Formation of interference-sensitive meiotic cross-overs requires sufficient DNA leading-strand elongation

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Meiosis halves diploid genomes to haploid and is essential for sexual reproduction in eukaryotes. Meiotic recombination ensures physical association of homologs and their subsequent accurate segregation and results in the redistribution of genetic variations among progeny. Most organisms have two classes of cross-overs (COs): interference-sensitive (type I) and -insensitive (type II) COs. DNA synthesis is essential for meiotic recombination, but whether DNA synthesis has a role in differentiating meiotic CO pathways is unknown. Here, we show that *Arabidopsis POL2A*, the homolog of the yeast *DNA polymerase-ε* (a leading-strand DNA polymerase), is required for plant fertility and meiosis. Mutations in *POL2A* cause reduced fertility and meiotic defects, including abnormal chromosome association, improper chromosome segregation, and fragmentation. Observation of prophase I cell distribution suggests that *pol2a* mutants likely delay progression of meiotic recombination. In addition, the residual COs in *pol2a* have reduced CO interference, and the double mutant of *pol2a* with *mus81*, which affects type II COs, displayed more severe defects than either single mutant, indicating that *POL2A* functions in the type I pathway. We hypothesize that sufficient leading-strand DNA elongation promotes formation of some type I COs. Given that meiotic recombination and DNA synthesis are conserved in divergent eukaryotes, this study and our previous study suggest a novel role for DNA synthesis in the differentiation of meiotic recombination pathways.

meiotic recombination | DNA synthesis | DNA polymerase-ε | Arabidopsis

Eukaryotic sexual reproduction requires meiosis to produce haploid gametes from parents. Meiotic recombination not only ensures physical association of homologs to form bivalents but also, promotes their accurate segregation, and it is initiated by the formation of SPO11-programmed DNA double-strand breaks (DSBs) (1). Repair of meiotic DSBs is evolutionarily important, because it redistributes genetic variants in regions flanking the site of recombination [cross-overs (COs)] or gene conversion without flanking exchange [non-COs (NCOs)], resulting in offspring that are genetically distinct from parents and siblings (2, 3). Although DNA synthesis is thought to be essential for DSB repair (DSBR) (4), molecular genetic studies are quite limited. Recent genome-wide studies provide evidence that meiotic COs and NCOs may require different amounts of DNA synthesis (2, 3, 5–7), but the underlying mechanism remains unknown.

Premeiotic chromosome replication requires the same DNA replication machinery as that for mitotic DNA replication (8), which is carried out by coordinated activities of DNA polymerase-α (Pol-α), -δ, and -ε (9) as well as three ancillary complexes: proliferating cell nuclear antigen, replication factor C (RFC), and the minichromosome maintenance complex. Pol-α and -δ are primarily required for lagging-strand DNA synthesis (9, 10), whereas Pol-ε carries out leading-strand DNA synthesis (11). Given that DNA synthesis is essential for S-phase replication during the mitotic cell cycle, complete disruption of these genes causes lethality, which was observed in yeast, plants, and humans (11–14), making it difficult to

study their functions in meiosis. In *Saccharomyces cerevisiae*, a partially defective mutation of *Pol-δ* causes no DNA replication defect but exhibits reduction in both the number of COs and the length of meiotic gene conversion tracts (15). In *Arabidopsis*, a partial loss of function of DNA replication factor C1 (*RFC1*) results in reduction of type I COs (16). These findings suggest that DNA synthesis likely affects the length of strand exchange intermediates and influences their resolution toward different CO pathways. Moreover, genome-wide analyses in both yeast and plants showed that meiotic gene conversion tracts vary in length, ranging from several hundreds to thousands of nucleotides, and that CO-associated tracts are usually longer than those of NCOs (2, 3, 5–7). This difference raises a question of whether there is any difference in DNA synthesis among CO-associated tracts with different lengths; however, this hypothesis has not been tested experimentally. Our previous research with *RFC1* supports the idea that lagging-strand synthesis promotes type I COs (16). Here, we test the hypothesis that sufficient leading-strand synthesis is important for type I COs.

To investigate the role of leading-strand DNA elongation in meiotic recombination, we analyzed two partial loss-of-function alleles of the largest subunit of DNA Pol-ε (hereafter designated as *POL2A*). They have normal vegetative development but show

