ZmMTOPVIB Enables DNA Double-Strand Break Formation and Bipolar Spindle Assembly during Maize Meiosis

Ju-Li Jing, Ting Zhang, Yu-Hsin Kao, Tzu-Han Huang, Chung-Ju Rachel Wang, and Yan He

Ministry of Education Key Laboratory of Crop Heterosis and Utilization, National Maize Improvement Center of China, College of Agronomy and Biotechnology, China Agricultural University, 100094 Beijing, China
Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan

The meiotic TopoVI B subunit (MTopVIB) plays an essential role in double-strand break formation in mouse (Mus musculus), Arabidopsis (Arabidopsis thaliana), and rice (Oryza sativa), and recent work reveals that rice MTOPVIB also plays an unexpected role in meiotic bipolar spindle assembly, highlighting multiple functions of MTopVIB during rice meiosis. In this work, we characterized the meiotic TopVIB in maize (Zea mays; ZmMTOPVIB). The ZmmtopVIB mutant plants exhibited normal vegetative growth but male and female sterility. Meiotic double-strand break formation was abolished in mutant meiocytes. Despite normal assembly of axial elements, mutants showed severely affected synapsis and disrupted homologous pairing. Importantly, we showed that bipolar spindle assembly was also affected in ZmmtopVIB, resulting in triad and polypad formation. Overall, our results demonstrate that ZmMTOPVIB plays critical roles in double-strand break formation and homologous recombination. In addition, our results suggest that the function of MTOPVIB in bipolar spindle assembly is likely conserved across different monocots.

Meiotic homologous recombination is a crucial step for ensuring proper chromosome segregation and generating genetic diversity in eukaryotes (Keeney and Neale, 2006). During this process, induction of programmed DNA double-strand breaks (DSBs), which is catalyzed by an evolutionary conserved topoisomerase-like translesion synthesis DNA polymerase SPO11, initiates homologous recombination (Bergerat et al., 1997; Keeney et al., 1997). Through a translesion polymerization reaction, the SPO11 dimer coordinate cleaves the double strands of a DNA molecule, forming an intermediate with one SPO11 molecule covalently linked with each 5’ end of the cleaved DNA strands (Keeney and Kleckner, 1995). The SPO11 proteins are then released with the single-strand DNA oligo by the Mre11/Rad50/Xrs2 (MRX) complex and Sae2/CtIP (Neale et al., 2005; Symington and Gautier, 2011). The 5’ ends are then resected to expose longer 3’ single-strand DNA tails that are subsequently associated with two recombinases, RAD51 and DMC1, to form nucleoprotein filaments that promote homologous pairing by single-strand invasion into homologous chromosomes (Bishop et al., 1992; Shinohara et al., 1992; Cloud et al., 2012). The strand invasion results in a nascent DNA joint molecule called the displacement loop (D-loop; Hunter and Kleckner, 2001). Consequently, joint molecule intermediates can be processed into crossovers (COs) with reciprocal exchanges between homologous chromosomes or noncrossovers (Allers and Lichten, 2001; Baudat and de Massy, 2007; Grelon, 2016).

DNA topoisomerases are essential for regulating DNA topology, such as decatenating/relaxing superhelicality and untangling DNA during replication, transcription, and recombination (Corbett and Berger, 2003; Graille et al., 2008). DNA topoisomerases are classified into two families based on the cleavages they make on single-strand (type I) or double-strand DNA (type II), respectively (Champoux, 2001; Wang, 2002). The type II topoisomerases are predominant and can be further categorized into two subfamilies based on their structural similarity: type IIA and type IIB (Gadelle et al., 2014). Type IIA subfamily members, such as DNA gyrase and topoisomerase II enzymes, are found throughout eubacteria and eukaryotic organisms (Forterre and Gadelle, 2009). One type IIB subfamily member, topoisomerase VI (topo VI), is ubiquitous in archaea for decatenating DNA and is also found in plants, where it is needed for successful progression of the endoreduplication cycle.

1This work was supported by the National Natural Science Foundation of China (grant nos. 31671277 and 31970524 to Y.H.) and the Taiwan Ministry of Science and Technology (grant no. 107–2923-B-001-001-MY4 to C.-J.R.W.).
2Senior authors.
3Author for contact: yh352@cau.edu.cn.
4The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Yan He (yh352@cau.edu.cn).

C.-J.R.W. and Y.H. conceived and supervised the project; J.L.J., T.Z., Y.-H.K., and T.-H.H. conducted the experiments; J.L.J., C.-J.R.W., and Y.H. prepared the manuscript; and all authors read and approved the final manuscript.

[OPEN]Articles can be viewed without a subscription.
www.plantphysiol.org/cgi/doi/10.1104/pp.20.00933

Plant Physiology, December 2020, Vol. 184, pp. 1811–1822, www.plantphysiol.org © 2020 American Society of Plant Biologists. All Rights Reserved.
Topo VI functions in a heterotetramer complex comprised of two A (TopVIA) and two B (TopVIB) subunits (Corbett and Berger, 2003; Gadelle et al., 2014). About two decades ago, research showed that SPO11, which shares general sequence homology with the TopVIA, plays a critical role in DSB formation in all eukaryotes (Bergerat et al., 1997; Keeney et al., 1997). Interestingly, the analogs of the corresponding TopVIB and their critical roles in meiotic DSB formation have only recently been identified in mouse (Mus musculus; Robert et al., 2016a), Arabidopsis (Arabidopsis thaliana; Vrielynck et al., 2016) and rice (Oryza sativa; Fu et al., 2016; Xue et al., 2016).

In eukaryotes, faithful chromosome segregation during cell division is mediated by the spindle, i.e., a complex protein superstructure composed of microtubules and associated proteins (Heald et al., 1996; Compton, 2000; Wittmann et al., 2001; Xue et al., 2019). After nuclear envelope breakdown in plant meiosis, randomly polarized microtubules self-organize into bipolar spindles during metaphase I (Vernos and Cande, 1998). In maize mutants defective in chromo-

Very recently, the rice MTOPVIB analog (OsMTOP-VIB) was found to play a key role in bipolar spindle assembly (Xue et al., 2019). The finding raises the critical question of whether this newly discovered role of OsMTOPVIB in meiosis is widely conserved across different plant species. In addition, a recent study detected few DSBs in the maize spo11-1 mutant (Ku et al., 2020), which suggested two possible scenarios: rare DSBs still occur in the absence of functional DSB machinery, or SPO11-2 is able to form a functional complex with MTOPVIB to execute DSBs. Here, we characterized functions of MTOPVIB in maize, one of the best model organisms for cytogenetic study. We found that normal DSB formation was disrupted in ZmMTOPVIB mutant meiocytes, similar to observations in the maize spo11-1 mutant (Ku et al., 2020). A deficiency of DSBs led to defective homologous recombination and synaptonemal complex (SC) assembly, which consequently caused univalents at diakinesis. Moreover, bipolar spindle assembly was abnormal in ZmMTOPVIB meiocytes, resulting in missegregated meiotic univalents which were aberrantly pulled by multipolarized spindles, yielding triads or polyads with micronuclei. Therefore, our results support the notion that MTOP-VIB displays a conserved function not only in DSB formation but also in bipolar spindle assembly among different monocots.

