombination differs among organisms.

Cytological and molecular genetic studies support the idea that homolog pairing, synapsis, and recombination are closely coupled events in normal meiosis. In particular, recombination and synapsis are often interdependent. In fact, a number of meiotic genes are required for both normal synapsis and recombination in yeast (*Saccharomyces cerevisiae*; Zickler and Kleckner, 1999). For example, in budding yeast, the SPO11 protein is required for both recombination and synapsis. SPO11 is a member of a family of type II topoisomerases and is required for the generation of double-stranded breaks (DSBs; Keeney et al., 1997) that initiate recombination. SPO11 homologs have been identified in both animals and plants, suggesting that the pathway for enzymatic generation of DSBs is conserved. In Arabidopsis (*Arabidopsis thaliana*), a *SPO11* homolog, *AtSPO11-1*, is required for meiotic recombination,

[1] This work was supported by the National Institutes of Health (grant no. R01 GM63871–01 to H.M.) and the National Science Foundation (grant nos. MCB–0092075 to H.M. and MCB–0322171 to C.A.M.). W.L. was partially supported by the Intercollege Graduate Program in Plant Physiology at the Pennsylvania State University. R.X. is supported by a University Graduate Fellowship from the Pennsylvania State University. H.M. gratefully acknowledges the support of the John Simon Guggenheim Memorial Foundation, the K.C. Wong Educational Foundation, and the Foreign Distinguished Young Scholar Award from the Chinese National Science Foundation.

* Corresponding author; e-mail hxm16@psu.edu; fax 814–863–1357.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.104.058347.

suggesting that there is an AtSPO11-1-dependent mechanism for DSB formation in plants (Grelon et al., 2001). On the other hand, *SPO11* homologs are required for recombination, but dispensable for synapsis in *Caenorhabditis elegans* and *Drosophila melanogaster* (Dernburg et al., 1998; McKim and Hayashi-Hagihara, 1998), indicating that requirements of specific genes for recombination and synapsis are not identical among organisms.

In the widely accepted DSB repair model for recombination (Szostak et al., 1983), DSBs facilitate the initiation of interaction between homologs via RAD51 and other proteins. RAD51, first discovered in yeast, is a homolog of the *Escherichia coli* RecA recombinase (Shinohara et al., 1992). Like RecA, RAD51 catalyzes DNA strand exchange in an ATP-dependent manner (Sung, 1994). In addition, a second yeast RecA homolog, DMC1, functions specifically in meiosis (Bishop et al., 1992). Biochemical studies indicate that RAD51/DMC1 binds to single-stranded DNA (ssDNA) that is formed following the generation of DSBs and then promotes strand invasion of the ssDNA into the intact homolog, producing a D-loop (Petukhova et al., 2000). Genetic studies in yeast indicate that a portion of DSBs leads to the formation of double Holliday junctions and crossover events, whereas other DSBs result in non-crossover events (Bishop and Zickler, 2004; Borner et al., 2004). Protein localization studies of RAD51 in maize (*Zea mays*) and Arabidopsis and molecular genetic analyses in Arabidopsis also support the idea that RAD51 is important for pairing, synapsis, and recombination (Franklin et al., 1999; Mercier et al., 2003; Pawlowski et al., 2003, 2004; Li et al., 2004).

Five RAD51 paralogs have been identified in mammals and birds, including RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 (Shinohara et al., 1993; Tebbs et al., 1995; Albala et al., 1997; Dosanjh et al., 1998; Liu et al., 1998; Pittman et al., 1998; Schild et al., 2000). In cell cultures, mutations in these genes cause defects in DSB repair, increased chromosome instability, and broad-spectrum mutagen sensitivity (Takata et al., 2001) similar to the effects of *rad51* mutations. These RAD51 paralogs have been found in two complexes, the RAD51C-XRCC3 complex and the RAD51B-RAD51C-RAD51D-XRCC2 complex, and RAD51 may interact with the RAD51C-XRCC3 complex (Masson et al., 2001; Liu et al., 2002). RAD51C is the only protein present in both complexes, suggesting an important role of RAD51C in recombination. The human RAD51C binds to ssDNA and double-stranded DNA and exhibits a DNA-stimulated ATPase activity (Lio et al., 2003), suggesting that it may have a similar biochemical function to that of RAD51. On the other hand, the fact that *RAD51* and *RAD51C* are both required for normal DNA repair and recombination indicates that they do not have identical functions. This is further supported by in vitro analysis showing that RAD51C promotes branch migration and the resolution of double Holliday junctions in homologous recombination (Liu et al., 2004). The critical role of *RAD51C* in mitotic recombination suggests that it might be involved in meiotic recombination as well. In Drosophila, a putative *RAD51C* homolog, *spnD*, is required for normal fertility and oogenesis, but its role in meiosis has not been analyzed in detail (Abdu et al., 2003). Therefore, the function of *RAD51C* in animal meiosis is still not clear.

A putative *RAD51C* ortholog, *AtRAD51C*, is present in the Arabidopsis genome (Osakabe et al., 2002). We have identified an insertional line in the *AtRAD51C* gene, *atrad51c-1*, and report our analysis of this mutant here. An independent analysis of this insertional line and other mutants has been published recently (Bleuyard et al., 2005) and these two studies both indicate that the *AtRAD51C* gene is required for male meiosis. We provide additional detailed characterization of the *atrad51c-1*mutant, including the result that this gene is also required for female meiosis, but it is not critical for mitosis under normal conditions. Fluorescence and transmission electron microscopy (TEM) results demonstrated that synapsis is abnormal and homolog juxtaposition might also be altered in this mutant, indicating that *AtRAD51C* may be required for both processes. Furthermore, *atrad51c-1* mutant cells exhibit chromosome fragmentation in an AtSPO11-1-dependent manner, indicating that AtRAD51C is required for proper processing of AtSPO11-1-generated DSBs. The number of breaks in the *atrad51c-1* cells is greater than the expected number of crossovers, suggesting that at least some of the unprocessed DSBs would have resulted in non-crossover events. Therefore, our results provide clues suggesting the involvement of non-crossover-generating DSBs in homolog juxtaposition. Here we also discuss a possible model for RAD51C function in early meiosis.

RESULTS

Identification of a T-DNA Insertion in the *AtRAD51C* Gene

Both Arabidopsis genomic and cDNA sequences reveal a putative homolog of the human *RAD51C* gene, At2g45280, which was named *AtRAD51C* (Osakabe et al., 2002). Sequence comparison indicates that the predicted AtRAD51C protein is more similar to the human RAD51C protein (37.7% identity and 59.8% similarity) than to other human and Arabidopsis RAD51 paralogs (21.3%–27.2% identities and 39.4%–48.8% similarities). Reverse transcription (RT)-PCR indicates that *AtRAD51C* is expressed widely, with the highest levels in floral buds (Fig. 1B). RNA in situ hybridization detected strong *AtRAD51C* expression only in meiocytes (Fig. 1C), suggesting a function of *AtRAD51C* in meiosis.

To understand the in vivo function of *AtRAD51C*, we obtained a T-DNA insertional line (SALK_021960) that contained an insertion in the second intron of the *AtRAD51C* gene (Fig. 1A). The position of the insertion, named *atrad51c-1*, was confirmed by PCR

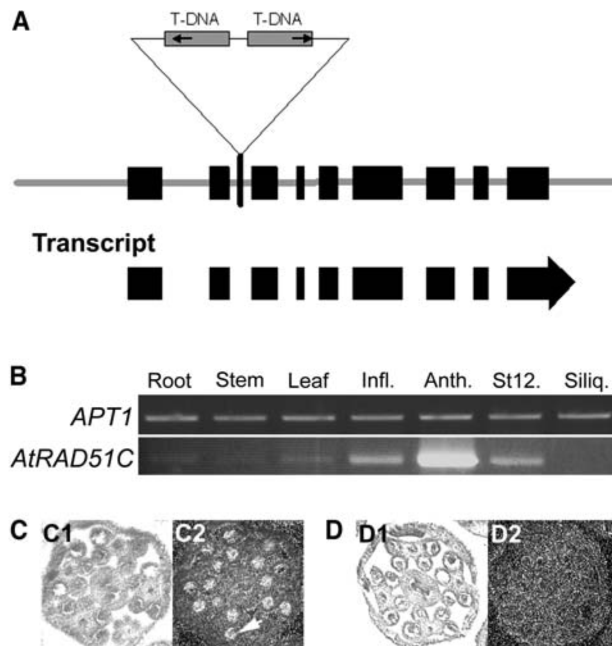

Figure 1. Analysis of the T-DNA insertion in the *AtRAD51C* gene and expression of *AtRAD51C*. A, Illustration of the *AtRAD51C* locus and the T-DNA insertion. Two copies of T-DNA fragments were present in the insertion. Therefore, the primer for the left border recommended by the SALK center (LBb1; designated as oMC 645 in this article) can amplify a product when combined with *AtRAD51C* gene-specific primers from either side of the T-DNA insertion. B, Detection of *AtRAD51C* expression in root, stem, leaf, young inflorescences (infl), anther (anth), stage 12 flowers (st12), and siliques (siliq). *APT1* expression was used as a positive control (Moffatt et al., 1994). C, In situ hybridization with an antisense AtRAD51C probe showing expression in the meiocytes (e.g. arrows). C1, Bright-field image; C2, dark-field image. D, Control with a sense probe. D1, Bright-field image; D2, dark-field image.

