Resolvase OsGEN1 Mediates DNA Repair by Homologous Recombination[1][OPEN]

Chong Wang, James D. Higgins, Yi He, Pingli Lu, Dabing Zhang, and Wanqi Liang*

Joint International Research Laboratory of Metabolic and Developmental Sciences, Shanghai Jiao Tong University-University of Adelaide Joint Centre for Agriculture and Health, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 20040, China (C.W., Y.H., D.Z., W.L.); Department of Genetics, University of Leicester, Leicester LE1 7RH, United Kingdom (J.D.H.); State Key Laboratory of Genetic Engineering and Collaborative Innovation Center of Genetics and Development, Institute of Plant Biology, School of Life Sciences, Fudan University, Shanghai 200433, China (P.L.); and School of Agriculture, Food and Wine, University of Adelaide, Urrbrae, South Australia 5064, Australia (D.Z.)

ORCID IDs: 0000-0001-9433-0112 (C.W.); 0000-0001-6027-8678 (J.D.H.); 0000-0002-5218-541X (Y.H.); 0000-0002-1764-2929 (D.Z.); 0000-0002-9938-5793 (W.L.).

Yen1/GEN1 are canonical Holliday junction resolvases that belong to the RAD2/XPG family. In eukaryotes, such as budding yeast, mice, worms, and humans, Yen1/GEN1 work together with Mus81-Mms4/MUS81-EME1 and Slx1-Slx4/SLX1-SLX4 in DNA repair by homologous recombination to maintain genome stability. In plants, the biological function of Yen1/GEN1 remains largely unclear. In this study, we characterized the loss of function mutants of *OsGEN1* and *OsSEND1*, a pair of paralogs of Yen1/GEN1 in rice (*Oryza sativa*). We first investigated the role of *OsGEN1* during meiosis and found a reduction in chiasma frequency by ~6% in *osgen1* mutants, compared to the wild type, suggesting a possible involvement of OsGEN1 in the formation of crossovers. Postmeiosis, OsGEN1 foci were detected in wild-type microspore nuclei, but not in the *osgen1* mutant concomitant with an increase in double-strand breaks. Persistent double-strand breaks led to programmed cell death of the male gametes and complete male sterility. In contrast, depletion of *OsSEND1* had no effects on plant development and did not enhance *osgen1* defects. Our results indicate that OsGEN1 is essential for homologous recombinational DNA repair at two stages of microsporogenesis in rice.

Homologous recombination (HR) is essential for maintaining genome stability by promoting the accurate repair of DNA lesions, such as double-strand breaks (DSBs) and stalled replication forks (Bzymek et al., 2010; Rass, 2013). During meiosis I, HR also facilitates the formation of chiasmata, the cytological manifestation of genetic crossovers, thus ensuring correct chromosome segregation at the first meiotic division. HR is initiated by a DSB that leads to the creation of 3′ single-stranded DNA tails, followed by Rad51-mediated strand-exchange between sister or homologous chromatids that form DNA joint molecules (JMs; Mimitou and Symington, 2009). Most JMs are repaired by the synthesis-dependent strand annealing pathway (Andersen and Sekelsky, 2010; Sarbajna et al., 2014). The remaining JMs may be ligated to form Holliday junctions (HJs), an important DNA intermediate consisting of four DNA strands of two homologous DNA helices (Mimitou and Symington, 2009). The resolution of HJs is crucial for the completion of recombination. In addition, HJs are toxic DNA structures if not processed appropriately because they can interfere with normal chromosome segregation as well as DNA replication. HJs are processed by two major mechanisms. One is through dissolution by the BLM-TopoisomeraseIIIα-RMI1-RMI2 complex (BTR in human, STR in yeast) to give rise to non-crossover (NCO) products (Wu and Hickson, 2003). Alternatively, HJs are resolved by structure-specific endonucleases to generate both crossover (CO) and NCO products (Mimitou and Symington, 2009).