Significance

Meiosis is essential for eukaryotic sexual reproduction, and meiotic recombination redistributes genetic variations among progeny. Homologous recombination is also important for DNA repair and genome stability. Current recombination models include DNA synthesis, particularly leading-strand synthesis, but its role has not been tested experimentally. Here, we showed that mutations in a DNA polymerase required for leading-strand elongation caused reduced fertility, defects in meiotic chromosome fragmentation and segregation, and reduction of interference-sensitive cross-overs (COs). Because the majority of meiotic COs are produced from the interference-sensitive pathway in budding yeast, mammals, and flowering plants, the discovery of leading-strand elongation in the differentiation of meiotic recombination pathways has important implications in understanding of human reproductive health and crop breeding.
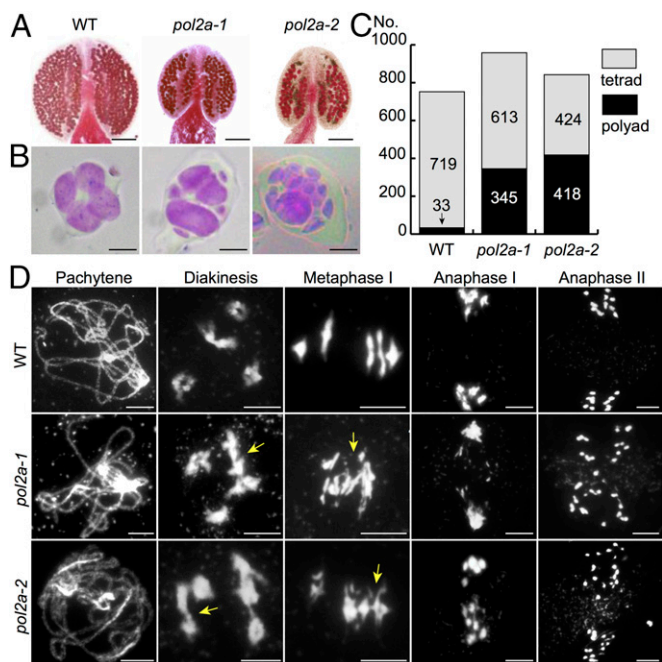
Fig. 1. Mutations in *POL2A* cause reduced fertility and meiotic defects in *Arabidopsis*. (*A*) Alexander red staining of WT, *pol2a-1*, and *pol2a-2* anthers. (*B*) Tetrad observation of the WT, *pol2a-1*, and *pol2a-2*. (*C*) A histogram of tetrads vs. polyads in the WT and mutants. (*D*) Chromosome morphologies of the WT, *pol2a-1*, and *pol2a-2* by DAPI staining. Arrows indicate probable nonhomologous associations. (Scale bars: *A*, 100 μm; *B*, 10 μm; *D*, 5 μm.)

defects in fertility and meiosis. Additional analyses show that *POL2A* has a role in the formation of normal bivalents and promoting formation of meiotic type I COs. Similar mutations in the yeast homolog showed reduced DNA replication efficiency in mitotic division (17–19), leading us to hypothesize that sufficient leading-strand DNA elongation could be a critical step for differentiation of types I and II COs. These observations provide a novel insight that meiotic types I and II COs are differentially dependent on leading-strand synthesis activity.

Results

***POL2A* Is Required for Normal Meiosis in *Arabidopsis*.** DNA Pol-ε is called POL2 or CDC20 in the budding and fission yeasts, respectively (20). Studies in yeast, flies, and humans showed that the POL2 N terminus is important for elongation and fidelity of DNA replication (19, 21), whereas the C-terminal region is essential for cell viability. *Arabidopsis* has two *POL2* homologs (*POL2A* and *POL2B*), and *POL2A* was expressed in male meiocytes, whereas *POL2B* was almost undetectable (22), suggesting a potential function of *POL2A* in meiosis. To test this hypothesis, we obtained three *pol2a* mutants (*SI Appendix*, Fig. S1*A*). Among them, *pol2a-1* (SALK_096341) is a transfer deoxyribonucleic acid (T-DNA) insertional allele in the 12th intron and has been shown to reduce abscisic acid (ABA) sensitivity and fertility (13) (Fig. 1*A* and *SI Appendix*, Fig. S1*A*), *pol2a-2* (formally named *til1-4*) is a point mutation and displays a prolonged cell cycle during embryo development (12), and *pol2a-3* (SALK_124217) also carries a T-DNA insertion affecting the conserved C-terminal domain (*SI Appendix*, Fig. S1 *A* and *B*) and exhibits aborted early embryo development (*SI Appendix*, Fig. S1*F*), consistent with an essential role of Pol-ε in DNA replication (19, 21). The lack of homozygous *pol2a-3* plants precluded the use of this mutant to analyze possible meiotic defects.

Both *pol2a-1* and *pol2a-2* plants had normal growth, including plant size and floral organ identity and number (*SI Appendix*, Fig. S1 *C* and *E*), indicating no obvious defects in their mitotic DNA

replication. However, they had greatly reduced fertility (*SI Appendix*, Fig. S1*D*). Alexander staining of pollen viability showed that viable pollen grains were obviously reduced in *pol2a-1* (294 ± 74; *n* = 16) and *pol2a-2* (181 ± 73; *n* = 16) in contrast to the WT (542 ± 38; *n* = 29) (Fig. 1*A*). In addition, unlike WT tetrads with four microspores (Fig. 1*B*), male meiocytes of *pol2a-1* and *pol2a-2* produced polyads with variable microspore numbers ranging from five to eight with abnormality rates of 36.0% and 49.6%, respectively (Fig. 1 *B* and *C*), suggesting meiotic defects. Pollination of either *pol2a* mutant pistil with WT pollen grains also showed a defect in female fertility (*SI Appendix*, Table S1). We examined the *POL2A* expression level (*SI Appendix*, Fig. S1*G*) and found a significant reduction in *pol2a-1*, indicating that *pol2a-1* is not a null mutant. In contrast, the mRNA level was slightly elevated in *pol2a-2*, indicating that the point mutation in the N-terminal region of *POL2A* likely diminishes its function, consistent with the observations in the budding and fission yeasts (19, 21).