## RESULTS

### Identification of ZmMTOPVIB

To identify a putative MTOPVIB gene in maize, the full-length amino acid sequence of Arabidopsis MTOPVIB was used as a query to search in the maize genome database (https://maizegdb.org/) using BLASTp analysis. We identified one candidate gene (Zm00001d014728) with the highest similarity to Arabidopsis MTOPVIB (At1G60460). By performing RACE, we obtained the 1,341-bp full-length ZmMTOPVIB complementary DNA (cDNA) sequence, which consists of 12 exons and 11 introns (Fig. 1A). The amino acid sequences of MTOPVIB from 10 different plant species obtained from the National Center for Biotechnology Information were subject to phylogenetic analysis. Two distinct clades of MTOPVIB homologs represented genetic divergence of monocots and dicots (Supplemental Fig. S1). In addition, alignment of MTOPVIB protein sequences from Arabidopsis, rice, and maize revealed that their MTOPVIB proteins are largely conserved, especially in three primary domains (GHKL, small, and transducer domains; Supplemental Fig. S2). We then investigated spatiotemporal expression patterns of ZmMTOPVIB by reverse transcription quantitative PCR (RT-qPCR) analyses and found that the gene was highly expressed in the developing tassel, moderately expressed in embryo, ear, and endosperm, and only weakly expressed in root, stem, and leaf (Supplemental Fig. S3).

To characterize functions of MTOPVIB in maize, we obtained one mutant line, UFMu-07260, from the UniformMu population (Liu et al., 2016), which has a Mutator inserted in the Zm00001d014728 gene. By PCR amplification and Sanger sequencing using the Mutator and ZmMTOPVIB-specific primers (Supplemental Table S1), we confirmed that the Mutator transposon was inserted into the first intron of ZmMTOPVIB (Fig. 1B). Although the insertion did not alter ZmMTOPVIB expression (Supplemental Fig. S3), it resulted in aberrant splicing, leading to two major splice variants. The longer transcript appeared more abundant relative to the shorter one, which was the same size as in the wild type (Fig. 1C). Sequence analyses revealed that the longer variant contains a fraction of Mutator transposon sequence, resulting in an in-frame premature stop codon (underlined tga; Fig. 1B). A second mutant allele (EMS4-0742ae) was obtained from the Maize EMS-induced Mutant Database (Lu et al., 2018). DNA sequence analysis of the EMS4-0742ae mutant confirmed a single base mutation from G to A at the splicing acceptor site of the eighth intron (Fig. 1D), which was predicted to abort splicing of the eighth intron and result in an in-frame premature stop codon (underlined taa; Fig. 1D). RT-PCR analysis showed a longer transcript in the mutant, confirming retention of this intron (Fig. 1E).
Both ZmmtopVIB mutants were completely male-sterile (Fig. 2A; Supplemental Fig. S4A), and their anthers appeared withered at the flowering stage (Fig. 2B; Supplemental Fig. S4B). Alexander staining showed that unlike the large, round, purple pollen grains of the wild type (Fig. 2C; Supplemental Fig. S4C), mutant pollen grains were empty, shrunken, and unable to stain (Fig. 2D; Supplemental Fig. S4D). In addition, mutant ears did not produce any seeds when pollinated with pollen from wild-type plants (Fig. 2E; Supplemental Fig. S4E). These results indicate that defective ZmMTOPVIB causes both male and female sterility. Hence, ZmmtopVIB-1 and ZmmtopVIB-2 mutant alleles were named UFMu-07260 and EMS4-0742ae lines, respectively.

Meiosis Is Disturbed in the ZmmtopVIB Mutants

Meiotic chromosome behavior in ZmmtopVIB mutants was analyzed by 4,6-diamidino-2-phenylindole (DAPI) staining. In the wild type, long, thin, threadlike chromosomes were first observed at the leptotene stage (Fig. 3A). Then, chromosomes rearranged next to a large and offset nucleolus and began to pair and synapse at the zygotene stage (Fig. 3B). During the pachytene stage, chromosomes completely synapsed to form thick chromosome threads (Fig. 3C). From the diplotene to diakinesis stage, chromosomes condensed into 10 short, rodlike bivalents distributed in the nucleus (Fig. 3D). At metaphase I, the 10 bivalents properly aligned on the equatorial plate (Fig. 3E), and equal numbers of chromosomes moved to the two opposite poles of the cell at anaphase I (Fig. 3F).

Chromosomal behaviors of ZmmtopVIB-1 and ZmmtopVIB-2 meiocytes were similar to the wild type in leptotene and zygotene (Fig. 3, G and H; Supplemental Fig. S5, A and B). However, in pachytene, when synapsis complete, chromosomes in ZmmtopVIB-1 and ZmmtopVIB-2 mutants are not appeared as thick thread as in the wild type (Fig. 3, C and I; Supplemental Fig. S5C). Subsequently, we observed 20 unpaired univalent chromosomes randomly scattered in the nuclei of mutant
meiocytes at diakinesis (Fig. 3J; Supplemental Fig. S5D), which never aligned on the equatorial plate at metaphase I (Fig. 3K; Supplemental Fig. S5E). At anaphase I, we recorded 20 univalents segregated randomly and asymmetrically, with lagging chromosomes often found in the center of the mutant nuclei (Fig. 3L; Supplemental Fig. S5F). Based on these obvious abnormalities in meiotic chromosome behavior, we concluded that the sterility of the ZmmtopVIB mutants was due to defective meiosis. Since both ZmmtopVIB-1 and ZmmtopVIB-2 exhibited similar meiotic phenotypes, all of our subsequent analyses were performed on ZmmtopVIB-1 as a representative mutant allele.