HJ resolvases have been identified from a wide variety of organisms, including bacteriophages, archaebacteria, bacteria, viruses, and eukaryotes. It has been demonstrated that Mus81-Mms4/MUS81-EME1 (MMS and UV-sensitive protein 81-Methyl Methane Sulfonate

[1] This work was supported by funds from the National Key Research and Development Program of China (grant no. 2016YFD0100804), the National Key Basic Research Developments Program, Ministry of Science and Technology (grant no. 2013CB126902), the National Transgenic Major Program (grant no. 2016ZX08009003-003-007), and the National Natural Science Foundation of China (grant no. 31322040). Research in the Higgins laboratory is funded by the Biotechnology and Biological Sciences Research Council.

* Address correspondence to wqliang@sjtu.edu.cn.

The 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: Wanqi Liang (wqliang@sjtu.edu.cn).

C.W., J.D.H., and W.L. designed the experiments; C.W. and Y.H. carried out the experiments; C.W., J.D.H., P.L., and W.L. analyzed the data; C.W., J.D.H., D.Z., and W.L. wrote the article.

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.16.01726

sensitivity4/Essential meiotic endonuclease1), Slx1-Slx4/SLX1-SLX4 (Synthetic lethal of unknown function), and Yen1/GEN1 (Holliday junction resolvase YEN1/Gen endonuclease homolog1) are key structure-specific endonucleases involved in HJ resolution in eukaryotes (Boddy et al., 2001; Schwartz and Heyer, 2011). Mus81-Mms4 and Slx1-Slx4 were identified from the same screen for proteins essential for the viability of yeast cells lacking the *Slow growth suppressor1* gene (Mullen et al., 2001). Mus81-Mms4 belong to the XPF endonuclease family and are capable of cutting several branched DNA structures, including 3′ flaps, nicked HJs, and D-loops (Boddy et al., 2001; Chen et al., 2001; Bastin-Shanower et al., 2003; Ciccia et al., 2003; Oğrünç and Sancar, 2003; Whitby et al., 2003; Taylor and McGowan, 2008). SLX1 is the catalytic subunit of the SLX1-SLX4 heterodimer endonuclease complex and belongs to the GIY-YIG nuclease family (Fricke and Brill, 2003; Dunin-Horkawicz et al., 2006). The SLX1-SLX4 complex prefers to cut 5′ flap structures in vitro while it is also able to cleave HJs (Fricke and Brill, 2003; Fekairi et al., 2009; Svendsen et al., 2009; Wyatt et al., 2013). MUS81-EME1 and SLX1-SLX4 act in the same pathway and cooperatively introduce asymmetric cuts on HJs. The gapped and flapped DNA intermediates generated by the asymmetric incisions require further processing before ligation (Fricke and Brill, 2003; Castor et al., 2013; Wyatt et al., 2013). Recent reports showed that *Saccharomyces cerevisiae* Yen1 and its human ortholog GEN1 introduce symmetric nicks on HJs in a MUS81-EME1 and SLX1-SLX4 independent pathway (Wyatt et al., 2013). Yen1/GEN1 were considered to be the canonical HJ resolvases in eukaryotes as they cleave HJs in a manner analogous to that of the *Escherichia coli* resolvase RuvC (crossover junction endodeoxyribonuclease RuvC; Ip et al., 2008; Rass et al., 2010).