***POL2A* Is Required for Normal Chromosome Interactions in Meiotic Prophase I.** The observation of polyads in *pol2a* suggests a meiotic defect; we, therefore, compared chromosome spreads of the WT and *pol2a*. Chromosome morphology showed no obvious differences up to the zygotene stage between the mutants and the WT (*SI Appendix*, Fig. S2). At pachytene, 73% (*n* = 100) *pol2a* meiocytes were similar to those of the WT (Fig. 1*D*), whereas the remaining 27% pachytene-like cells showed abnormal chromosome condensation and association (*SI Appendix*, Fig. S2). After the dissociation of the synaptonemal complex (SC) after the pachytene stage, WT meiocytes showed continued association at diakinesis of condensed homologs in five bivalents, which aligned along the equatorial plane at metaphase I, and subsequent separation of homologs at anaphase I. By contrast, both *pol2a-1* and *pol2a-2* meiocytes had entanglement between three or more chromosomes indicative of abnormal associations from diakinesis to metaphase I (Fig. 1*D*) and chromosome fragmentation at anaphase I and II, indicating the importance of *POL2A* for normal chromosome interactions. These findings also provide an explanation for the formation of polyads and reduced pollen viability.

To test whether the associated chromosomes in *pol2a-1* are homologs or nonhomologs, we performed chromosome-specific FISH using bacterial artificial chromosome (BAC) probe F1N21 for chromosome 1 (16). In the WT and *pol2a-1*, the F1N21 signals were similar at leptotene and zygotene (*SI Appendix*, Fig. S3). At pachytene, the WT had only one signal representing the closely synapsed homologous chromosome 1 arms (Fig. 2). In the *pol2a-1* pachytene-like cells (*n* = 31), 77.4% had one signal similar to that in the WT, but 22.6% had two signals (Fig. 2), consistent with the results from DAPI-stained chromosome spreads described above. At metaphase I, 53.1% of *pol2a-1* meiocytes (*n* = 32) had two signals that were on widely separate chromosomes; one of the signals appeared to be on a bivalent, whereas the other was often in a cluster of chromosomes in contrast to the one focus observed on one bivalent in the WT (Fig. 2). Similar results were also obtained using another BAC probe F4P13 located in the chromosome 3 left arm, with 13.2% of pachytene cells (*n* = 76) and 15.8% of metaphase I cells (*n* = 19) exhibiting abnormal separation of BAC signals (*SI Appendix*, Fig. S4). These results indicate that the two homologous chromosome 1 or 3 arms were sometimes separated in the mutant and suggest that they could associate with nonhomologs.

To determine whether the observed abnormal association and chromosome fragmentation in *pol2a-1* are dependent on programmed meiotic DSBs, we tested for possible genetic interaction of *POL2A* with genes for DSB generation and repair. *Arabidopsis* SPO11-1 is required for DSB formation, chromosome pairing, and bivalent formation (Fig. 3*C*) (23). Repair of the DSBs requires RAD51 (24), and failed repair in *atrad51* results in chromosome fragmentation (Fig. 3*E*), which is absent in the *spo11-1 rad51* double mutant (24). Because meiotic DNA synthesis is proposed to occur after RAD51-dependent single-strand invasion, we hypothesized that chromosome fragmentation in *pol2a-1* depends on SPO11-1 and that *pol2a-1* would not enhance the defects of *rad51*. To test these
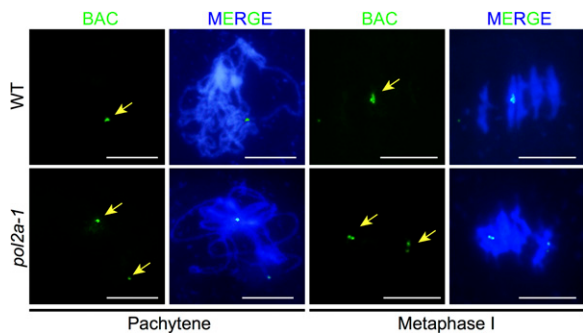
PLANT BIOLOGY

Fig. 2. *POL2A* is required for homologous chromosome interactions in meiotic prophase I. BAC FISH with F1N21 probe at pachytene and metaphase I in the WT and *pol2a-1* are shown. Arrows indicate the BAC probe signals. (Scale bar: 5 μm.)

hypotheses, we analyzed the double mutants with *spo11-1* and *rad51*. As expected, the chromosome fragmentation in *pol2a-1* was dependent on SPO11-1 (Fig. 3*D*), whereas the *pol2a-1 rad51-3* double-mutant meiocytes resembled the *rad51-3* single mutant, lacking distinctive pachytene chromosomes and bivalents (Fig. 3*F*). These results are consistent with the idea that POL2A acts downstream of RAD51.