**ZmMTOPVIB Is Essential for Normal Meiotic DSB Formation**

Formation and repair of programmed meiotic DSBs during meiosis prophase I is an essential prerequisite for homologous recombination (de Massy, 2013). Despite important roles during meiosis, DSBs represent one of the most deleterious lesions, and such DNA damage rapidly induces phosphorylation of the histone variant H2AX at its S139 residue (γH2AX; Nakamura et al., 2010; Yuan et al., 2010). Consequently, the occurrence of γH2AX foci is routinely used as a biomarker to monitor DSB formation (Dickey et al., 2009; Löbrich et al., 2010). To investigate whether DSB formation is defective in maize ZmmtopVIB mutants, we used an antibody that specifically recognizes γH2AX for immunofluorescence analysis in wild-type and ZmmtopVIB-1 meiocytes. We found that γH2AX foci appeared as punctate-like signals scattered throughout the nuclei of wild-type meiocytes, reaching a peak at the early zygotene and decreasing at the late zygotene stage (Fig. 4, A–D and H). In contrast, very few γH2AX signals were detected at the early zygotene stage of ZmmtopVIB-1 meiocytes (Fig. 4, F and H), suggesting that normal DSB formation is defective in the ZmmtopVIB-1 mutant. Interestingly, at the late zygotene stage, we found a few clusters of γH2AX signals in ZmmtopVIB-1 meiocytes that were often associated with chromosome tangles (Fig. 4G). In magnified images, multiple chromosome axes appeared to become knotted around the γH2AX signals. On average, we found 19.9 γH2AX foci that often formed a few clusters in mutant cells (n = 31; Fig. 4H).

To further investigate ectopic detection of γH2AX in mutants, we analyzed distribution of recombinases RAD51 and DMC1. While RAD51 is required for DNA repair in somatic and meiotic recombination, DMC1 functions specifically during meiotic recombination (Bishop et al., 1992; Cloud et al., 2012). In the wild type, both RAD51 and DMC1 manifested as numerous punctate foci distributed on chromosomes at the zygotene stage. At the late zygotene stage, their signals were significantly reduced (Fig. 5, A–D). In ZmmtopVIB-1 meiocytes, RAD51 and DMC1 were not detected in...
the early zygotene stage (Fig. 5, E and G). Similar to gH2AX results, mutant meiocytes at the late zygotene stage displayed two to six clusters of RAD51 and DMC1 signals (Fig. 5, F and H). In magnified images, both signals were clearly decorated around these entangled chromosome axes (Fig. 5, I and J). Although DSBs were detected at a low level in mutant meiocytes, they were likely DNA damage associated with chromosomal entanglements. Given that no bivalent chromosomes were observed, these aberrant DSBs may not be repaired through the canonical meiotic recombination pathway. Nevertheless, these results suggest that ZmMTOPVIB is essential for normal meiotic DSB formation.

ZmMTOPVIB Is Critical for Homologous Pairing But Not Required for Telomere Bouquet Formation

To investigate whether defective ZmMTOPVIB disrupts homologous chromosome pairing, we performed fluorescence in situ hybridization (FISH) using the 5S ribosomal DNA (rDNA) probe in the wild type and the ZmmtopVIB-1 mutant. The 5S rDNA is a tandem repeat sequence located on the long arm of chromosome 2 that is routinely used to evaluate chromosome pairing and segregation in maize (Wang et al., 2018). In wild-type meiocytes, two 5S signals gradually paired with each other during the zygotene stage (Fig. 6, A and B). At the pachytene stage, the paired 5S signal was observed in all cells examined ($n = 48$; Fig. 6C). In contrast, two separate 5S rDNA signals were consistently detected in ZmmtopVIB-1 meiocytes (Fig. 6, F and G), indicating that homologous chromosome pairing is defective in the ZmmtopVIB-1 mutant.

The telomere bouquet is an evolutionarily conserved chromosome arrangement that clusters telomeres in a small region on the nuclear envelope (Tomita and Cooper, 2007; Moiseeva et al., 2017). This specialized structure is thought to promote initiation of homologous pairing during early prophase I (Loidl, 1990; Scherthan, 2001; Harper et al., 2004). To explore whether defective ZmmtopVIB affects telomere bouquet formation, we conducted FISH analysis using the pAtT4 probe (Richards and Ausubel, 1988; Prieto et al., 2004) in wild-type and ZmmtopVIB-1 meiocytes. In wild-type ($n = 44$;...
Fig. 6, A and B) and ZmmtopVIB-1 (n = 51; Fig. 6F) meiocytes at the early zygotene stage, telomere signals were clustered and attached to the nuclear envelope, indicating that ZmMTOPVIB is not required for telomere bouquet formation. As mutant meiocytes did not show a normal pachytene stage, telomere signals become diffused at the late zygotene (Fig. 6G).

The observation of γH2AX signals in ZmmtopVIB mutants was not expected. Although homologous pairing assessed by 5S rDNA signals suggested that pairing is defective, we sought to analyze more loci in the genome. Therefore, we adapted the recently developed chromosome painting method in meiosis (Albert et al., 2019). By using chromosome 3- and chromosome 8-specific probes, wild-type meiocytes clearly showed 10 bivalent chromosomes at diakinesis (Fig. 6D). Bivalents labeled with the chromosome-specific probes were correctly separated at anaphase I (Fig. 6E). In contrast, ZmmtopVIB mutants showed a complete failure of bivalent formation by chromosome painting (Fig. 6, H and I).

**Defective Installation of Central Element ZYP1 in ZmmtopVIB-1 Meiocytes**

The SC is a meiosis-specific chromosomal structure that connects homologous chromosomes by a transverse filament to promote efficient CO formation (Page and Hawley, 2004; Argunhan et al., 2017). To investigate
the pattern of SC localization in wild-type and ZmmtopVIB-1 mutant meiocytes, we performed immunostaining using AFD1, ASY1, and ZYP1 antibodies. Maize AFD1 is homologous to Arabidopsis and rice REC8 (Cai et al., 2003; Shao et al., 2011). It is a vital component of the cohesion complex associated with axial and lateral elements and is required for axial element (AE) elongation (Golubovskaya et al., 2006). AFD1 signals appeared as threads along entire chromosomes of wild-type meiocytes at the zygotene stage \((n = 21;\) Fig. 7A). AFD1 signals in ZmmtopVIB-1 meiocytes was consistent with that of the wild type \((n = 24;\) Fig. 7B), indicating that ZmMTOPVIB is not required for cohesion complex assembly.