amplification with *AtRAD51C*-specific (oMC1451) and T-DNA-specific (oMC 645; LBb1) primers (Fig. 1A). Molecular characterization indicated that the *atrad51c-1* insertion contains two copies of T-DNAs with their left border-*AtRAD51C* junctions. This insertion is also associated with the loss of a 141-bp sequence in the second *AtRAD51C* intron (Fig. 1A). RT-PCR with gene-specific primers did not detect *AtRAD51C* transcripts even after 43 cycles of amplification (Fig. 2M), indicating that *atrad51c-1* is likely a null mutation. Segregation analyses indicated that *atrad51c-1* was tightly linked with a sterility phenotype (see below): Of the 359 plants examined, every plant (92) that was homozygous for *atrad51c-1* was found to be sterile. In addition, no sterile plants were found that were not homozygous for *atrad51c-1*.

atrad51c-1 Is Normal in Vegetative Development, But It Is Female and Male Sterile

Plants containing the *atrad51c-1* mutation resembled wild-type plants during vegetative development under standard growth conditions (Fig. 2, A and B). Flowers from *atrad51c-1* plants had normal morphology, including normal floral organ identity and number (Fig. 2, C and D). However, the *atrad51c-1* plants were completely sterile, with small, empty seedpods (Fig. 2, G and H). Examination of the mutant flowers indicated that they were male sterile and mutant anthers failed to produce functional pollen grains (Fig. 2, D, F, and J). Some pollen grains were produced in the mutant, but they had abnormal sizes (Fig. 2F) and stained blue with Alexander's solution, indicating that they were not viable (Fig. 2J). At the tetrad stage, *atrad51c-1* anthers contained polyads with five to 10 microspores (Fig. 2L) instead of four in the wild type (Fig. 2K). When the stigmas of *atrad51c-1* flowers were pollinated with wild-type pollen (16 flowers crossed), they failed to set seed, indicating that the *atrad51c-1* mutant is also female sterile.

Because mutations in human *RAD51C* affect the DNA repair in somatic cells, and our RT-PCR results as well as microarray data (Obayashi et al., 2004; Zimmermann et al., 2004) indicate that *AtRAD51C* is also expressed in leaves and roots, we examined mitosis in root tips of wild-type and *atrad51c-1* mutant plants to detect possible minor defects that may not be obvious at the plant or organ levels. Analysis in root tips of 4-d-old mutant plants indicated that mitosis in the mutant is very similar to that in wild-type plants, with no obvious differences being detected (Fig. 3; over 350 cells analyzed). Therefore, unlike the vertebrate *RAD51C* genes, *AtRAD51C* function appears to be dispensable for somatic growth in Arabidopsis under normal conditions.

Male and Female Meiosis Is Disrupted in *atrad51c-1* Plants

Our morphological and genetic analyses suggest that *atrad51c-1* may be defective in both male and female meiosis. To test this hypothesis, we compared both male and female meioses in wild-type and *atrad51c-1* plants grown under standard conditions, using chromosome spreads stained with 4′,6-diamidino-2-phenylindole (DAPI). Meiotic abnormalities were identified in both male and female meiocytes (Fig. 4). In wild-type plants, male meiosis I starts with the condensation of chromatin, forming distinct thin chromosome threads at leptotene (Fig. 4A). After further condensation and pairing of homologous chromosomes during zygotene (Fig. 4B), juxtaposed homologs are observed at pachytene (Fig. 4C). Following further condensation (Fig. 4, D and E), the chromosomes then aligned at the division plane (Fig. 4F). No obvious alterations were observed in *atrad51c-1* meiocytes at leptotene (Fig. 4G); however, dramatic abnormalities were observed from zygotene through telophase I. In contrast to the wild type (Fig. 4B), close association of homologs was rarely detected in mutant meiocytes (Fig. 4H). In addition, typical pachytene images could not be found in *atrad51c-1* meiocytes (Fig. 4I), suggesting that fully juxtaposed chromosomes may not be

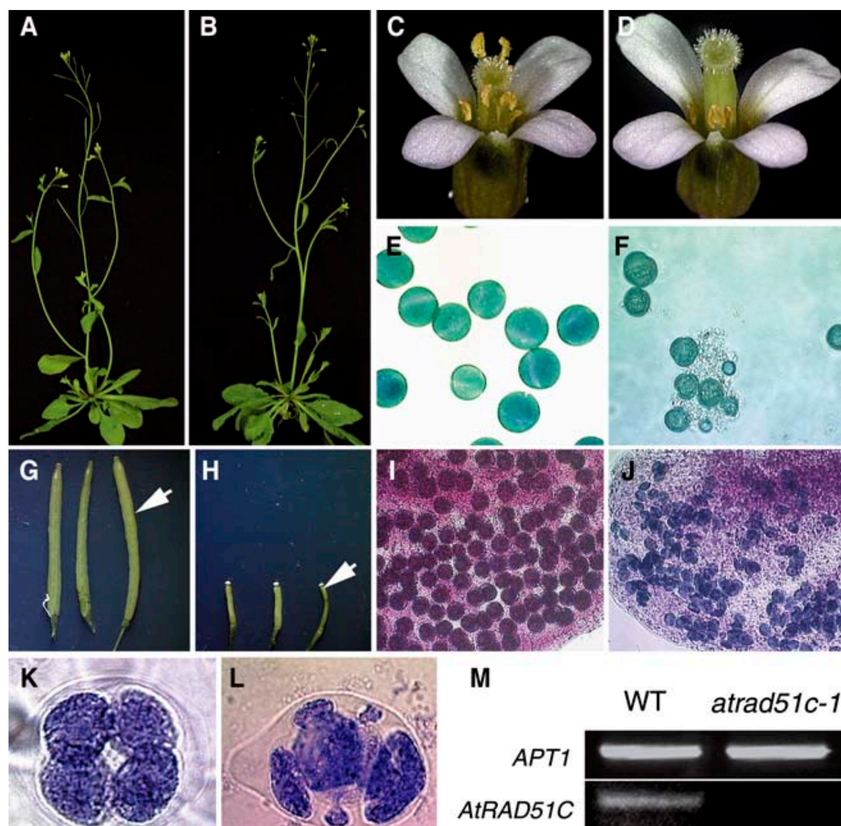

Figure 2. Phenotypes of wild-type (A, C, E, G, I, and K), and *atrad51c-1* (B, D, F, H, J, and L) plants. Arabidopsis plants (A and B), opened flowers (C and D), pollen grains (E and F), siliques (G and H), anthers stained with Alexander's solution (I and J), and tetrads (K and L) are shown. M, RT-PCR result of *AtRAD51C* expression in wild-type and *atrad51c-1* plants. Expression of *APT1* was used as a positive control (Moffatt et al., 1994).

present. Subsequent abnormalities were also observed during diplotene (Fig. 4J) and diakinesis (Fig. 4K). A striking feature of the *atrad51c-1* mutant is the presence of numerous brightly stained spots. The abnormally large number of chromosomal entities was detected beginning at approximately diakinesis, suggesting that some DSBs were not repaired and subsequently were manifest as broken chromosomes. The chromosome fragments became more obvious at metaphase II, when they are highly condensed (*atrad51c-1*, an average of 28.7 $\pm$ 4.2 spots from 29 cells; for comparison, 31 *atrad51-1* mutant cells have an average of 32.4 $\pm$ 4.9 spots; only well-spread cells were counted, although $>$1,000 were observed with fragmentation for each mutant). To test the possibility that the observed chromosome fragmentation might be resulting in part due to the force of chromosome spreading, we also examined intact *atrad51c* meiocytes (n = 324) and found similar chromosome fragments (data not shown), whereas wild-type cells had the expected five bivalents at late diakinesis. Other abnormalities observed in *atrad51c-1* meiosis II were likely the consequence of defects in meiosis I (Fig. 4, S–X).