Because the primary function of POL2A is DNA replication, several studies have shown that disruption of the DNA Pol-ε exonuclease domain causes reduced efficiency of mitotic DNA synthesis in yeast and plants (12, 13, 25). Thus, the *pol2a-1* phenotype may be caused by abnormal premeiotic DNA replication. However, the normal vegetative morphology of *pol2a-1* plant suggests that its mitotic DNA replication is unaffected (*SI Appendix*, Fig. S1*C*). Furthermore, analysis of meiotic chromosomes in *pol2a-1 spo11-1–1* showed that premeiotic replication produces 20 chromatids (*SI Appendix*, Fig. S5), suggesting normal premeiotic DNA replication in *pol2a-1*. The lethality of *pol2a-3* provides a means to test the phenotype of plants with possibly even less *POL2A* activity than *pol2a-1* by generating the *pol2a-1/pol2a-3* heteroallelic plants, which showed similar developmental morphology to *pol2a-1* (*SI Appendix*, Fig. S6). Likewise, the *pol2a-1/pol2a-3* meiocytes showed meiotic phenotypes similar to those of *pol2a-1* and *pol2a-2* (*SI Appendix*, Fig. S7). We also generated transgenic plants with a *POL2A* RNAi construct driven by the meiosis-specific *DMC1* promoter and observed a meiotic defect similar to those of both single mutants (*SI Appendix*, Fig. S7). These results show that the meiotic phenotypes in *pol2a* are probably caused by loss of meiosis-specific function rather than partial loss of a general function.

POL2A Is Not Required for DSB Formation and Synapsis but Promotes Progression of Meiotic Recombination. If these mutations in *POL2A* do not affect premeiotic DNA replication, another possible role for POL2A in meiosis is DNA synthesis for meiotic recombination (4). Defects in DNA synthesis could result in abnormal persistence of meiotic DSBs (16). To detect DSBs in *pol2a-1*, we examined the distribution of phosphorylated histone H2AX (γ-H2AX), which specifically marks DSBs (26). At zygotene, when DSBs are formed, no significant difference in γ-H2AX foci between the WT (189 ± 26; $n = 39$) and *pol2a-1* (185 ± 16; $n = 32$) was observed (Fig. 4 *A* and *B*). We also examined the RAD51 recombinase, which mediates ssDNA invasion during recombination (24). No obvious difference was found between the WT (188 ± 24; $n = 15$) and *pol2a-1* (187 ± 11; $n = 14$) at zygotene (Fig. 4 *A* and *B*). Together, the presence of γ-H2AX and RAD51 foci provides strong evidence that *POL2A* does not influence meiotic DSB formation. In contrast, at pachytene, the numbers of γ-H2AX and RAD51 foci in the WT decreased to 57 ± 12 ($n = 55$) and 51 ± 14 ($n = 70$), respectively, indicating completion of the majority of DSBR. At the equivalent stage, the *pol2a-1* meiocytes maintained significantly more foci of γ-H2AX (113 ± 27; $n = 19$; $P = 1.4E-08$) and RAD51 (87 ± 15; $n = 5$; $P = 0.003$) (Fig. 4 *A* and *B*), suggesting delayed DSBR.

After formation of DSBs, synapsis is critical for stabilizing homolog interactions during meiotic prophase I (27). We examined the distributions of ASY1 and ZYP1, which mediate formation of the axial element and the SC, respectively (27). At zygotene, ASY1 showed linear signals with chromosomes in the WT and *pol2a-1*, and initiation of ZYP1 signals was also similar (*SI Appendix*, Fig. S8 *A–H*). At pachytene, the ASY1 signals in *pol2a-1* seemed normal, and ZYP1 also had a linear distribution on the chromosomes in *pol2a-1* ($n = 128$), similar to that of the WT (*SI Appendix*, Fig. S8 *I–P*). We conclude that ASY1 axis formation is normal in *pol2a-1*, and the normal localization of ZYP1 also suggests no obvious defect for SC formation in *pol2a-1*. However, the earlier described separate FISH signals for two BAC probes on different chromosomes suggest that sometimes synapsis occurred between nonhomologs.

Although there are no molecular markers for DNA synthesis during meiotic recombination, it presumably takes place in prophase I (5) soon after DSB formation. In *Arabidopsis*, DSBs are formed from leptotene to early zygotene, and their repair is completed by pachytene. We, therefore, compared the distribution of meiocytes from leptotene to pachytene at the four most relevant time points between the WT, *pol2a-1*, and *pol2a-2* (Fig. 4*C*). Leptotene cells in the WT and mutants showed a higher level at zeitgeber time 4 (ZT4) (Fig. 4*C*). Zygotene cells in the WT also peaked at ZT4 and showed a gradual decrease to ZT5, whereas mutants displayed a gradual increase from ZT4 to reach the peak at ZT5 (Fig. 4*C*). In addition, unlike WT pachytene cells with a peak at ZT5, both mutant pachytene cells presented lower peaks with 1- or 2-h delays at ZT6 and ZT7, respectively (Fig. 4*C*). These results are consistent with the proposed delay in DSBR mentioned earlier and support a role of *POL2A* in progression of meiotic recombination.