Maize ASY1, a homolog of Arabidopsis ASY1 and rice PAIR2, is a basic element of AE, and it is essential for SC assembly and homologous recombination (Armstrong et al., 2002; Golubovskaya et al., 2011). In wild-type meiocytes, ASY1 loading manifested as linear signals along entire chromosomes in the zygotene stage \((n = 22;\) Fig. 7C). Again, ASY1 signals in ZmmtopVIB-1 meiocytes were very similar to the pattern observed for the wild type \((n = 20;\) Fig. 7D), confirming that ZmMTOPVIB is not required for AE installation.

Maize ZYP1, a homolog of Arabidopsis ZYP1 and rice ZEP1, is the central element (CE) of the SC and is assembled between AEs to regulate chromosome synapsis and CO formation (Higgins et al., 2005; Wang et al., 2010; Barakate et al., 2014). In wild-type meiocytes, we observed elongated filaments of ZYP1 signals along the entire length of synapsed chromosomes at the pachytene stage \((n = 25;\) Fig. 7E). In contrast, only short branched or punctate ZYP1 signals were observed in ZmmtopVIB-1 meiocytes at the same stage \((n = 27;\) Fig. 7F), supporting the finding that ZmMTOPVIB is crucial for ZYP1 loading. Taken together, these results indicate that ZmMTOPVIB is not required for AE installation, but it is indispensable for CE assembly during maize meiosis.

**ZmMTOPVIB Is Required for Meiotic Bipolar Spindle Assembly in Maize**

The recently identified function of OsMTOPVIB in regulating meiotic spindle assembly in rice prompted us to investigate whether ZmMTOPVIB has the same role in maize meiosis. To do so, we performed immunostaining using \(\alpha\)-tubulin antibody in wild-type and ZmmtopVIB-1 meiocytes. \(\alpha\)-Tubulin heterodimerizes and polymerizes with \(\beta\)-tubulin to form microtubule walls (Hunter and Kleckner, 2001; Blume et al., 2009; Gunning et al., 2015; Higgins et al., 2016). Microtubule filaments in wild-type meiocytes gradually extended and attached to chromosomes during diakinesis \((n = 13;\) Fig. 8A). At metaphase I, spindle fibers linked to the kinetochores and were arranged into two arrays, forming a canonical bipolar spindle structure \((n = 25;\) Fig. 8B). At anaphase I, as the spindles extended, equal numbers of chromosomes were pulled by the bipolar spindles toward the opposite poles of the cell \((n = 23;\) Fig. 8C). Finally, dyads formed, and the remaining spindles stayed at the equatorial plate \((n = 18;\) Fig. 8D). In contrast, we consistently observed abnormal spindle structures in the ZmmtopVIB-1 mutant at different stages of meiosis. In diakinesis, extension and aggregation of microtubule filaments was incomplete, with only one polar spindle forming, so that the opposite cell pole lacked a polar spindle \((n = 11;\) Fig. 8, E and I). At metaphase I, spindle fibers of mutant meiocytes became entangled and distorted, lacking the typical bipolar spindle structure upon separation \((n = 12;\) Fig. 8, F

---

*Figure 7. Immunofluorescence localization of AFD1 (A), ASY1 (C), and ZYP1 (F) in wild-type (A, C, and E) and ZmmtopVIB-1 (B, D, and F) meiocytes. Scale bars = 10 \(\mu\)m.*
and J). These distorted spindles dragged chromosomes randomly in multiple directions across the cell at anaphase I \((n = 13;\) Fig. 8, G and K), resulting in multinuclear dyads with abnormal equatorial plates in telophase I \((n = 9;\) Fig. 8, H and L).

To ascertain the outcomes of these abnormal spindle structures, we examined spore products in wild-type and \textit{ZmmtopVIB-1} lines. In the wild type, we consistently observed symmetric dyads at telophase I \((n = 55;\) Fig. 9A) and tetrads at telophase II \((n = 52;\) Fig. 9E). Similarly, we also observed dyads at telophase I \((54.13\%, n = 59;\) Fig. 9B) and tetrads at telophase II \((36.76\%, n = 36;\) Fig. 9F) in \textit{ZmmtopVIB-1}. In contrast, we frequently detected triads at telophase I \((25.69\%; n = 28;\) Fig. 9C) and polyads at telophase I and II \((63.24\%; n = 62;\) Fig. 9, D, G, and H) in \textit{ZmmtopVIB-1}. Taken together, these results demonstrate that \textit{ZmMTOPVIB} plays a critical role in proper bipolar spindle assembly during maize meiosis.

**DISCUSSION**

**Evolutionary Conservation of \textit{ZmMTOPVIB} Function in Meiotic DSB Formation**

We have shown that disruption of \textit{ZmMTOPVIB} causes defects in the formation of meiotic DSBs and results in severe abnormalities of DSB-induced events, such as homologous synapsis, meiotic recombination, and chromosome segregation. Although we detected few \(\gamma\)H2AX signals in the late zygotene stage, often associated with entangled chromosomes in mutant meiocytes, these sites are unlikely to be canonical meiotic DSBs. One possible scenario is that DNA damage resulted from chromosome knotting, which may be more frequent in a large genome with long chromosomes in the absence of recombination initiation. In support of this possibility, a similar observation was reported in maize \textit{spo11-1} mutants (Ku et al., 2020). Nevertheless, our results demonstrate that \textit{ZmMTOPVIB} is essential for normal meiotic DSB formation in maize, consistent with studies in other organisms, including mouse (Robert et al., 2016a), Arabidopsis (Vrielynck et al., 2016), and rice (Fu et al., 2016; Xue et al., 2016), strengthening the notion that the role of TopoVIB in meiotic DSB formation is evolutionarily conserved among plants and mammals.