Yen1/GEN1 homologs belong to the fourth clade of the RAD2/XPG family of structure-specific nucleases and contain three functional domains: N-terminal XPG domain, internal XPG nuclease domain (XPGI), and helix-hairpin-helix (HhH) domain (Rass et al., 2010; Bauknecht and Kobbe, 2014). It was reported that Yen1/GEN1 homologs from human, yeast, *Caenorhabditis elegans*, Arabidopsis (*Arabidopsis thaliana*), and rice (*Oryza sativa*) have the ability to cleave 5′-flaps, replication forks (RFs), and HJs in vitro (Ip et al., 2008; Bailly et al., 2010; Yang et al., 2012; Bauknecht and Kobbe, 2014; Lee et al., 2015). The Yen1/GEN1 HJ resolution pathway shows a complicated interplay with other HJ resolution pathways and functional variation in different organisms. In *S. cerevisiae* the *yen1* mutant lacks an obvious phenotype, while the *yen1 mms4* double mutant is severely compromised in meiotic joint molecule resolution and fails to complete meiosis, suggesting that Yen1 acts in a Mus81-Mms4 independent pathway (Ip et al., 2008). In addition, although the *yen1* mutant in budding yeast is proficient in DNA repair, the *yen1 mus81* double mutant is extremely sensitive to a variety of DNA damaging agents and accumulates toxic recombination intermediates after MMS treatment, indicating that Yen1 can process recombination intermediates that arise in the absence of Mus81 after replication fork damage (Blanco et al., 2010). *Kluyveromyces lactis* mutants lacking a functional Mus81 are severely compromised in sporulation efficiency and crossover frequency, but lacking both Mus81 and Yen1 showed no further reduction in spore formation. Overexpression of Yen1 partially rescued the crossover defect in *mus81* mutant and the DNA damage sensitivity of *mus81* and *sgs1* mutants. These results suggest that Yen1 is redundant with Mus81 and SGS1 in meiosis and DNA repair processes (Chen and Aström, 2012). In *C. elegans*, GEN-1 facilitates the repair of DNA DSBs only when other structure-specific nucleases are absent, and is not essential for homologous recombination during meiosis (Saito et al., 2012, 2013). Mutational analysis reveals that GEN-1's function in DNA damage signaling is separated from its role in DNA repair. GEN-1 promotes germ cell cycle arrest and apoptosis via a pathway parallel to canonical DNA damage response pathways and acts redundantly with the 9-1-1 complex to ensure genome stability. This suggests that GEN-1 acts as a dual function Holliday junction resolvase that coordinates DNA damage signaling with a late step in DNA DSB repair (Bailly et al., 2010). In *Drosophila*, *mus81 gen* double mutants have elevated levels of apoptosis. In contrast to yeast, *gen* mutants are more hypersensitive to DNA damage than *mus81* mutants, indicating that GEN plays a more dominant role than MUS81-MMS4 in responding to DNA damage (Andersen et al., 2011). Depletion of GEN1 by RNA interference-mediated gene silencing in human cells disrupts mitotic progression and increases the number of cells with multinuclei, increased apoptosis, and elevated levels of spontaneous DNA damage (Gao et al., 2012; Rodrigue et al., 2013). These phenotypes indicate that human GEN1 is required for DNA repair and recombination, which may indirectly lead to centrosome abnormalities (Rodrigue et al., 2013). Human cells depleted of GEN1 exhibit mild sensitivity to DNA damage after MMS and CPT treatment (Svendsen et al., 2009). Recent studies indicate that GEN1 and SLX-MUS81 act in two distinct pathways in sister chromatid exchange formation, chromosome abnormalities, and cell death (Wechsler et al., 2011; Garner et al., 2013; Wyatt et al., 2013). Human cells lacking SLX, MUS81, and GEN1 simultaneously exhibited impaired movement of the replication fork, endogenous checkpoint activation, chromosome instability, and multinucleation, indicating that GEN1 acts together with SLX4 and MUS81 to assure the timely and faithful completion of mitosis and to increase genome stability throughout the cell cycles (Sarbajna et al., 2014).

Although the roles of Yen1/GEN1 homologs have been extensively studied in yeast and animals, their biological functions remain largely unknown in plants. There is only one Yen1/GEN1 type of resolvase in yeast, animals, and moss. On the contrary, two Yen1/GEN1 homologs, named GEN1 and SINGLE-STRAND

DNA ENDONUCLEASE1 (SEND1), exist in the genome of most plants (Bauknecht and Kobbe, 2014; Olivier et al., 2016). It was reported that in Arabidopsis, SEND1 but not GEN1 acts as a functional Yen1/GEN1 homolog and plays an important role in the repair of toxic replication intermediates and telomere homeostasis, but not in meiotic recombination (Olivier et al., 2016), suggesting a functional diversification of these two paralogs. In rice, RNA interference-mediated gene silencing of rice GEN1 results in complete male sterility, which indicates that OsGEN1 plays an essential role in male microspore development in rice (Moritoh et al., 2005). OsSEND1 is induced by UV and DNA damage agents, indicating that it may play a role in DNA repair in somatic cells (Furukawa et al., 2003). However, it remains unclear how OsGEN1 regulates microspore development and whether OsSEND1 and OsGEN1 have redundant or divergent functions.