Female meiosis in *atrad51c-1* exhibited similar, but less severe, defects (Fig. 4EE–JJ) as those observed during male meiosis. In *atrad51c-1* female meiocytes, association of homologs at zygotene was also altered (Fig. 4FF), and fully juxtaposed chromosomes as seen in normal pachytene could not be observed (Fig. 4GG). Mutant female meiocytes also exhibited abnormal chromosome alignment and the presence of more than 10 chromosomal entities. However, fewer fragments were detected at metaphase I (Fig. 4II) and anaphase I (Fig. 4JJ) in female meiocytes than male meiocytes (13–15 chromosomal entities in 23 cells), suggesting that female meiosis may be less affected by the mutation.

atrad51c-1 Is Defective in Homolog Juxtaposition and Synapsis

The absence of normal pachytene chromosomes suggested that the *atrad51c-1* mutant may be defective in synapsis and/or in homolog juxtaposition. To test the latter, we examined both wild-type and *atrad51c-1* meiocytes using fluorescence in situ hybridization (FISH). From interphase to early zygotene, eight to 10 centromere signals were observed in wild-type and *atrad51c-1* meiocytes (Fig. 5, A, B, E, and F). By late zygotene, 72% of the wild-type male meiocytes had five or fewer centromere signals ($>$100 cells); at pachytene and diplotene/early diakinesis (Fig. 5, C and D), 100% of the cells had five or fewer centromere signals ($>$100 cells for each stage). In contrast, about one-half of the *atrad51c-1* meiocytes had more than six centromere signals, with an average of 5.7, during mid-to-late stages of prophase I (approximately equivalent to the wild-type pachytene, diplotene, and early

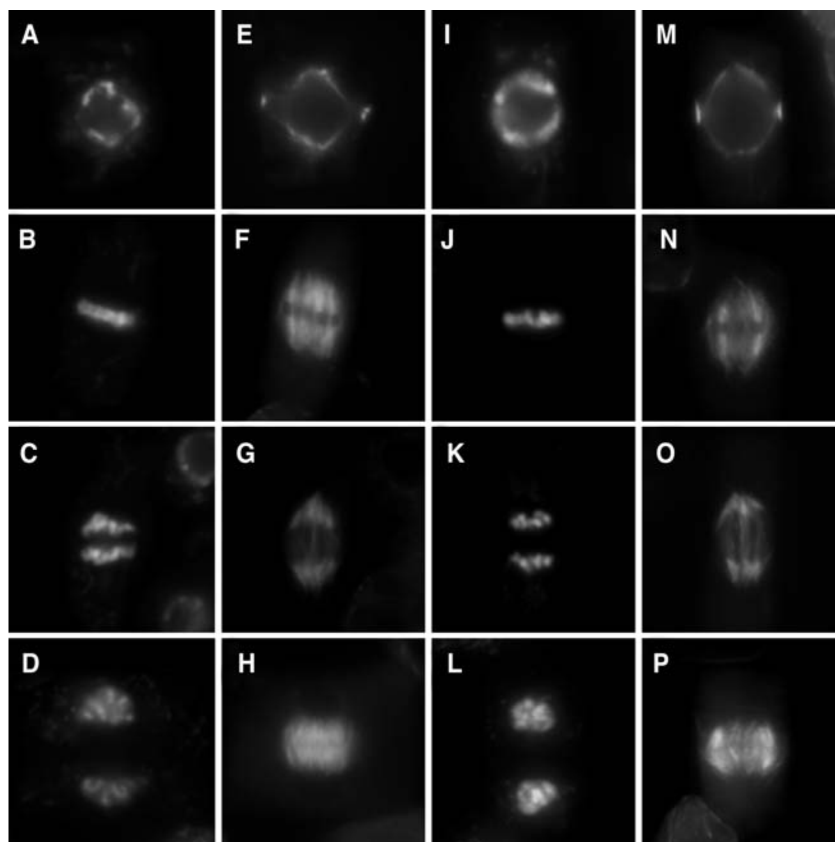

Figure 3. Mitosis in root tips of 4-d-old wild-type (A–H) and *atrad51c-1* (I–P) seedlings. Chromosomes (left) and microtubule structures (right) from the same cell were visualized with DAPI and antibodies against β-tubulin, respectively. Cells at different stages during the mitotic cell cycle were analyzed (preprophase, A, E, I, and M; metaphase, B, F, J, and N; anaphase, C, G, K, and O; and telophase, D, H, L, and P).

diakinesis stages, >100 cells for each stage; Fig. 5, G and H). This suggests that the *atrad51c-1* mutation affects the juxtapositioning of the centromere regions of some chromosomes. Furthermore, most *atrad51c-1* cells observed had one or two centromere signals that were larger than the rest and those in the wild type, whereas other centromere signals were often smaller and not as brightly stained as wild-type centromeres. These observations suggest that centromere regions in *atrad51c-1* meiocytes may have abnormal structure.

Because paired centromeres could be nonhomologous, we next used a telomere-derived probe that labels, in addition to telomeres, an arm sequence adjacent to the centromere only on chromosome 1 (Armstrong et al., 2001), in order to further assess homolog interaction in *atrad51c-1* meiocytes. Two strong signals corresponding to chromosome 1 were observed during interphase, leptotene, and early zygotene in both wild-type and *atrad51c-1* meiocytes (Fig. 6). Beginning at late zygotene and extending through pachytene, diplotene, and diakinesis, one signal was observed in wild-type meiocytes (Fig. 6). In contrast, two sometimes adjacent signals were observed in the meiocytes of *atrad51c-1* plants at the stages corresponding to the normal pachytene, diplotene, and diakinesis stages (Fig. 6). *atrad51c-1* cells containing a single chromosome 1 signal were rarely (<20/300) observed. These results are consistent with our observations from DAPI staining and centromere FISH and confirm that, while there may be some association of homologs, the normal homolog juxtaposition is altered in the mutant. In addition to the chromosome 1 signal(s), similar numbers of telomere signals were observed in both wild type and *atrad51c-1* (Fig. 6). However, the overall sizes of the telomere signals seemed to be smaller in *atrad51c-1* meiocytes than those in wild-type cells (Fig. 6). In some instances, a telomere signal was not detected at some chromosome ends, possibly due to chromosome breakage.

To investigate possible *atrad51c-1* defects in synapsis, SC formation was analyzed using TEM (Fig. 7). Wild-type meiotic chromosomes start to synapse at early zygotene (Fig. 7, A and B). Two lateral elements, aligned in parallel, become connected by transverse filaments to the central element forming the SC. Early recombination nodules associated with the SC structures can be observed in wild-type nuclei (Fig. 7B). In mutant meiocytes, at the stages corresponding to zygotene or early pachytene in wild-type meiocytes, SC structures can be seen as short stretches (less than 10% in total length of that in wild type), indicating that most chromosome regions remain unsynapsed (Fig. 7, D–F). Recombination nodules were not observed on the mutant SC central element among 73 cells that were examined with 25 to 30 sections for each cell. Furthermore, abnormal SCs consisting of more than two lateral elements (Fig. 7E) were sometimes observed (approximately one-third of the SCs in the