TopoVI is a heterotetramer comprised of two A subunits and two B subunits (Corbett and Berger, 2003; Gadelle et al., 2014). To generate DSBs on DNA, the A2 dimers catalyze the transesterification reaction for DNA cleavage (Bergerat et al., 1997), whereas the B2 subunits are responsible for A2 conformation and DNA binding (Dutta and Inouye, 2000; Corbett et al., 2007; Graille et al., 2008). Although the basic setup of the TopoVI complex seems conserved among organisms, the arrangement and operational mode of each component may vary. For instance, in mouse, the sole SPO11 gene produces two major SPO11 isoforms, i.e. a short SPO11\(\alpha\) and a longer SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b). In contrast, all land plants encode at least two SPO11 proteins, referred to as SPO11\(\alpha\) and SPO11\(\beta\), by alternative splicing (Bellani et al., 2010). SPO11\(\beta\) interacts with TopoVIB to form a symmetrical heterotetramer for creating meiotic DSBs (Robert et al., 2016b).
SPO11-1/SPO11-2 heterodimers, though not of SPO11-1 or SPO11-2 homodimers (Vrielynck et al., 2016; Jing et al., 2019b). Hence, this function of Arabidopsis MTOPVIB as a linker to promote the assembly of SPO11 dimers may not necessarily be displayed by its counterpart in mouse. These results highlight that analogs of TopoVIB involved in meiotic DSB formation are evolutionarily conserved but subject to variation among different organisms.

Although we detected low levels of γH2AX, RAD51, and DMC1 signals in the ZmMTOPVIB mutant, these signals appear to aggregate abnormally around chromosomal entangles. These knotted chromosome axes may reflect difficulties of pairing due to lack of normal DSB formation. Perhaps these entanglements may trigger TOPII activity for resolution, which ectopically creates chromosomal breaks. The recent study of Arabidopsis TOPII and its role in unknotting entangled chromosomes also supports this possibility (Martinez-Garcia et al., 2018). In addition, a similar phenotype of aberrant DSB formation was also reported in the maize spo11-1 mutant (Ku et al., 2020), which may imply that species with long meiotic chromosomes endure a higher risk of chromosome damage when DSBs fail to initiate. However, given the absence of normal bivalent chromosomes and chromosome fragmentation in ZmMTOPVIB mutant cells, these aberrant DSBs were likely repaired but did not give rise to meiotic crossovers.

### ZmTOPVIB Is Involved in Meiotic Bipolar Spindle Assembly

Proper morphogenesis and orientation of microtubule-based spindles are critical processes for ensuring the fidelity of chromosome segregation and cell division in eukaryotes (Gadde and Heald, 2004; Zhang and Dawe, 2011). In both monocot and dicot plants, construction of a meiotic bipolar spindle occurs by converting multipolar into bipolar spindle poles (Nannas and Dawe, 2016; Xue et al., 2019). However, the mechanism underlying meiotic bipolar spindle assembly remains unclear, with only a few contributory genes reported to date. Arabidopsis ATK1 and ATK5, which encode kinesins, are motor proteins that typically advance along microtubules and presumably generate the force for microtubule movement, thereby affecting spindle organization (Chen et al., 2002; Ambrose and Cyr, 2007). PRD2, an essential gene for meiotic DSB formation in Arabidopsis, was originally
named Multipolar Spindle 1 (MPS1) based on the unequal bipolar or multipolar spindles present in mps1 mutant meioocytes (Jiang et al., 2009). Intriguingly, OsMTOPVIB interacts with OsPRD2 in rice, indicating that these proteins may function in the same pathway, though the function of OsPRD2 has not yet been characterized (Xue et al., 2019). Since both PRD2 and OsMTOPVIB are essential for DSB formation, it is conceivable that the processes of DSB formation and bipolar spindle assembly may be coordinated. However, this speculation was proven untrue upon discovery that bipolar spindles at metaphase I occur normally in three rice mutants exhibiting defects in DSB formation, i.e. the pair2, Osspo11-1, and crc1 mutants (Xue et al., 2019). Given these results, it is evident that meiotic spindle assembly is independent of DSB formation. Additionally, bipolar spindle assembly also seems to be uncoupled from homologous pairing, since it was not perturbed in the dsl1, dsl2, and afi1 mutants, three maize mutants defective in homologous pairing (Chan and Cande, 1998). Therefore, the molecular mechanism that integrates these homologous recombination-related genes in meiotic spindle assembly remains to be resolved.

As reported for the OsmtopVIB mutant, we observed a substantial proportion of triads at telophase I and polyads at telophase II in ZmmtopVIB meioocytes, demonstrating that maize ZmMTOPVIB is also required for meiotic bipolar spindle assembly. This finding confirms functional conservation of the MTOPVIB role in bipolar spindle assembly between rice and maize. However, we observed fewer abnormal triads or polyads in ZmmtopVIB than were found in the OsmtopVIB mutant line. That discrepancy may be due to differential allelic effects on phenotypes. The two mutant alleles we considered in this study exhibited defective transcript splicing, hypothetically resulting in production of truncated protein. Although the complete ablation in DSB formation indicates that two alleles are at least severe hypomorphs, we cannot rule out the possibility that neither of these ZmmtopVIB mutant alleles was completely null for the specific role in bipolar spindle assembly, unlike the previously studied OsmtopVIB mutant. Therefore, it would be worth investigating spindle defects in other ZmmtopVIB mutants in future studies.

Overall, our study demonstrates that ZmMTOPVIB is essential for meiotic DSB formation and that it plays a critical role in bipolar spindle assembly in maize. Our findings shed light on the evolutionary conservation of the dual functions of MTOPVIB in meiosis, though the mechanisms by which it plays a moonlighting role in bipolar spindle assembly await further investigation.

MATERIALS AND METHODS

Plant Material and Genotyping

The maize (Zea mays) UFMu-07260 mutant line (ZmmtopVIB-1) in the W22 inbred background was obtained from the UniformMu stock center of MaizeDB (https://maizegdb.org/; McCarty et al., 2013). Another mutant allele, EMS4-0742ae (ZmmtopVIB-2) in the B73 inbred background, was obtained from the Maize EMS Induced Mutant Database (http://www.elabcaas.cn/memd/; Lu et al., 2018). All plants were cultivated and fertilized under normal field conditions during the growing season or in a growth chamber (16 h light at 28°C, 8 h dark at 22°C, 60% to 70% humidity). Maize genomic DNA was extracted using a method previously described (Li et al., 2013). Primers used for genotyping and sequencing of the two mutant alleles are listed in Supplemental Table S1.

Pollen Viability

Pollen viability was assessed using the Alexander staining method (Alexander, 1969; Johnson-Brosseau and McCormick, 2004). Mature pollen grains were dissected out of anthers from the wild type and ZmmtopVIB mutants during the pollination stage and then stained with 10% Alexander solution. Images of stained pollen grains were taken using a Leica EZ4 HD stereo microscope equipped with a Leica DM2000 LED illumination system.