POL2A Functions in Meiotic Interference-Sensitive CO Formation. Similar to budding yeast and mammals, meiotic DSBR in *Arabidopsis* also yields NCOs and two types of COs (types I and II). The type I COs require ZMM proteins, such as MSH4 and MER3/RCK (27), and type II COs require the MUS81-MMS4 endonuclease (28, 29). The remaining fertility and bivalent-like cells in *pol2a-1* (Fig. 1*D*) suggest formation of some COs. RFC1 (16) and MER3/RCK (30, 31) are required for formation of type I COs, whereas MUS81
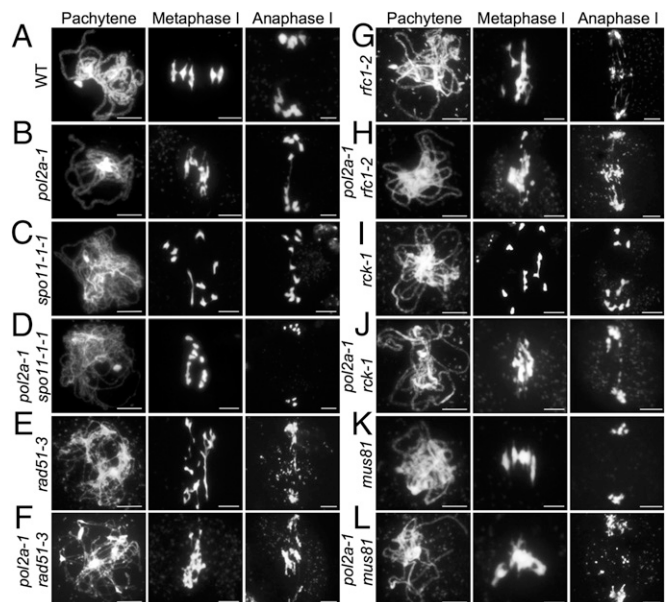

Fig. 3. Genetic analyses of *POL2A* with other meiotic recombination pathway genes. Chromosome morphologies shown by DAPI staining at pachytene, metaphase I, and anaphase I in (*A*) the WT, (*B*) *pol2a-1*, (*C*) *spo11-1–1*, (*D*) *pol2a-1 spo11-1–1*, (*E*) *rad51-3*, (*F*) *pol2a-1 rad51-3*, (*G*) *rfc1-2*, (*H*) *pol2a-1 rfc1-2*, (*I*) *rck-1*, (*J*) *pol2a-1 rck-1*, (*K*) *mus81*, and (*L*) *pol2a-1 mus81*. (Scale bar: 5 μm.)
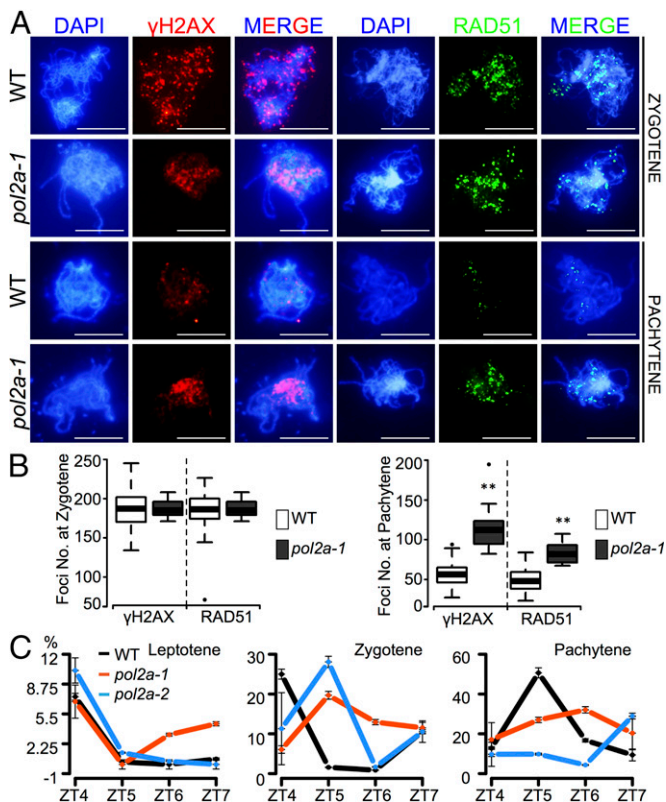
Fig. 4. POL2A is not required for DSB formation but promotes progression of meiotic recombination. (A) Immunostaining with γ-H2AX (red), RAD51 (green), and DAPI counterstain (blue) at zygotene and pachytene in the WT and pol2a-1. (Scale bar: 5 μm.) (B) Variation of the γ-H2AX and RAD51 foci numbers above shown by boxplot in the WT and pol2a-1. **$P \leq 0.01$ with a one-tailed Student t test. (C) Percentages of leptotene, zygotene, and pachytene cells at ZT4 (4 h after lights on), ZT5, ZT6, and ZT7, with error bars from analyses of three to five biological replicates in the WT, pol2a-1, and pol2a-2.