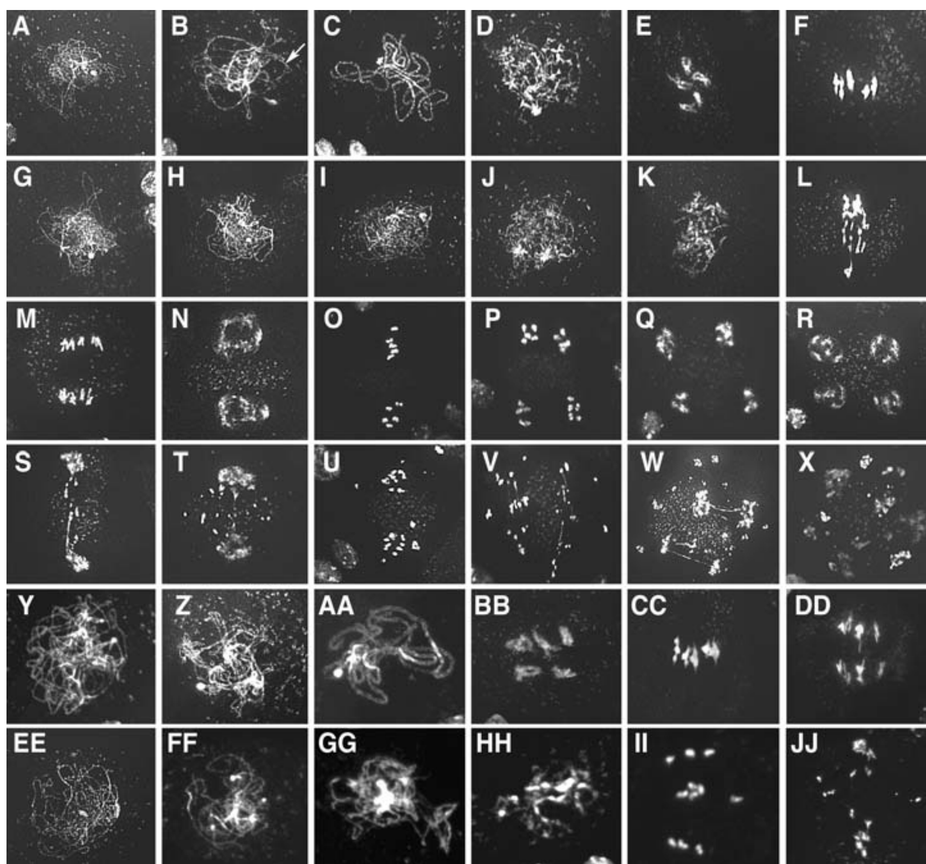

Figure 4. Male meiosis and female meiosis in wild-type (male, A–F, M–R; female, Y–DD) and *atrad51c-1* (male, G–L, S–X; female, EE–JJ) plants. For male meiosis I, stages included for wild-type (A–F) and *atrad51c-1* (G–L) meiosis are leptotene (A and G), zygotene (B and H), pachytene (C and I), diplotene (D and J), diakinesis (E and K), and metaphase I (F and L). During wild-type male meiosis I, after alignment at the division plane of metaphase I, the homologs are segregated at anaphase I (M) and form two clusters of chromosomes at telophase I (data not shown). Meiosis II starts with prophase II (N), followed by the alignment of chromosomes at metaphase II (O), sister chromatid separation at anaphase II (P), and segregation at telophase II (Q), resulting in four nuclei (R). In *atrad51c-1*, multiple chromosome fragments were present at anaphase I (S), and these fragments were distributed abnormally at telophase I (H). The abnormal meiosis II (T–X) seems to be the consequence of defects in meiosis I. For female meiosis, stages included for wild-type (Y–DD) and *atrad51-1* (EE–JJ) meiosis are leptotene (Y and EE), zygotene (Z and FF), pachytene (AA and GG), diakinesis (BB and HH), metaphase I (CC and II), and anaphase I (DD and JJ). Arrow in B indicates a possible site of homolog interaction.

mutant), suggesting that synapsis might have occurred between nonhomologous chromosomes. However, no SC or SC-like structures were observed in mutant nuclei at late pachytene, suggesting that partially synapsed chromosomes did not proceed to become fully synapsed and that the partial SCs precociously disassembled during the stage corresponding to wild-type pachytene.

AtSPO11-1 Is Important for Chromosome Fragmentation in *atrad51c-1* Meiocytes

It is known that, in yeast, SPO11-generated DSBs are required for normal recombination and synapsis and that the Arabidopsis *AtSPO11-1* gene is required for pairing and recombination. The chromosome fragmentation observed in *atrad51c-1* meiocytes suggests that they may represent a defect to process DSBs generated by the AtSPO11-1 protein. To test the relationship between AtSPO11-1-generated DSBs and chromosome fragmentation in *atrad51c-1* plants, we generated the *atspo11-1 atrad51c-1* double mutant and analyzed its meiosis (Fig. 8). The meiotic chromosome behavior in the double mutant was similar to that of the *atspo11-1* single mutant, including the presence of many univalents and occasional bivalents (Fig. 8, D and J). This was very different from that in the *atrad51c-1* mutant (Fig. 4). In the vast majority of cells (>110), no more than 10 chromosomes were observed in male meiocytes of the double mutant from metaphase I through metaphase II, and 20 newly separated sister chromatids were observed at anaphase II (Fig. 4V), although, in a few cells (3 out of 110 examined), there was an extra DAPI-staining spot in the double mutant. Therefore, chromosome fragmentation was

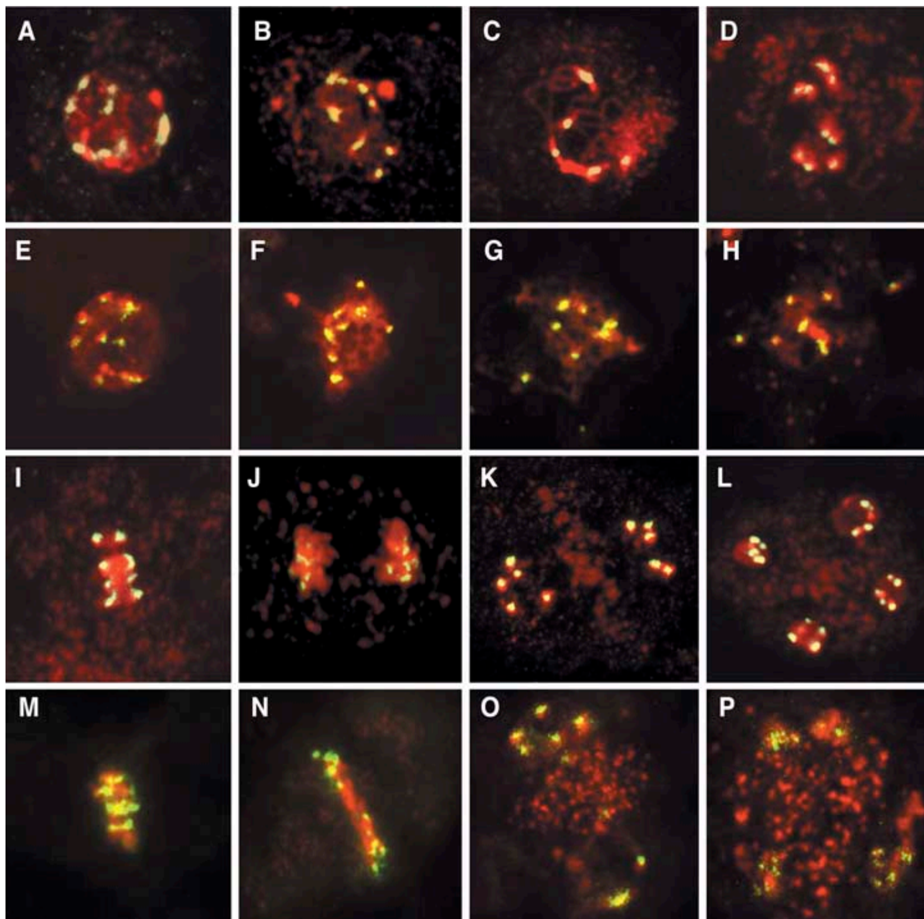

Figure 5. FISH using a centromere DNA probe in wild-type (A–D, I–L) and *atrad51c-1* (E–H, M–P) male meiocytes. The centromere DNA was labeled with biotin and detected with FITC-labeled streptavidin (green) and chromosomes were stained with DAPI (red). Stages included are interphase I (A and E), early zygotene (B and F), pachytene (C and G), late diakinesis (D and H), metaphase I (I and M), anaphase I (J and N), premetaphase II (K and O), and telophase II (L and P).

nearly absent in the double mutant, indicating that *AtRAD51C* acts downstream of *AtSPO11-1*. We conclude that *AtRAD51C* likely plays an important role in processing AtSPO11-1-generated DSBs.

DISCUSSION

Our results clearly show that *AtRAD51C* is required to repair the meiotic DSBs generated by the AtSPO11-1 during meiosis I, consistent with the function of vertebrate RAD51C in mitosis. The *atrad51c-1* mutant plants can undergo normal vegetative and flower development with apparently normal mitosis, although mutations in *AtRAD51C* and other *RAD51* paralogs have recently been shown to cause increased sensitivity to the DNA cross-linking agent mitomycin C (Bleuyard et al., 2005), suggesting that *AtRAD51C* plays a role in DNA repair in the mitotic cell cycle. In vitro studies showed that mammalian RAD51C promotes branch migration and the processing of double Holliday junctions in homologous recombination (Liu et al., 2004). Our results also show that *AtRAD51C* is required for normal synapsis and may be involved in homolog juxtaposition, suggesting that *AtRAD51C* may be required for early meiosis before the formation of double Holliday junctions. Therefore, analyses reported here and from others indicate that RAD51C is likely involved in multiple processes during the meiotic prophase I and mitotic cell cycle in animals and plants.

Conservation and Divergence of *RAD51C* and Other *RAD51* Paralogs

Although *RAD51C* orthologs have not been identified in yeast and other fungal organisms, putative orthologs are found in plants and animals. In Drosophila, the *spnD* gene encodes a putative *RAD51C* homolog (Abdu et al., 2003), but the levels of amino acid sequence similarity between spnD and mammalian RAD51C are lower (approximately 30% identity and 50% similarity) than those between AtRAD51C and mammalian RAD51C proteins (approximately 40% identity and 60% similarity). Mutations in the *spnD* gene affect meiosis and oogenesis (Abdu et al., 2003), suggesting that RAD51C may have a conserved function in meiosis in both animals and plants. It is possible that vertebrate *RAD51C* also functions during meiosis; this will need to be tested using mutants, such as mouse knockouts or zebrafish mutants, if they are not embryonic lethal.