RT-qPCR Analysis

Total RNA was isolated from root, stem, leaf, developing embryo, endosperm, young tassel, and young ear using a TRNzol-A Kit Reagent (TIANGEN) according to the manufacturer’s instructions. Reverse transcription was performed using a PrimeScript II first strand cDNA Synthesis Kit (TaKaRa) with Oligo-T primers to obtain cDNA. Quantitative PCR was conducted with a 7500 Fast Real-Time PCR System (Applied Biosystems) using SYBR Green Master Mix (TaKaRa). All reactions were performed with three biological replicates and technical repeats. Gene-specific primers and reference gene (Ubiquitin) primers for internal control are listed in Supplemental Table S1.

Meiotic Chromosome Preparation and DAPI Staining

Young tassels were fixed in Carnoy’s solution (ethanol:glacial acetic acid [3:1]) for 1 d at room temperature and then stored in 70% (v/v) ethanol at 4°C. Anthers were dissected in 45% (v/v) acetic acid solution. Meioocytes were squeezed from anthers and squashed onto slides using coverslips. Slides were frozen in liquid nitrogen and the coverslips were removed immediately. After serial dehydration in 70%, 90%, and 100% (v/v) ethanol, the air-dried slides were stained and mounted with DAPI in Vectashield antifade medium (Vector Laboratories).

FISH and Chromosome Painting

Chromosome spreads were prepared by the method described previously (Wang et al., 2006). Three repetitive DNA probes were used, including the pTa294 clone containing 55 rDNA repeats (Li and Arumuganathan, 2001), the pAtT4 clone containing telomere-specific repeats (Richards and Ausubel, 1988), and c5-conjugated 180-bp knob oligonucleotides. The rDNA and telomere probes were labeled by the Nick Translation Kit (Roche). The chromosome 3 painting probe was labeled with ATTO-550 as previously described (Albert et al., 2019). Slides were counterstained using DAPI in antifade mounting medium (Vector Laboratories). Chromosome images were captured under a Ci-S-Fl. fluorescence microscope (Nikon) equipped with a DS-Q2i microscope camera (Nikon) or under a Delta Vision ELITE system (GE Healthcare) equipped with an Olympus IX71 microscope.

Immunofluorescence Assay

Immunofluorescence was performed as described previously (Pawlowski et al., 2003), with minor modifications. After being dissected and permeabilized in 1× buffer A solution with 4% (w/v) paraformaldehyde for 30 min at room temperature, fresh young anthers were washed twice in 1× buffer A at room temperature and then stored in 1× buffer A at 4°C. Meioocytes were squeezed from anthers and squashed onto slides. After freezing in liquid nitrogen, coverslips were removed immediately. The meioocytes were incubated in blocking buffer diluted with primary antibodies for 1 h in a 37°C humidity chamber, then washed three times in 1× phosphate-buffered saline. Goat anti-rabbit antibodies conjugated with Fluor 555 diluted in blocking buffer were added to the slides. After incubation at 37°C for 1 h, the slides were washed three times in 1× phosphate-buffered saline. Finally, cells were counterstained with DAPI in
antifade mounting medium (Vector Laboratories). The antibodies against ASY1, ZVP1, and yHAX were prepared as described previously (Jing et al., 2019a). Antibodies against AFD1, RAD51, and DMC1 were gifts from collaborators. All primary and secondary antibodies were diluted at 1:100. Images of meiocytes were observed and captured using a Zeiss FL microscope (Nikon) equipped with a DS-Q2 microscope camera (Nikon). Two-dimensional projected images were generated using NIH-Elements software. Further image processing was conducted using ImageJ software (https://imagej.nih.gov/ij/index.html).

Accession Numbers

Genes referenced in this article can be found in GenBank/National Center for Biotechnology Information data libraries under accession numbers Zm0000101041728 (ZmMTOPViB); AT1G06400 (Arabidopsis MTOPViB); GSRNA2T0026842001 (Brassica napus MTOPViB); GLYMA (Glycine max MTOPViB _01G029900); LOC107872855 (Capsicum annuum MTOPViB); LOC107787690 (Nicotiana tabacum MTOPViB); BRAD1, JgMYT1 (Brachypodium distachion MTOPViB); Os06g0768200 (O. sativa MTOPViB); SETIT1_00822zeng (Setaria italica MTOPViB); and SOR_BI010C279900 (Sorghum bicolor MTOPViB).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Phylogenetic analysis of MTOPViB homologs in different plant species.

Supplemental Figure S2. Multiple sequence alignment analysis of MTOPViB proteins in maize, Arabidopsis, and rice.

Supplemental Figure S3. Tissue-specific expression patterns of ZmMTOPViB revealed by RT-qPCR.

Supplemental Figure S4. Sterility phenotypes in ZmmtopViB-2.

Supplemental Figure S5. Meiotic chromosome behavior in ZmmtopViB-2.

Supplemental Table S1. Primers used in this study.

ACKNOWLEDGMENTS

We thank all members of our laboratories for helpful discussion and assistance during this research. We greatly appreciate Changbin Chen (University of Minnesota), Wojciech Pawlowski (Cornell University), and Huabang Chen (Chinese Academy of Science) for their gifts of AFD1, RAD51, and DMC1 antibodies, respectively. We are also grateful to James Birchler (University of Missouri) for the generous gift of chromosome-specific painting probes. We thank the cell biology core laboratory in Institute of Plant and Microbial Biology, Academia Sinica, for microscope imaging technical support. Received July 28, 2020; accepted October 1, 2020; published October 19, 2020.

LITERATURE CITED

Albert PS, Zhang T, Semrau K, Rouillard JM, Kao YH, Wang CR, Daniilova TV, Jiang J, Birchler JA (2019) Whole-chromosome paints in maize reveal rearrangements, nuclear domains, and chromosomal relationships. Proc Natl Acad Sci USA 116: 1679–1685

Alexander MP (1969) Differential staining of aborted and nonabortcd pollen. Stain Technol 44: 117–122

Allers T, Lichten M (2001) Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106: 47–57

Ambrose JC, Cyr R (2007) The kinesin ATTK5 functions in early spindle assembly in Arabidopsis. Plant Cell 19: 226–236

Argunhan B, Leung WK, Afshar N, Terentьев Y, Subramanian VV (2017) Fundamental cell cycle kinases collaborate to ensure timely destruction of the synaptonemal complex during meiosis. 36: 2488–2509

Armstrong SJ, Caryl AP, Jones GIH, Franklin FC (2002) Asy1, a protein required for meiotic chromosome synopsis, localizes to axis-associated chromatin in Arabidopsis and Brassica. J Cell Sci 115: 3645–3655