Here we describe the characterization of *osgen1*, *ossend1* single mutants, and *osgen1 ossend1* double mutants. Our data revealed that OsGEN1 but not OsSEND1 plays an essential role in male meiosis and mitotic DNA replication processes. We conclude that OsGEN1 is a functional homolog of Yen1/GEN1 in rice and that it has indispensable roles in chiasmata formation and DNA lesion repair in rice male gametophytes.

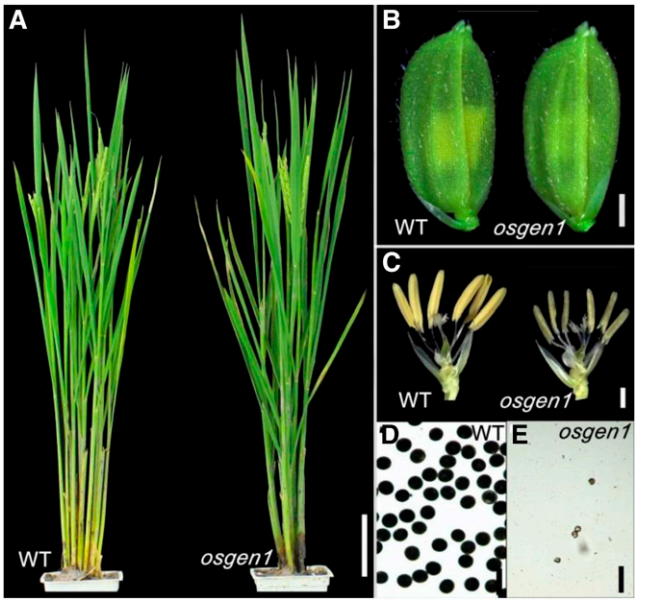

Figure 1. *osgen1* plants exhibit normal vegetative growth but severe fertility defects. A, Wild-type and *osgen1* plants after heading. B, Wild-type and *osgen1* spikelets before anthesis. C, Wild-type and *osgen1* spikelets after removal of the palea and lemma. D and E, I_2-KI staining of the pollen grains within the anther of the wild type (D) and *osgen1* (E). Bars = 5 cm in A, 1 mm in B and C, and 100 μm in D and E.

RESULTS

Identification of the *osgen1* Mutant

We isolated a male sterile mutant designated *osgen1* from our rice mutant library constructed by using ^{60}Co γ-ray radiated *O. sativa* ssp. *japonica* cv 9522 (Chu et al., 2005; Chen et al., 2006). The *osgen1* mutant showed normal vegetative growth and floral development (Fig. 1, A and B), except the stamen was smaller (Fig. 1C) and did not produce normal pollen (Fig. 1, D and E). When the *osgen1* mutant was pollinated with wild-type pollen, they could set seeds normally and all F1 progeny displayed a normal phenotype, indicating that *osgen1* female fertility is normal. F2 progeny segregated 75 sterile plants from 288 plants totally ($\chi^2 = 0.1667$, $P > 0.05$), indicating monofactorial recessive inheritance of the mutation. To identify the stage responsible for the male fertility defect in *osgen1*, we prepared transverse sections of pollen mother cells (PMCs) in the mutant and the wild type. The wild-type PMCs appeared normal and were able to complete meiosis and produce microspores, whereas the mutant microspores arrested at the early microspore stage (Zhang et al., 2011a) and then degenerated (Supplemental Fig. S1).