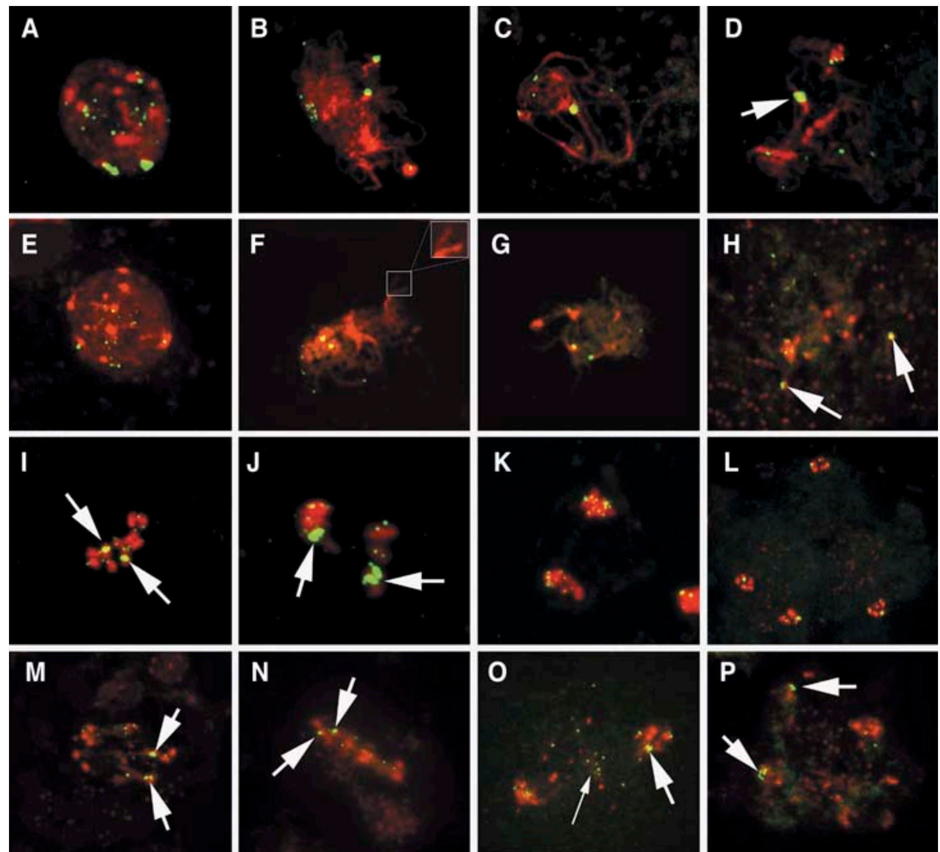

Figure 6. FISH of a telomere probe in wild-type (A–D, I–L) and *atrad51c-1* (E–H, M–P) male meiocytes. A FITC-labeled telomere probe (green) was hybridized to chromosome spreads. Chromosomes were counterstained with DAPI (red). Thick arrows denote the telomere-associated chromosome 1 signals at the centromere region. Stages included are interphase I (A and E); zygotene (B and F; inset at the top right corner of F shows the broken chromosome ends with no telomere signals in *atrad51c-1*); pachytene (C and G); diplotene (D and H; note no interaction occurred between the homologous chromosome I signals); early anaphase I (I and M); late anaphase I (J and N); telophase I (K and O; note that the telomere signals are detected at the center of the mutant cell, as indicated by a thin arrow), and telophase II (L and P).

In contrast to mammalian and chicken *RAD51C*, *AtRAD51C* and *spnD* are not required for mitotic growth under normal conditions. A similar situation exists for *RAD51* homologs. The mammalian and chicken *RAD51* gene is required for the mitotic cell cycle and a mouse *rad51* knockout is embryonic lethal (Lim and Hasty, 1996; Sonoda et al., 1999). In contrast, the Arabidopsis *atrad51-1* mutant exhibits normal vegetative and flower development (Li et al., 2004). Similarly, Drosophila *rad51/spnA* and *C. elegans rad51* mutants are also both viable, although they have elevated sensitivity to radiation (Rinaldo et al., 2002; Staeva-Vieira et al., 2003). Furthermore, the Arabidopsis *atxrcc3* and Drosophila *spnB* (a putative *XRCC3*

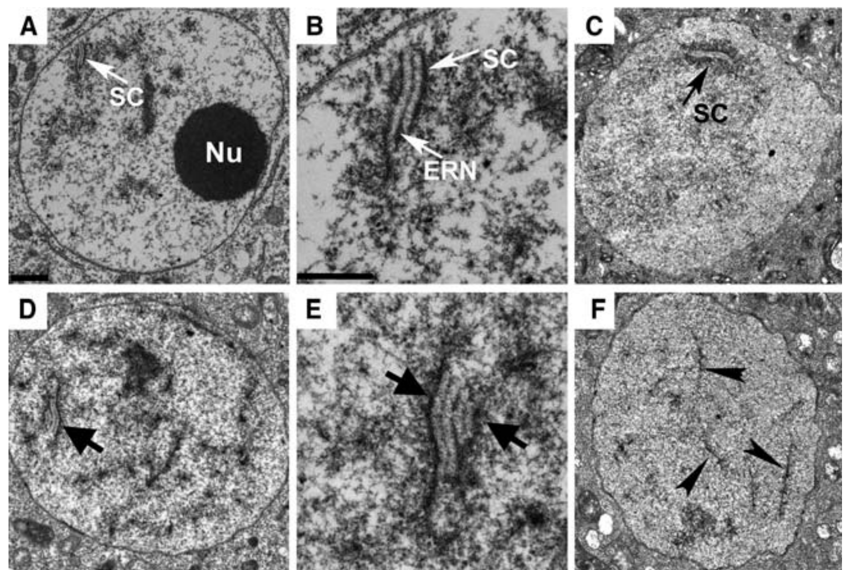

Figure 7. TEM of male meiocyte nuclei in wild-type (A–C) and *atrad51c-1* (D–F) plants. SCs (arrows) were observed at zygotene (A and B) and pachytene (C) stages in the wild type. In *atrad51c-1*, the majority of the axial elements (arrowheads) remained unpaired in nuclei corresponding to zygotene (D) or pachytene (F) stages; an occasional abnormal SC (thick arrows) was observed (D and E). Nu, Nucleolus; ERN: early recombination nodule. Bar in A equals 100 nm (same scale for C, D, and F); bar in B equals 500 nm (same scale for E).

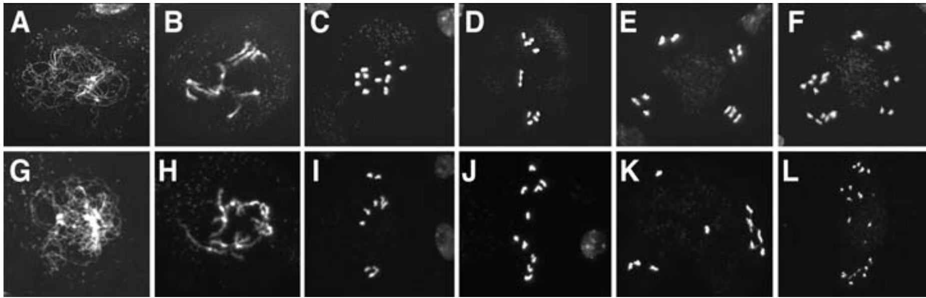

Figure 8. Male meiosis in *atspo11-1* (A–F) and *atspo11-1 atrad51c-1* (G–L). Stages included are zygotene (A and G), diakinesis (B and H), metaphase I (C and I), anaphase I (D and J), metaphase II (E and K), and anaphase II (F and L). (See Figure 4 for a comparison of the wild-type and *atrad51c-1* meioses.)

homolog) mutants are viable (Abdu et al., 2003; Bleuyard and White, 2004). Therefore, *RAD51* orthologs and paralogs seem to be less critical for the mitotic cell cycle than their counterparts in vertebrates. It is thought that *RAD51* and its paralogs are required to maintain genome integrity during the cell cycle because even a small number of DSBs that can occur during DNA replication trigger checkpoint control and cell cycle arrest. The fact that *RAD51* and some of its paralogs are not as critical in plants and Drosophila suggests that mitotic cell cycle regulation might be different in these organisms or that DSBs formed in mitosis could be repaired by alternative pathways (Li et al., 2004).