Barakate A, Higgins JD, Vivera S, Stephens J, Perry RM, Ramsay L, Colas I, Oakley H, Waugh R, Franklin FC, et al (2014) The synaptonemal complex protein ZVP1 is required for imposition of meiotic crossovers in barley. Plant Cell 26: 729–740

Baudat F, de Massy B (2007) Regulating double-stranded DNA break repair towards crossover or non-crossover during mammalian meiosis. Chromosome Res 15: 565–577

Bellani MA, Boataeng KA, McLeod D, Camerini-Otero RD (2010) The expression profile of the major mouse Spo11 isoforms indicates that Spo11P1 introduces double strand breaks and suggests that Spo11P1 has an additional role in prophage in both spermatocytes and oocytes. Mol Cell Biol 30: 4391–4403

Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P (1997) An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature 386: 414–417

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

Blume Y, Lloyd C, Yemets A (2009) Plant tubulin phosphorylation and its role in cell cycle progression. In YB Blume, WV Baird, AI Yemets, and D Breviario, eds, The Plant Cytoskeleton: A Key Tool for Agro-Biotechnology, NATO Science for Peace and Security Series C: Environmental Security, no 2008. Springer, Dordrecht, Germany, pp 145–159

Cai X, Dong F, Edelmann RE, Makaroff CA (2003) The Arabidopsis SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. J Cell Sci 116: 2999–3007

Champoux JJ (2001) DNA topoisomerases: Structure, function, and mechanism. Annu Rev Biochem 70: 369–413

Chan A, Cande WZ (1998) Maize meiotic spindles assemble around chromosomes and do not require paired chromosomes. J Cell Sci 111: 3507–3515

Chen C, Marcus A, Li W, Hu Y, Calzada JP, Grossniklaus U, Cyr RJ, Ma H (2002) The Arabidopsis ATK1 gene is required for spindle morphogenesis in male meiosis. Development 129: 2401–2409

Cloud V, Chan YL, Grubb J, Budke B, Bishop DK (2012) Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. Science 337: 1222–1225

Compton DA (2000) Spindle assembly in animal cells. Annu Rev Biochem 69: 95–114

Corbett KD, Benedetti P, Berger JM (2007) Holokaryonyse assembly and ATP-mediated conformational dynamics of topoisoerase VI. Nat Struct Mol Biol 14: 611–619

Corbett KD, Berger JM (2003) Structure of the topoisoerase VI-B subunit: Implications for type II topoisoerase mechanism and evolution. EMBO J 22: 151–163

de Massy B (2013) Initiation of meiotic recombination: How and where? Conservation and specificities among eukaryotes. Annu Rev Genet 47: 563–599

Dickey AN, Yim WS, Faller R (2009) Using ergosterol to mitigate the deleterious effects of ethanol on bilayer structure. J Phys Chem B 113: 2388–2397

Duita R, Ionuｙe M (2000) GHKL, an emergent ATPase/kinase superfamily. Trends Biochem Sci 25: 24–28

Forterre P, Gadelle D (2009) Phylogenomics of DNA topoisomerases: Their origin and putative roles in the emergence of modern organisms. Nucleic Acids Res 37: 679–692

Forterre P, Gribaldo S, Gadelle D, Serre MC (2007) Origin and evolution of DNA topoisomerases. Biochimie 89: 427–446

Fu M, Wang C, Xue F, Higgins J, Chen M, Zhang D, Liang W (2016) The DNA topoisomerase VI-B subunit OsMTOPViB is essential for meiotic recombination initiation in rice. Mol Plant 9: 1539–1541

Gadde S, Heald R (2004) Mechanisms and molecules of the mitotic spindle. Curr Biol 14: R797–R805

Gadelle D, Krupovic M, Raymann K, Mayer C, Forterre P (2014) DNA topoisoerase VII-E: A novel subfamily of type IIB topoisoerases encoded by free or integrated plasmids in Archaea and Bacteria. Nucleic Acids Res 42: 8578–8591

Golubovskaya IN, Hamant O, Timofejeva L, Wang CJ, Braun D, Meeley R, Cande WZ (2006) Alleles of Rad51 affect fertility, sterility, and crossover recombination during meiosis. J Cell Sci 119: 3306–3315

Golubovskaya IN, Wang CJ, Timofejeva L, Cande WZ (2011) Maize meiotic mutants with improper or non-homologous synapsis due to problems in pairing or synaptonemal complex formation. J Exp Bot 62: 1533–1544

Graille M, Cladière L, Durand D, Leconte F, Gadelle D, Quevillon-Cheruel S, Vachette P, Forterre P, van Tilbeurgh H (2008) Crystal
structure of an intact type II DNA topoisomerase: Insights into DNA
transfer and recombination. Structure 14: 360–370

Grelon M (2016) Meiotic recombination mechanisms. C R Biol 339: 247–251

Gunw PJ, Ghoshdastider U, Whitaker S, Popp D, Robinson RC (2015)
The evolution of compositionally and functionally distinct actin fila-
ments. J Cell Sci 128: 2009–2019

Harper L, Golubovskaya I, Cande WZ (2004) A bouquet of chromosomes.
J Cell Sci 117: 4025–4032

Hartung F, Angles J, Meister A, Schubert I, Osman K, Darbyshire A, Sanchez-
Loidl J (1996) Self-organization of microtubules into bipolar spindles
around artificial chromosomes in Xenopus egg extracts. Nature 382: 420–425

Higgins DM, Nannas NJ, Dawe RK (2016) The maize divergent spindle
1 (dvi1) gene encodes a kinesin-14A motor protein required for meiotic
spindle pole organization. Front Plant Sci 7: 1277

Higgins JD, Sanchez-Moran E, Armstrong SJ, Jones GH, Franklin FC
(2005) The Arabidopsis synaptonemal complex protein ZYP1 is required
for chromosome synopsis and normal fidelity of crossing over. Genes Dev 19:
2488–2500

Hunter N, Kleckner N (2001) The single-end invasion: An asymmetric in-
termediate at the double-strand break to double-holliday junction
transition of meiotic recombination. Cell 106: 59–70

Jiang H, Wang FF, Wu YT, Zhou X, Huang XY, Zhu J, Gao JF, Dong RB,
Cao KM, Yang ZN (2009) MULTIPOLAR SPINDLE 1 (MPS1), a novel
coiled-coil protein of Arabidopsis thaliana, is required for meiotic spindle
organization. Plant J 59: 1001–1010