To isolate the mutated gene that controls the sterile phenotype, a F2 mapping population was generated by crossing the mutant plants with the *Indica* rice variety 9311. Sterile plants segregated in F2 populations were collected for genotyping. We initially mapped the mutation to chromosome 9. The mutation locus was further narrowed between two InDel markers (907-3 and 907-6), with a genetic distance of 78.5 and 78.8 centimorgans, respectively (Supplemental Fig. S2A). By resequencing the mutant genomic DNA, we found a 2 bp deletion in the seventh exon of *OsGEN1* gene (LOC Os09g35000), leading to a frame shift and premature translation termination (Supplemental Fig. S2B). A genomic DNA fragment containing the *OsGEN1* gene and 2 Kb upstream sequence was translationally fused to the β-glucuronidase (GUS) reporter and eGFP reporter separately and were introduced into *osgen1* mutant plants. Both primary transgenic lines were fertile (Supplemental Fig. S4), indicating that *OsGEN1* is responsible for the male sterile phenotype in *osgen1*. The full-length OsGEN1 protein sequence was used as a query to search the National Center for Biotechnology Information protein database and identified 41 homologs belonging to the RAD2/XPG family (Supplemental Fig. S2C). Of these, OsGEN1 clustered with 10 homologs in the Yen1/GEN1 subfamily, proteins that have similar structures but are variable in length and isoelectric points (Supplemental Fig. S3). The XPGI and N-terminal XPG domains are conserved in the Yen1/GEN1 subfamily (Moritoh et al., 2005; Yang et al., 2012), whereas the C terminus is highly variable. As the *osgen1* mutation is present in the C terminus, the sterile phenotype suggests that this is important for OsGEN1 function.

We further investigated the spatio-temporal expression pattern of the *OsGEN1* by quantitative RT-PCR (qRT-PCR) analyses. *OsGEN1* is highly expressed in the shoot, young leaves, and anthers at stage 8b (tetrad stage; Supplemental Fig. S5).

OsGEN1 Mutation Influences Meiotic Progression in the Pollen Mother Cell

It has been reported that OsGEN1 has HJ resolvase activity in vitro (Yang et al., 2012). To investigate a potential role for OsGEN1 in recombination, we analyzed the chromosome behavior in wild-type and *osgen1* PMCs. During leptotene to pachytene of meiosis, chromosomes appeared normal in the *osgen1* mutant, whereas at diakinesis, a small number of cells with univalents were observed (9.62%, n = 156; Fig. 2I). Randomly distributed DAPI bodies were also observed at later stages, including metaphase I (12.5%, n = 113; Fig. 2J), anaphase I (8.33%, n = 120; Fig. 2K), telophase I (9%, n = 111; Fig. 2L), prophase II (13.2%, n = 47; Fig. 2M), metaphase II (11.2%, n = 63; Fig. 2N), anaphase II (7%, n = 56; Fig. 2O), and tetrad (1.70%, n = 176; Fig. 2P). These observations suggested that the number of crossovers was affected in the mutant PMCs.

To further investigate whether OsGEN1 deficiency affects recombination, we quantified the frequency and distribution of chiasmata in *osgen1* and the wild type by studying the shape of bivalents at diakinesis and metaphase I. At diakinesis, the X-shaped and ring-shaped bivalents were treated as having one and two chiasmata, respectively, and the 8-shaped bivalents were treated as having three chiasmata. At metaphase I, the rod-shaped bivalents were treated as having one chiasmata and the ring-shaped having two or three chiasmata depending on the different characteristic as described in Sanchez Moran et al. (2001). The mean chiasma frequency in the wild type was 20.3 per cell (n = 168), while in *osgen1* mutant the mean chiasma frequency was 18.8 per cell (n = 170; Fig. 3A). Thus, mutation of *osgen1* led to an ~6% reduction in chiasmata compared to the wild type. Because a small reduction in chiasma frequency could be more easily detected against a background with low numbers of residual chiasmata, we generated an *osgen1 hei10* double mutant (Supplemental Fig. S6) and compared chiasma frequency to each of the single mutants. The chiasmata frequency in the *hei10* single mutant was 6.1 per cell (n = 167) A significant reduction in the number of chiasmata was observed in the *osgen1 hei10* double mutant (reduced to 4.0 per cell, n = 149; $t_{[314]} = 7.2$, $P < 0.01$; Fig. 3B). Furthermore, *OsGEN1* mutation led to a decrease in the number of bivalents having two and three chiasmata, but higher numbers of univalents and bivalents with one chiasmata in both the wild type and *hei10* background (Fig. 3C).