(28) is important for type II COs. To determine which types of COs are formed in pol2a-1, we generated double mutants of pol2a-1 with rfc1-2 (16), rck-1 (30, 31), or mus81-1 (28) and compared their meiotic chromosome morphologies. The pol2a-1 rfc1-2 double mutant also displayed entangled chromosomes, resembling those found in either single mutant (being slightly more similar to those in rfc1-2) (Fig. 3H) and suggesting that they may act in the same pathway, whereas RFC1 has a greater role. In contrast to the rck-1 single mutant with one to two bivalents (Fig. 3I), the pol2a-1 rck-1 double mutant had entangled chromosomes (Fig. 3J), similar to those of the pol2a-1 single mutant (Figs. 1D and 3B), suggesting that POL2A acts upstream of RCK. In contrast, the pol2a-1 mus81-1 double mutant lacked typical bivalents but had more severely entangled chromosomes than those of the pol2a-1 single mutant, unlike the mus81-1 single mutant with normal chromosome morphology (Fig. 3 K and L). These data suggest that formation of bivalents in pol2a-1 depends on MUS81. Together, these results indicate that POL2A functions in the ZMM-dependent type I CO pathway, independent of the MUS81-dependent type II CO pathway.

Because pol2a-1 plants produce some viable pollen grains, a fluorescence assay system was used to investigate the effect of the mutation on recombination frequency using three different fluorescent lines (SI Appendix, Fig. S9) (32). Four of five genetic intervals assayed showed significant increases in genetic distance (Fig. 5A and SI Appendix, Table S2). Because pol2a-1 had inviable pollen grains (Fig. 1A), to minimize biased estimation of recombination using viable pollen grains, we also counted triads with three viable pollen grains. The trend was in general

agreement with the results from tetrad analysis (Fig. 5A and SI Appendix, Table S2). A possible explanation for the increased COs in pol2a-1 is that CO interference may be affected. To test this hypothesis, we estimated chiasma distributions in the WT and pol2a-1. The chiasma distribution in the WT deviated significantly from Poisson distribution ($P = 3.1E-06$; $n = 59$), indicative of interference, in contrast to that in pol2a-1 being statistically no different from Poisson ($P = 0.16$; $n = 66$) (Fig. 5B), suggesting reduced interference in pol2a-1. To verify the interference phenotype in pol2a-1, we calculated the genetic distance of interval I2a with or without CO in the adjacent I2b in the WT and pol2a-1. The WT interference ratio in I2a was 0.47 [$P = 0.03$ for hypothesis $P^{(\text{interference ratio} = 1)}$], indicating a significant CO interference, whereas the interference ratio in pol2a-1 I2a was 0.69 [$P = 0.15$ for hypothesis $P^{(\text{interference ratio} = 1)}$], indicating weaker CO interference (Fig. 5C). Together, these results provide evidence that POL2A functions in the type I CO pathway, and the residual COs in pol2a were type II, which depends on MUS81.

To obtain independent validation that the observed COs in pol2a-1 were type II, we examined the MLH1 foci, which mark type I COs but not type II COs (33) at diakinesis in the WT and pol2a-1. As expected, pol2a-1 had significantly reduced MLH1 foci, with an average of 4.5 ($P = 9.1E-05$; $n = 16$) compared with the WT (an average of 9.0; $n = 61$) (Fig. 5D). Interestingly, the distribution of residual COs among meiocytes was bimodal: one group (62.5%) of pol2a-1 meiocytes had several (average of 7.2) MLH1 foci ($n = 10$), whereas another group (37.5%) of pol2a-1 meiocytes had one or no MLH1 foci ($n = 6$) (Fig. 5 D and E). Although the cause of bimodality is unknown, the clustering of COs in the second group of meiocytes is consistent with interference-insensitive type II COs.

Discussion

POL2A Plays a Role in DNA Synthesis During Meiotic Recombination. DNA synthesis is required for both the mitotic cell cycle and meiosis. Because factors for DNA replication are essential for the
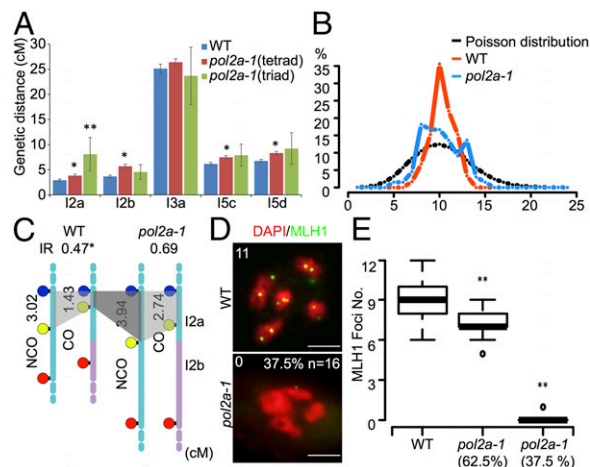