Jing J, Zhang T, Wang Y, Cui Z, He Y (2019a) ZmRAD51C is essential for
double-strand break repair and homologous recombination in maize meiosis.
Int J Mol Sci 20: 5513

Jing JI, Zhang T, Wang YZ, He Y (2019b) Advances towards how meiotic
recombination is initiated: A comparative view and perspectives for
plant meiosis research. Int J Mol Sci 20: 4718

Johnson-Brousseau SA, McCormick S (2004) A compendium of methods
useful for characterizing Arabidopsis pollen mutants and gametophytically-expressed genes. Plant J 39: 761–775

Keeney S, Gioux CN, Kleckner N (1997) Meiotic-specific DNA double-
strand breaks are catalyzed by Spo11, a member of a widely conserved
protein family. Cell 86: 375–384

Keeney S, Kleckner N (1995) Covalent protein-DNA complexes at the 5’
strand termini of meiosis-specific double-strand breaks in yeast. Proc Natl Acad Sci USA 92: 11274–11278

Keeney S, Neale MJ (2006) Initiation of meiotic recombination by forma-
tion of DNA double-strand breaks: Mechanism and regulation. Biochem
Soc Trans 34: 523–525

Ku JC, Roncetti G, Golubovskaya I, Lee DH, Wang C, Timofeeva L, Kao YH,
Gomez Angapa AK, Kremling K, Williams-Carrier R, et al (2020)
Dynamic localization of SPO11-1 and conformational changes of meiotic
axial elements during recombination initiation of maize meiosis. PLoS One
14: e0245138

Li H, Li J, Cong XH, Duan YB, Li L, Wei PC, Lu XZ, Yang JB
(2019) Molecular and functional analyses of UnornuMa transposon insertion
lines. Methods Mol Biol 1057: 157–166

Moiseeva V, Amelina H, Collop LC, Armstrong CA, Pearson SR, Tomita K
(2017) The telomere bouquet facilitates meiotic prophase progression
and exit in fission yeast. Cell Discov 3: 17041

Nakamura AJ, Rao VA, Pommier Y, Bonner WM (2010) The complexity
of phosphorylated H2AX foci formation and DNA repair assembly at DNA
double-strand breaks. Cell Cycle 9: 389–397

Nannas NJ, Dawe RK (2016) Live-cell imaging of meiotic spindle and chro-
mosome dynamics in maize (Zea mays). Curr Proto Plant Biol 1: 546–565

Neale MJ, Pan J, Keeney S (2005) Endonucleolytic processing of covalent
protein-linked DNA double-strand breaks. Nature 436: 1053–1057

Page SL, Hawley RS (2004) The genetics and molecular biology of the
synaptonemal complex. Annu Rev Cell Dev Biol 20: 525–558

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

Prieto P, Martin A, Cabrera A (2004) Chromosomal distribution of telo-
meric and telomere-associated sequences in Hordeum chilense by in situ
hybridization. Hereditas 141: 122–127

Richards EJ, Ausubel FM (1988) Isolation of a higher eukaryotic telomere
drom Arabidopsis thaliana. Cell 53: 127–136

Robert T, Nore A, Brun C, Mafre C, Grimi B, Bourbon HM, de Massy B
(2016a) The TopoVIB-like protein family is required for meiotic DNA
double-strand break formation. Science 351: 943–949

Robert T, Vielynych N, Mézard C, de Massy B, Grelon M (2016b) A new light
on the meiotic DSB catalytic complex. Semin Cell Dev Biol 54: 165–176

Schertahan H (2001) A bouquet makes ends meet. Nat Rev Mol Cell Biol 2:
621–627

Shao T, Tang D, Wang K, Wang M, Che L, Qin B, Yu H, Li M, Gu M, Zhong C
(2017) OsREC8 is essential for chromatic cohesion and meiotic
phase I monopolar orientation in rice meiosis. Plant Physiol 175:
1386–1396

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

Sprink T, Hartung F (2014) The splicing fate of plant SPO11 genes. Front
Plant Sci 5: 214

Syntingston LS, Gautier J (2011) Double-strand break end resection and
repair pathway choice. Annu Rev Genet 45: 247–271

Tomita K, Cooper JP (2007) The telomere bouquet controls the meiotic
spindle. Cell 130: 113–126

Vernos I, Karnesti E (1995) Chromosomes take the lead in spindle as-
sembly. Trends Cell Biol 5: 397–399

Vieleynych N, Chambon A, Vezon D, Pereira L, Cheysheva L, De Muaty A,
Mézard C, Mayer C, Grelon M (2016) A DNA topoisomerase VI-like complex
initiates meiotic recombination. Science 351: 939–943

Wang CJ, Harper L, Cande WZ (2006) High-resolution single-copy gene
fluorescence in situ hybridization and its use in the construction of a
cytogenetic map of maize chromosome 9. Plant Cell 18: 529–544

Wang JC (2002) Cellular roles of DNA topoisomerases: A molecular per-
spective. Nat Rev Mol Cell Biol 3: 430–440

Wang M, Wang K, Tang D, Wei C, Li M, Shen Y, Chi Z, Gu M, Cheng Z
(2010) The central element protein ZEP1 of the synaptonemal complex
regulates the number of crossovers during meiosis in rice. Plant Cell 22:
417–430

Wang Y, Jiang L, Zhang T, Jing J, He Y (2018) ZmComl is required for both
mitotic and meiotic recombination in maize. Front Plant Sci 9: 1005

Wittmann T, Hyman A, Desai A (2001) The spindle: A dynamic assembly
of microtubules and motors. Nat Cell Biol 3: E28–E34

Xue Z, Li Y, Zhang L, Shi W, Zhang C, Feng M, Zhang F, Tang D, Yu H,
Gu M, et al (2016) OsMTP0VIB promotes meiotic DNA double-strand break
formation in rice. Mol Plant 9: 1535–1538

Xue Z, Liu C, Shi W, Miao Y, Shen Y, Tang D, Li Y, You A, Xu Y, Chong K,
et al (2019) OsMTP0VIB is required for meiotic bipolar spindle assem-
blage. Proc Natl Acad Sci USA 116: 15967–15972

Yuan J, Adamski R, Chen J (2010) Focus on histone variant H2AX: To be or
to be. FEBS Lett 584: 3177–3172

Zhang H, Dawe RK (2011) Mechanisms of plant spindle formation. Chro-
mosome Res 19: 335–344