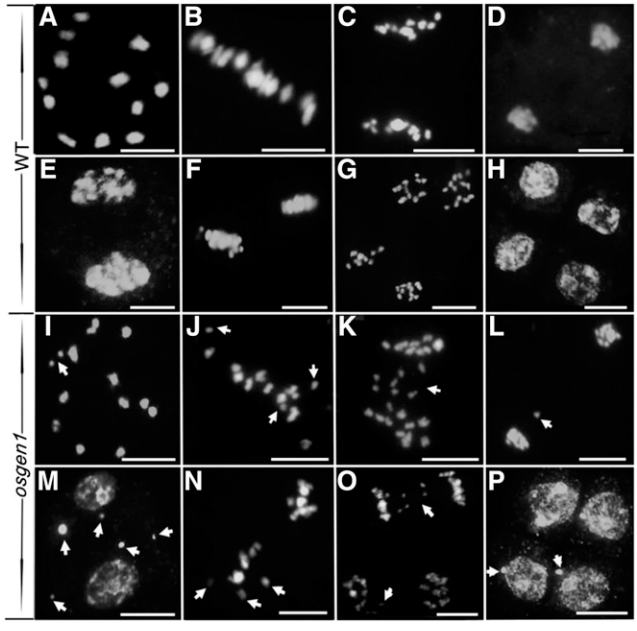

Figure 2. Meiotic progression in wild-type and *osgen1* PMCs. Meiotic progression was assayed in wild-type (A–H) and *osgen1* PMCs (I–P). A and I, Late diakinesis; B and J, metaphase I; C and K, anaphase I; D and L, telophase I; E and M, prophase II; F and N, metaphase II; G and O, anaphase II; H and P, tetrad. Bar = 5 μm.

OsGEN1 Is Dispensable for Formation of Synaptonemal Complex and Late Recombination Intermediates

To investigate whether OsGEN1 is required for synaptonemal complex (SC) formation, we performed dual-immunolocalization analysis of axis-associated proteins OsREC8, PAIR2, and PAIR3 and the transverse filament protein ZEP1 (Nonomura et al., 2006; Wang et al., 2010, 2011; Shao et al., 2011). In *osgen1*, no difference in the localization of OsREC8, PAIR2, PAIR3, and ZEP1 was observed in the mutant PMCs (Fig. 4; Supplemental Fig. S7), indicating that SC assembly is normal in *osgen1*.

We further analyzed male meiosis in *osgen1* by using a panel of antibodies that mark recombination sites. DSB formation is the first step in meiotic recombination and in rice can be assayed indirectly by detection of phosphorylated γH2AX (Mahadevaiah et al., 2001; Che et al., 2011). OsCOM1 and RPA2c are required for DSB end-processing and 3′-single-strand invasion after DSB generation (Ji et al., 2012; Li et al., 2013) and DMC1 and RAD51C indicate the number of potential strand invasion events (Neale and Keeney, 2006; Tang et al., 2014). Our analysis showed that the localization of γH2AX, OsCOM1, RPA2c, DMC1, and RAD51C in *osgen1* was indistinguishable to wild-type male meiocytes (Fig. 5, A–H, J, and K; Supplemental Fig. S8, A–E). At zygotene, the average number of DMC1 foci was 64.9 (n = 18, range 48–98) in the wild type, and 61.3 (n = 18, range 54–84) in *osgen1*; the average number of RAD51C foci was 56.2 (n = 20, range 46–80) in the wild type, and 58.7 (n = 20, range 46–69) in *osgen1*. These results indicated that DSB formation and the initial steps of recombination were unaffected in the *osgen1* mutant. HEI10 is the rice homolog of budding yeast Zip3 and *C. elegans* ZHP-3. It has been reported that HEI10 is required for Class I CO formation and used as a marker for COs during late prophase I in rice (Wang et al., 2012). At pachytene, the average number of HEI10 foci was 23.9 (n = 10, range