The Relationship between Pairing, Synapsis, and Recombination

Analyses in yeast, *C. elegans*, and Drosophila indicate that the relationship between synapsis and recombination is not conserved between yeast and invertebrate animals (Keeney et al., 1997; Dernburg et al., 1998; McKim and Hayashi-Hagihara, 1998; Peoples et al., 2002). Previously, phenotypic analysis of the *atspo11-1* mutant suggests that a SPO11-dependent linkage between recombination and synapsis may exist in plants, but synapsis was not described in the *atspo11-1* mutant (Grelon et al., 2001). Our results on synapsis in the *atrad51-1* (Li et al., 2004) and *atrad51c-1* (this study) mutants support a close relationship between recombination and synapsis. Our analysis strongly suggests that yeast and plants share highly conserved mechanisms for coupled recombination and synapsis. Furthermore, the Arabidopsis *syn1* mutant defective in a Rec8 homolog also exhibits chromosome fragmentation. It is possible that the loading of AtRAD51 is affected in the *syn1* mutant, resulting in a failure to process SPO11-1-induced DSBs. Although the relationship between SYN1 and RAD51 in Arabidopsis is not clear, recent studies revealed that Rec8 may promote the assembly of RAD51 foci in rat and possibly in yeast (Eijpe et al., 2003; Zierhut et al., 2004).

The relationship between pairing and recombination is much less clear than that for synapsis and recombination (Page and Hawley, 2003). It is known that the processing of SPO11-dependent DSBs has two possible outcomes—crossovers and non-crossovers—but the role of non-crossovers is not understood (Bishop and Zickler, 2004; Haber et al., 2004). RAD51 is localized in wild-type maize meiocytes initially to a very large number of foci, about 10 times the number of crossover sites, and then the number is reduced to be comparable to that of crossovers (Franklin et al., 1999). Recently, similar observations have been made from localization studies in mouse and Arabidopsis meiocytes (Moens et al., 2002; Mercier et al., 2003). Therefore, RAD51 seems to mark sites of DSBs for both crossovers and non-crossovers in early prophase I. Further analysis of RAD51 foci in pairing defective maize mutants provides strong evidence that RAD51 sites, including the non-crossover sites, are pairing sites (Pawlowski et al., 2003). The possible defect in homolog juxtaposition in the *atrad51c-1* mutant suggests that it might be abnormal in pairing. It is possible that, in plants, RAD51C may also function in a complex with XRCC3, consistent with the observation that both *atrad51c* and *atxrcc3* mutations exhibit similar abnormalities in meiosis (this study; Bleuyard and White, 2004; Bleuyard et al., 2004, 2005; see below). In addition, the number of chromosome fragments in *atrad51c-1* cells exceeds those expected if only the subset of DSBs leading to crossovers is affected by these mutations, indicating that at least some of the non-crossover DSBs also are not repaired. Therefore, strand invasion following the generation of DSBs seems to be a critical mechanism for homology recognition and pairing. It is possible that processing of the DSB sites mediated by RAD51 and its paralogs ensure correct pairing between homologs.

A Model for AtRAD51, AtRAD51C, and AtXRCC3 Functions

Recent reports (Bleuyard and White, 2004; Li et al., 2004; Bleuyard et al., 2005) and this study showed that *atrad51-1*, *atrad51c-1*, and *atxrcc3* mutants have related meiotic phenotypes, suggesting that they are involved in the same process. This is also supported by the observations that chromosome fragmentation in *atrad51-1*, *atxrcc3*, and *atrad51c-1* mutants requires the function of AtSPO11-1 (Bleuyard et al., 2004; Li et al., 2004), although AtXRCC3 may have an additional function during meiosis II because *atxrcc3 atspo11-1* exhibited severe chromosome fragmentation during anaphase II and telophase II (Bleuyard et al., 2004). At

the same time, the fact that each of these three mutants has very severe defects indicates that these genes have distinct functions, even though they code for homologous proteins.

It is known that RAD51 can bind to ssDNA and catalyze strand invasion by the ssDNA into a double-stranded DNA, forming a D-loop (Petukhova et al., 2000). In vitro studies indicate that mammalian XRCC3 and RAD51C can form a complex and RAD51C can complex with other RAD51 paralogs and at least some of these complexes can promote the loading of RAD51 onto DNA (Masson et al., 2001; Sigurdsson et al., 2001; Wiese et al., 2002; Lio et al., 2003). In addition, analysis of mutant cell lines revealed that the formation of RAD51 foci is reduced in both *xrcc3* and *rad51c* mutant cells and the formation of XRCC3 foci on chromosomes depends on RAD51C, but not on RAD51 (Lio et al., 2004; Yoshihara et al., 2004). Therefore, we propose that AtRAD51C and AtXRCC3 form a complex that facilitates the formation of AtRAD51 foci during meiosis. It is possible that RAD51 foci can still form in the absence of AtRAD51C or AtXRCC3, but in reduced numbers. If this is true, it would explain the remaining homolog interaction that was observed in the *atrad51c-1* mutant. In addition, a smaller number of chromosome fragments was found in *atrad51c-1* mutant meiocytes than in *atrad51-1* meiocytes, consistent with the idea that some DSBs might be processed in the *atrad51c-1* mutant.

Mutant analysis has also been reported for the Arabidopsis *DMC1* homolog, *AtDMC1* (Couteau et al., 1999); the *atdmc1* mutant exhibits abnormal chromosome segregation, but not chromosome fragmentation, suggesting that AtDMC1 is not required for the processing of meiotic DSBs. In addition, Drosophila does not possess a *DMC1* ortholog, suggesting that its function may be provided by another *RAD51* paralog. This is different from the situation in budding yeast in which both RAD51 and DMC1 are required for processing SPO11-generated meiotic DSBs (Shinohara et al., 1997). The phenotypic difference of *atrad51-1*, *atrad51c-1*, and *atxrcc3* mutants from that of the *atdmc1* mutant suggests that the *AtRAD51*/*AtRAD51C*/*AtXRCC3* genes may function upstream of *AtDMC1*. Recently, it was observed that a Chinese hamster mutant cell line defective in the *RAD51C* gene exhibits abnormal sister chromatid cohesion (Godthelp et al., 2002), suggesting a possible role for RAD51C in cohesion.

The model proposed above for AtRAD51, AtRAD51C, and AtXRCC3 function may also explain why these proteins are dispensable for the mitotic cell cycle. It is likely that, during normal mitotic growth, only a small number of DSBs are generated, far fewer than the number of DSBs generated by SPO11. If so, the number of RAD51 foci needed during the mitotic cell cycle would be far fewer than during meiosis. Indeed, the *RAD51* mRNA levels are higher in meiotic cells than other cells (Li et al., 2004). Therefore, whereas AtRAD51C and AtXRCC3 may be important for the loading of RAD51 at numerous meiotic foci, they might not be needed when only a few RAD51 foci are needed during the mitotic cell cycle. This idea is supported by the observation that *atrad51c* and *atxrcc3* mutant cells are sensitive to DNA cross-linking agents (Bleuyard and White, 2004; Bleuyard et al., 2005), which presumably induce a large number of DNA lesions. The fact that *atrad51-1* is also normal during mitotic growth suggests that some RAD51 paralog(s) might even be able to substitute for AtRAD51 when relatively little AtRAD51 activity is needed. The hypothesis that RAD51 is the primary player in the homology search and that RAD51C and XRCC3 play supporting roles is also supported by phenotypes of Drosophila mutants. The Drosophila *spnA* (*rad51*) mutant is hypersensitive to radiation, but *spnB* and *spnD* (*xrcc3* and *rad51c*) mutants are completely normal during mitotic development (Abdu et al., 2003; Staeva-Vieira et al., 2003), suggesting that SpnB and SpnD are less important than SpnA. It is possible that the molecular interactions between RAD51 and its paralogs RAD51C and XRCC3 are conserved between meiotic recombination in plants and mitotic DNA repair in Drosophila and mammals. Further experiments are needed to test the possible functional overlap between and distinct biochemical activities of AtRAD51, AtRAD51C, and AtXRCC3, as well as other RAD51 paralogs.

MATERIALS AND METHODS

Plant Materials

The *atrad51c-1* T-DNA insertional line was obtained from the SALK stock of Arabidopsis (*Arabidopsis thaliana*) T-DNA insertion lines. Both the wild-type and the *atrad51c-1* mutant plants are of the Columbia ecotype. Unless otherwise indicated, the plants were grown under long-day conditions (16-h day/8-h night) at 22°C ± 2°C.

Phenotypic Analysis

Plants were photographed using a Sony (Tokyo) digital camera DSC-F707, and photographs of other fresh plant samples (flowers and siliques) were obtained using a Nikon (Tokyo) dissecting microscope with an Optronics (Goleta, CA) digital camera. Developing microspores from fresh floral buds and anthers prior to dehiscence were processed as reported for examining gametophyte development (Azumi et al., 2002). Chromosome spreads were prepared as previously described (Ross et al., 1996) and stained with DAPI (1 μg/mL). Digital images were obtained using a Nikon E400 fluorescence microscope with an Optronics camera. Female meiosis was analyzed essentially as described (Armstrong et al., 2001), using floral buds at stages 10 to 11 (Smyth et al., 1990) and photographed using a Nikon E800 fluorescence microscope with ImagePro software (Media Cybernetics, Silver Spring, MD) and a Hamamatsu CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan). Mitosis images were obtained from root tip cells of 4-d-old seedlings (Li et al., 2004).