Fig. 5. Estimation of meiotic recombination frequency and CO interference in pol2a. (A) Meiotic recombination frequency in five intervals from analyses of fluorescent tetrad or triad in the WT and pol2a-1. Positions of these intervals on chromosomes are indicated in SI Appendix, Fig. S9. Significant differences are shown by *$P \leq 0.05$ and **$P \leq 0.01$. (B) A graph of the chiasma frequency per nucleus in the WT and pol2a-1. Poisson distribution was based on 10,000 random samplings with an average of 10. (C) Genetic distance of interval I2a without or with CO in adjacent I2b in the WT and pol2a-1, respectively. Interference ratios are shown above with *$P \leq 0.05$ against $P^{(\text{interference ratio} = 1)}$. (D) MLH1 foci (green) with DAPI (red) counterstain by immunostaining in the WT and pol2a-1. Numbers in the upper left indicate MLH1 foci numbers. (Scale bar: 5 μm.) (E) Statistics of MLH1 foci distribution in WT and pol2a-1 meiocytes. **$P \leq 0.01$ with a one-tailed Student t test. IR, interference ratio.
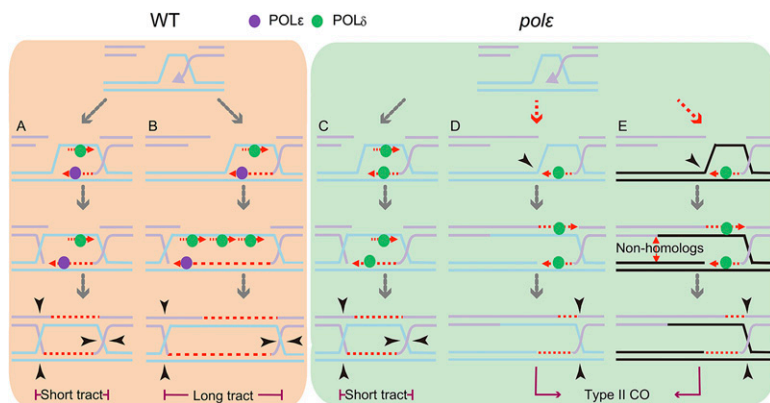
Fig. 6. A model for POL-ε participation in meiotic type I COs. Our previous study presented that meiotic type I CO likely requires both leading- and lagging-strand DNA synthesis. In this model, we propose that, (*A* and *B*) in the WT, both short and long tracts involve POL-ε and POL-δ for leading- and lagging-strand synthesis, respectively. In the absence of POL-ε, for the COs with short tracts, POL-δ can replace POL-ε to carry out initiation and limited synthesis, such as (*C*) one Okazaki fragment, whereas for COs with long tracts, POL-δ cannot fully replace the elongation function of POL-ε. The failure of dHJ formation may result in the formation of MUS81-dependent type II COs between (*D*) homologs and (*E*) nonhomologs.

mitotic S phase, null mutations in corresponding genes cause lethality in mammals, yeast, and plants (11–14, 34), making it difficult to study their functions. Nevertheless, characterization of weak mutations and/or generation of conditional mutations of DNA synthesis genes provide a feasible approach to study their functions, such as *DNA Pol-α* (35), *Pol-δ* (15, 36), *Pol-ε* (12, 13), and *RFC1* (16) in multiple organisms. Genetic studies in yeast showed that the N-terminal region of Pol-ε is dispensable for DNA replication, DNA repair, and cell viability, whereas the noncatalytic C-terminal domain is essential (19–21). Consistently, both a previous study (12) and this study showed that disruption of the C-terminal region of *POL2A* also causes lethality in *Arabidopsis*, but a mutant affecting its N-terminal region is viable (*SI Appendix*, Fig. S1 *C and F*), consistent with previous observation in *Drosophila* (37) and humans (14), suggesting functional conservation of DNA Pol-ε among major eukaryotic groups (20).

Meiotic DSBR models invoke leading-strand DNA synthesis for yielding both COs and NCOs, but the role of DNA Pol-ε in meiosis has not been characterized. Here, we showed that two mutations in *Arabidopsis POL2A* and meiosis-specific knockdown of *POL2* (*pDMC1-POL2^RNAi^*) cause similar defects in fertility and meiosis (Fig. 1 and *SI Appendix*, Fig. S7 *K–O*), supporting the idea that the observed meiotic defects in *pol2a* are caused by the *POL2A* mutation. Because POL2A is a DNA Pol, an obvious possibility of POL2A function in meiosis is in premeiotic DNA replication. Although we found no evidence for the mutants used here being defective in premeiotic DNA replication, we present several lines of evidence that support a crucial role of POL2A in DNA synthesis during meiotic recombination. First, *pol2a* mutants showed normal vegetative growth and produced 20 chromatids, indicating that these mutants could still replicate chromosomes. Second, observation of the delayed peak in pachytene cell distribution in mutant suggests that *POL2A* likely plays a role in a late stage of meiotic recombination (Fig. 4*C*). Third, the formation of DSBs seemed normal in *pol2a* (Fig. 4*A*). Fourth, the possible nonhomolog association and chromosome fragmentation in *pol2a* depend on SPO11-1–generated DSBs. Fifth, genetic analysis showed that POL2A acts downstream of RAD51 and upstream of RCK/MER3. Moreover, genetic studies of *POL2A* with *RFC1* also support the idea that they function in a similar process in meiotic recombination. The weaker meiotic defect in *pol2a* than that in *rfc1* suggests that lagging-strand synthesis is likely more important for double Holliday junction (dHJ) formation (Fig. 6), because Pol-δ, a DNA Pol required for lagging-strand elongation, can partially substitute the role for elongation in leading-strand synthesis (see below).