RT-PCR and RNA In Situ Hybridizations

RNA samples were extracted from young inflorescence tissues of wild-type and *atrad51c-1* mutant plants. One microgram of total RNA from each sample was treated with RNase-free DNase I (Invitrogen, Carlsbad, CA) followed by inactivation of the DNase I. Reverse transcription was performed using Supertranscriptase II (Invitrogen) and synthesized cDNA was used as

the template for PCRs. The primers used for the PCRs were oMC 1594 (5′-ATGATTTCATTTGGGCGGCGTA-3′) and oMC 1593 (5′-CCTCCA-AGACCACCACACTCA-3′). As a control, the *APT1* cDNA in the same samples was amplified using primer pair oMC 571 (5′-TCCCAGAATCGCTA-AGATTGCC-3′) and oMC 572 (5′-CCTTTCCCTTAAGCTCTG-3′), based on a report that this gene is expressed constitutively in different Arabidopsis organs (Moffatt et al., 1994). For RNA in situ hybridization, sections of wild-type immature floral buds were hybridized with ^{35}S-labeled *AtRAD51C* antisense and sense probes synthesized from a cDNA clone (amplified with primers oMC 1605 [5′-CAACGATAGGGAGTTAAAAGACTTTCATCG-3′], and oMC 1595 [5′-CGGCGCGCCGGATCCCGGGACCGAATATTTGCCCAT-TTGGGC-3′]), following previously described procedures (Drews et al., 1991; Flanagan et al., 1996). The similarities of *AtRAD51C* to other *RAD51* paralogs are too low (44.4%–49.1%) for cross-hybridization.

Analysis of the *atspo11-1 atrad51c-1* Double Mutant

Crosses were made between *AtSPO11-1/atspo11-1* and *AtRAD51C/atrad-51c-1* plants and resulting double-heterozygous F_1 plants (*AtRAD51C/atrad51c-1*; *AtSPO11-1/atspo11-1*) were identified with gene-specific primers. The progeny of the double-heterozygous plants were genotyped with gene-specific primers and chromosome behavior in male meiosis of double-mutant plants was analyzed following DAPI staining. The primers used for genotyping the plants include oMC 1662 (5′-TGAACTCCTCCTTGGAGCTAT-GTTGG-3′); oMC 1451 (5′-CCAATGCGATTTGCTCGGTGTAACT-3′); oMC 645 (5′-GCGTGGACCGCTTGCTGCAACT-3′); oMC 703 (5′-ACGTATCGG-GCCTAAATTCC-3′); oMC 704 (5′-TTTGGAGATCTTCCTTCAGCC-3′); and oMC 705 (5′-ACTGGGATTCGTCTTGGACA-3′).

FISH

Inflorescences were fixed in acetic alcohol (ethanol:glacial acetic acid, 3:1) for 2 h at room temperature and stored at −20°C after replenishing the fixative. Staged anthers were spread onto slides and subjected to FISH using previously published procedures (Fransz et al., 1996; Caryl et al., 2000) with modifications. The samples on slides were treated with a solution of 70% formamide in 2× SSC for 2 min at 80°C and dehydrated immediately through a graded ethanol series (5 min each in 70%, 90%, and 100%) at −20°C. The slides were dried at room temperature before the application of the probe. The pAL1 clone containing a pericentromeric 180-bp repeat (pAL1-CEN) was used to detect centromere sequences (Martinez-Zapater et al., 1986). Primary PCR amplification of pAL1-CEN was conducted using the M13 forward and reverse primers followed by random primer labeling in the presence of biotin high prime-labeled dUTP (Roche, Indianapolis). The biotin-labeled probe was used in hybridization solution at 5 μg/mL and detected with 10 μg/mL fluorescein-conjugated streptavidin. Chromosome 1-associated telomere sequences were detected by hybridization with the 5′-end fluorescein isothiocyanate (FITC)-labeled oligonucleotide probe, FITC-$(CCCTAAA)_6$ at 5 μg/mL. Slides were counterstained with DAPI, mounted, and viewed as above.

TEM

For TEM, the fixation and infiltration procedures were as described previously (Owen and Makaroff, 1995) with minor modifications (Li et al., 2004). 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 (JEOL, Tokyo). At least 50 ultrathin sections were analyzed for each bud.

ACKNOWLEDGMENTS

We thank J. Ecker and the Arabidopsis Biological Resource Center (ABRC) for providing the T-DNA insertional line, M. Grelon for the *atspo11-1* seeds, Y. Hu for RNA in situ experiments, R. Cyr, G. Ning, M. Hazen, R. Haldeman, W. Hu, and A. Wijeratne for advice and technical assistance, A. Omeis and J. Wang for plant care, and C. Chen, O. Hamant, C. Hendrix, S.J. Hord, A. Wijeratne, and the reviewers for helpful comments on this manuscript.

Received December 14, 2004; revised March 27, 2005; accepted March 27, 2005; published May 27, 2005.

LITERATURE CITED

Abdu U, Gonzalez-Reyes A, Ghabrial A, Schupbach T (2003) The Drosophila *spn-D* gene encodes a RAD51C-like protein that is required exclusively during meiosis. Genetics **165:** 197–204

Albala JS, Thelen MP, Prange C, Fan W, Christensen M, Thompson LH, Lennon GG (1997) Identification of a novel human RAD51 homolog, RAD51B. Genomics **46:** 476–479

Armstrong SJ, Franklin FC, Jones GH (2001) Nucleolus-associated telomere clustering and pairing precede meiotic chromosome synapsis in *Arabidopsis thaliana.* J Cell Sci **114:** 4207–4217

Azumi Y, Liu D, Zhao D, Li W, Wang G, Hu Y, Ma H (2002) Homolog interaction during meiotic prophase I in *Arabidopsis* requires the *SOLO DANCERS* gene encoding a novel cyclin-like protein. EMBO J **21:** 3081–3095

Bishop DK, Park D, Xu L, Kleckner N (1992) DMC1: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:** 439–456

Bishop DK, Zickler D (2004) Early decision; meiotic crossover interference prior to stable strand exchange and synapsis. Cell **117:** 9–15

Bleuyard JY, Gallego ME, Savigny F, White CI (2005) Differing requirements for the *Arabidopsis* Rad51 paralogs in meiosis and DNA repair. Plant J **41:** 533–545

Bleuyard JY, Gallego ME, White CI (2004) The *atspo11-1* mutation rescues *atxrcc3* meiotic chromosome fragmentation. Plant Mol Biol **56:** 217–224

Bleuyard JY, White CI (2004) The *Arabidopsis* homologue Xrcc3 plays an essential role in meiosis. EMBO J **23:** 439–449

Borner GV, Kleckner N, Hunter N (2004) Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell **117:** 29–45

Caryl AP, Armstrong SJ, Jones GH, Franklin FC (2000) A homologue of the yeast *HOP1* gene is inactivated in the *Arabidopsis* meiotic mutant *asy1.* Chromosoma **109:** 62–71

Couteau F, Belzile F, Horlow C, Grandjean O, Vezon D, Doutriaux MP (1999) Random chromosome segregation without meiotic arrest in both male and female meiocytes of a *dmc1* mutant of Arabidopsis. Plant Cell **11:** 1623–1634

Dernburg AF, McDonald K, Moulder G, Barstead R, Dresser M, Villeneuve AM (1998) Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell **94:** 387–398

Dosanjh MK, Collins DW, Fan W, Lennon GG, Albala JS, Shen Z, Schild D (1998) Isolation and characterization of *RAD51C*, a new human member of the *RAD51* family of related genes. Nucleic Acids Res **26:** 1179–1184

Drews GN, Bowman JL, Meyerowitz EM (1991) Negative regulation of the Arabidopsis homeotic gene *AGAMOUS* by the *APETALA2* product. Cell **65:** 991–1002

Eijpe M, Offenberg H, Jessberger R, Revenkova E, Heyting C (2003) Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1beta and SMC3. J Cell Biol **160:** 657–670

Flanagan CA, Hu Y, Ma H (1996) Specific expression of the *AGL1* MADS-box gene suggests regulatory functions in *Arabidopsis* gynoecium and ovule development. Plant J **10:** 343–353

Franklin AE, McElver J, Sunjevaric I, Rothstein R, Bowen B, Cande WZ (1999) Three-dimensional microscopy of the Rad51 recombination protein during meiotic prophase. Plant Cell **11:** 809–824

Fransz PF, Alonso-Blanco C, Liharska TB, Peeters AJ, Zabel P, de Jong JH (1996) High-resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence in situ hybridization to extended DNA fibres. Plant J **9:** 421–430

Godthelp BC, Wiegant WW, van Duijn-Goedhart A, Scharer OD, van Buul PP, Kanaar R, Zdzienicka MZ (2002) Mammalian Rad51C contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability. Nucleic Acids Res **30:** 2172–2182

Grelon M, Vezon D, Gendrot G, Pelletier G (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. EMBO J **20:** 589–600

Haber JE, Ira G, Malkova A, Sugawara N (2004) Repairing a double-strand chromosome break by homologous recombination: revisiting Robin Holliday's model. Philos Trans R Soc Lond B Biol Sci **359:** 79–86

Keeney S, Giroux CN, Kleckner N (1997) Meiosis-specific DNA double-

strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384

Li W, Chen C, Markmann-Mulisch U, Timofejeva L, Schmelzer E, Ma H, Reiss B (2004) The *Arabidopsis AtRAD51* gene is dispensable for vegetative development but required for meiosis. Proc Natl Acad Sci USA **101:** 10596–10601

Lim DS, Hasty P (1996) A mutation in mouse *rad51* results in an early embryonic lethal that is suppressed by a mutation in p53. Mol Cell Biol **16:** 7133–7143

Lio YC, Mazin AV, Kowalczykowski SC, Chen DJ (2003) Complex formation by the human Rad51B and Rad51C DNA repair proteins and their activities in vitro. J Biol Chem **278:** 2469–2478

Lio YC, Schild D, Brenneman MA, Redpath JL, Chen DJ (2004) Human Rad51C deficiency destabilizes XRCC3, impairs recombination, and radiosensitizes S/G2-phase cells. J Biol Chem **279:** 42313–42320

Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR, Brookman KW, Siciliano MJ, Walter CA, Fan W, et al (1998) XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol Cell **1:** 783–793

Liu N, Schild D, Thelen MP, Thompson LH (2002) Involvement of Rad51C in two distinct protein complexes of Rad51 paralogs in human cells. Nucleic Acids Res **30:** 1009–1015

Liu Y, Masson JY, Shah R, O'Regan P, West SC (2004) RAD51C is required for Holliday junction processing in mammalian cells. Science **303:** 243–246

Martinez-Zapater J, Estelle MA, Somerville CR (1986) A highly repeated DNA sequence in *Arabidopsis thaliana.* Mol Gen Genet **204:** 417–423

Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC (2001) Identification and purification of two distinct complexes containing the five RAD51 paralogs. Genes Dev **15:** 3296–3307

McKim KS, Hayashi-Hagihara A (1998) *mei-W68* in *Drosophila melanogaster* encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. Genes Dev **12:** 2932–2942

Mercier R, Armstrong SJ, Horlow C, Jackson NP, Makaroff CA, Vezon D, Pelletier G, Jones GH, Franklin FC (2003) The meiotic protein SWI1 is required for axial element formation and recombination initiation in *Arabidopsis.* Development **130:** 3309–3318

Moens PB, Kolas NK, Tarsounas M, Marcon E, Cohen PE, Spyropoulos B (2002) The time course and chromosomal localization of recombination-related proteins at meiosis in the mouse are compatible with models that can resolve the early DNA-DNA interactions without reciprocal recombination. J Cell Sci **115:** 1611–1622

Moffatt BA, McWhinnie EA, Agarwal SK, Schaff DA (1994) The adenine phosphoribosyltransferase-encoding gene of *Arabidopsis thaliana.* Gene **143:** 211–216

Obayashi T, Okegawa T, Sasaki-Sekimoto Y, Shimada H, Masuda T, Asamizu E, Nakamura Y, Shibata D, Tabata S, Takamiya K, et al (2004) Distinctive features of plant organs characterized by global analysis of gene expression in Arabidopsis. DNA Res **11:** 11–25

Osakabe K, Yoshioka T, Ichikawa H, Toki S (2002) Molecular cloning and characterization of RAD51-like genes from Arabidopsis thaliana. Plant Mol Biol **50:** 71–81

Owen HA, Makaroff CA (1995) Ultrastructure of microsporogenesis and microgametogenesis in *Arabidopsis thaliana* (L.) Heynh. ecotype *Wassilewskija* (Brassicaceae). Protoplasma **185:** 7–21

Page SL, Hawley RS (2003) Chromosome choreography: the meiotic ballet. Science **301:** 785–789

Pawlowski WP, Golubovskaya IN, Cande WZ (2003) Altered nuclear distribution of recombination protein RAD51 in maize mutants suggests the involvement of RAD51 in meiotic homology recognition. Plant Cell **15:** 1807–1816

Pawlowski WP, Golubovskaya IN, Timofejeva L, Meeley RB, Sheridan WF, Cande WZ (2004) Coordination of meiotic recombination, pairing, and synapsis by PHS1. Science **303:** 89–92

Peoples TL, Dean E, Gonzalez O, Lambourne L, Burgess SM (2002) Close, stable homolog juxtaposition during meiosis in budding yeast is dependent on meiotic recombination, occurs independently of synapsis, and is distinct from DSB-independent pairing contacts. Genes Dev **16:** 1682–1695

Petukhova G, Sung P, Klein H (2000) Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. Genes Dev **14:** 2206–2215

Pittman DL, Weinberg LR, Schimenti JC (1998) Identification, characterization, and genetic mapping of *Rad51D*, a new mouse and human *RAD51/RecA*-related gene. Genomics **49:** 103–111

Rinaldo C, Bazzicalupo P, Ederle S, Hilliard M, La Volpe A (2002) Roles for *Caenorhabditis elegans* Rad51 in meiosis and in resistance to ionizing radiation during development. Genetics **160:** 471–479

Ross KJ, Fransz P, Jones GH (1996) A light microscopic atlas of meiosis in *Arabidopsis thaliana.* Chromosome Res **4:** 507–516

Schild D, Lio YC, Collins DW, Tsomondo T, Chen DJ (2000) Evidence for simultaneous protein interactions between human Rad51 paralogs. J Biol Chem **275:** 16443–16449

Schwarzacher T (2003) Meiosis, recombination and chromosomes: a review of gene isolation and fluorescent in situ hybridization data in plants. J Exp Bot **54:** 11–23

Shinohara A, Gasior S, Ogawa T, Kleckner N, Bishop DK (1997) *Saccharomyces cerevisiae* recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. Genes Cells **2:** 615–629

Shinohara A, Ogawa H, Matsuda Y, Ushio N, Ikeo K, Ogawa T (1993) Cloning of human, mouse and fission yeast recombination genes homologous to *RAD51* and *recA.* Nat Genet **4:** 239–243

Shinohara A, Ogawa H, Ogawa T (1992) Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell **69:** 457–470

Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, Sung P (2001) Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. Genes Dev **15:** 3308–3318

Smyth DR, Bowman JL, Meyerowitz EM (1990) Early flower development in Arabidopsis. Plant Cell **2:** 755–767

Sonoda E, Sasaki MS, Morrison C, Yamaguchi-Iwai Y, Takata M, Takeda S (1999) Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. Mol Cell Biol **19:** 5166–5169

Staeva-Vieira E, Yoo S, Lehmann R (2003) An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. EMBO J **22:** 5863–5874

Sung P (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science **265:** 1241–1243

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983) The double-strand-break repair model for recombination. Cell **33:** 25–35

Takata M, Sasaki MS, Tachiiri S, Fukushima T, Sonoda E, Schild D, Thompson LH, Takeda S (2001) Chromosome instability and defective recombinational repair in knockout mutants of the five *Rad51* paralogs. Mol Cell Biol **21:** 2858–2866

Tebbs RS, Zhao Y, Tucker JD, Scheerer JB, Siciliano MJ, Hwang M, Liu N, Legerski RJ, Thompson LH (1995) Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the *XRCC3* DNA repair gene. Proc Natl Acad Sci USA **92:** 6354–6358

Wiese C, Collins DW, Albala JS, Thompson LH, Kronenberg A, Schild D (2002) Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells. Nucleic Acids Res **30:** 1001–1008

Yoshihara T, Ishida M, Kinomura A, Katsura M, Tsuruga T, Tashiro S, Asahara T, Miyagawa K (2004) XRCC3 deficiency results in a defect in recombination and increased endoreduplication in human cells. EMBO J **23:** 670–680

Zickler D, Kleckner N (1999) Meiotic chromosomes: integrating structure and function. Annu Rev Genet **33:** 603–754

Zierhut C, Berlinger M, Rupp C, Shinohara A, Klein F (2004) Mnd1 is required for meiotic interhomolog repair. Curr Biol **14:** 752–762

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol **136:** 2621–2632