Elongation of DNA Synthesis Likely Promotes dHJ Formation. Meiotic recombination is initiated from programmed DSBs (4), and DNA synthesis is required for DSBR during meiotic recombination. However, the underlying mechanism has not been characterized. Recently, we showed that formation of meiotic type I CO likely requires lagging-strand DNA synthesis (16) and proposed that lagging-strand synthesis promotes dHJ formation. This idea is

consistent with the observation that more DNA synthesis accompanies CO formation than NCO formation in yeast (5). In mitotic DNA replication, POL-ε and POL-δ perform elongation of leading and lagging strands, respectively (8, 9). Unlike POL-ε for continuous elongation of the leading strand, POL-δ is only responsible for elongation of Okazaki fragments on the lagging strand (9). Studies in yeast showed that POL-δ can partially replace POL-ε for leading-strand synthesis (8, 9). This finding raises a question of whether there is a threshold of amount of DNA synthesis to differentiate the requirement for distinct DNA synthesis factors. In yeast mitosis, mutations in the N-terminal exonuclease domain of Pol-ε have no obvious defects in DNA replication initiation but greatly reduce efficiency of leading-strand elongation by 10- to 100-fold compared with the WT (17–19, 38), providing a possible means to study the role of elongation in meiosis. Here, we provided the following pieces of evidence to support the idea that POL2A participates in type I CO formation. First, the *pol2a* mutants have reduced type I COs, but the residual COs are more than those of *rfc1* and the *zmm* mutants (27), indicating that the defect in elongation affects a portion of type I COs. Second, genetic analyses of *pol2a* with factors involved in the type I pathway, such as *rfc1* and *mer3/rck* (Fig. 3 *H* and *J*), show similar chromosome morphologies to *pol2a*, whereas the *pol2a* double mutant with a mutation in the type II gene *MUS81* has more severe phenotype than either single mutant (Fig. 3*L*). Third, CO interference in *pol2a* is significantly reduced (Fig. 5 *B* and *C*).

Why did POL2A only affect some type I COs? Recent genome-wide analyses showed that CO-associated tracts are usually longer than those of NCO in both yeast and *Arabidopsis*, ranging from several hundreds to thousands of base pairs (2, 6, 7, 39). In *Arabidopsis*, the mean length of CO-associated tracts is ~500 bp (2, 7), whereas an Okazaki fragment for discontinuous lagging-strand synthesis is about 100–200 bp (40). It is reasonable to assume that only COs with long conversion tracts require elongation to fulfill the need for more extensive leading-strand synthesis. We, therefore, hypothesize that the CO-associated conversion tracts with >200 bp may require POL2A, providing an explanation for why POL2A affects some type I COs.

Previous models for meiotic recombination are not explicit regarding leading-strand elongation (4). The *pol2a* mutations that we analyzed here are similar to a mutation in yeast *Pol-ε* that showed no obvious defects in DNA replication initiation but caused greatly reduced leading-strand elongation efficiency (17–19, 38). The extensive phenotypic analyses presented here are consistent with these *Arabidopsis* mutations, affecting only elongation of leading strands but not initiation during meiotic recombination. Therefore, we present a revised model for describing the meiotic type I CO pathway (Fig. 6), incorporating both lagging-strand synthesis and leading-strand elongation. In this model, we suggest a threshold of DNA synthesis (say a length of 200 bp for discussion) to discriminate two different kinds of CO-associated conversion tracts: long tracts with >200 bp and short tracts with <200 bp. In the WT, both long and short tracts involve the functions of both Pol-δ and Pol-ε

(Fig. 6 *A* and *B*). In the absence of POL2A (Pol-ε), for the COs with short tracts, Pol-δ can replace Pol-ε to carry out initiation and limited synthesis, similar to that for an Okazaki fragment (Fig. 6*C*), whereas for COs with long tracts, Pol-δ cannot perform the elongation function of Pol-ε. For these COs, a failure of dHJ formation in the *pol2a* mutation might result in formation of MUS81-dependent COs (Fig. 6*D*). Alternatively, the single invasion of the 3′ end may withdraw to find a nonhomolog to yield a CO as resolved by MUS81 (Fig. 6*E*). This model is further supported by the finding that a weak mutation of yeast *Pol-δ* causes a reduction in the length of meiotic gene conversion tracts and CO number (15, 36).

In summary, meiotic recombination is essential for sexual reproduction in eukaryotes, and most organisms have at least two distinct types I and II COs (41, 42), including the budding yeast, animals, and flowering plants; whereas DNA Pol is also highly conserved in eukaryotes (10), we hypothesize that discrimination of meiotic types I and II COs by the amount of associated DNA synthesis may be a general mechanism. However, the precise threshold length of the conversion tract that is required for factors to coordinate leading- and lagging-strand synthesis might be different in different organisms.

Materials and Methods

Materials used in this study are all Columbia (Col-0) background. Details of plant materials and growth conditions are described in *SI Appendix, SI Materials and Methods*. Assays of characterization of mutant phenotypes, like Alexander red staining, tetrad and chromosome morphology analyses, FISH and immunofluorescence observation, statistics of chiasmata distribution, and others are also described or follow the references listed in *SI Appendix, SI Materials and Methods*. Statistical analyses of *t* tests were performed in Microsoft Excel 2013 or Rstudio v0.98.501. Statistical significance was showed as $P < 0.05$ and $P < 0.01$.

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