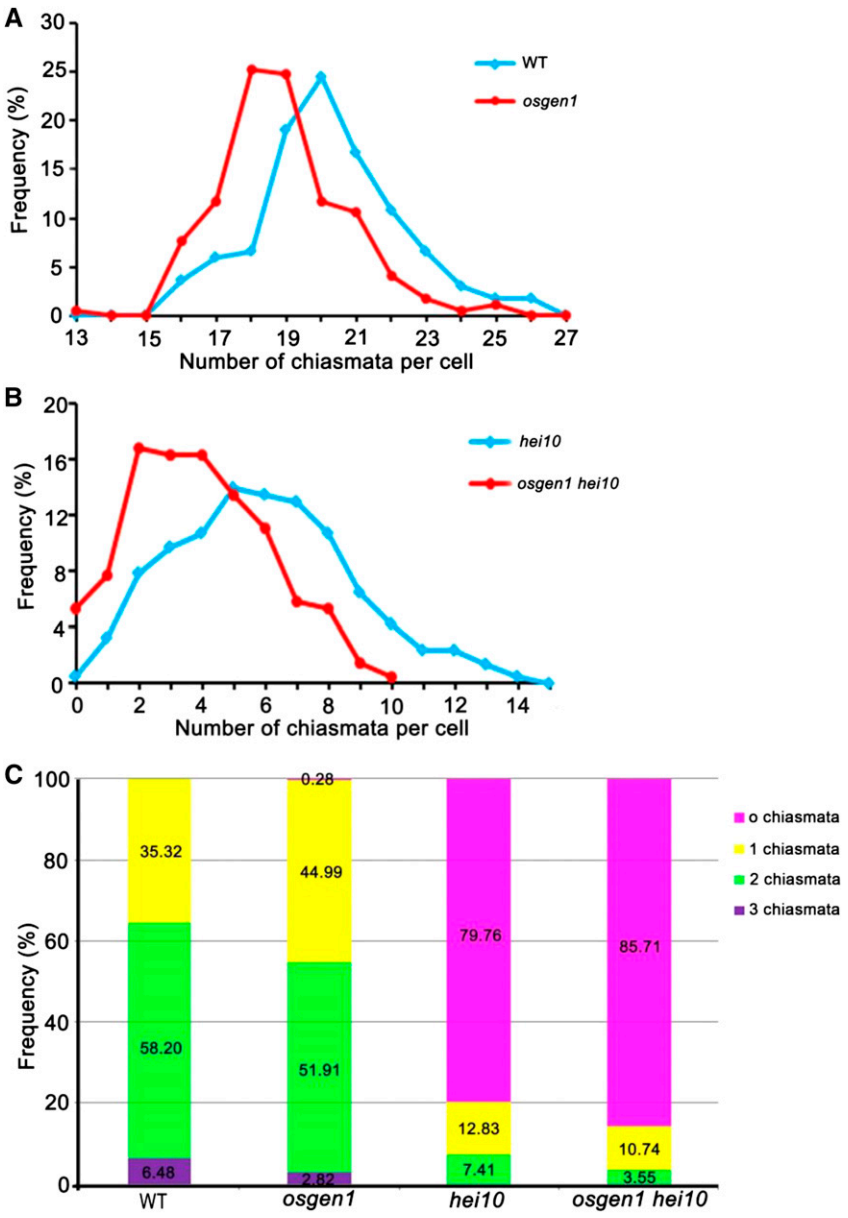

Figure 3. Chiasma distribution in wild-type and *osgen1* plants compared to *hei10* and *osgen1 hei10*. A, Chiasma distribution in wild-type (blue; n = 168) and *osgen1* (red; n = 170) PMCs. B, Chiasma distribution in *hei10* (blue; n = 167) and *osgen1 hei10* (red; n = 149) PMCs. C, Chiasmata frequency in the wild type (n = 63), *osgen1* (n = 59), *hei10* (n = 50), and *osgen1 hei10* double mutant (n = 59).

21–27) in the wild type, and 23.2 (n = 10, range 22–28) in *osgen1* (Fig. 5, I and L; Supplemental Fig. S8F), suggesting that HEI10 localization was not influenced by *OsGEN1* mutation.

OsGEN1 Dynamically Localizes to Meiotic Chromosomes during Meiosis

To define OsGEN1 distribution during meiosis, dual immunolocalization assays were performed using OsGEN1 and OsREC8 antibodies. At leptotene, 147.5 punctate OsGEN1 foci were observed (n = 4, range 127–173; Fig. 6, A–C). At early zygotene, the numbers of OsGEN1 foci began to decline (79.7, n = 7, range 67–89; Fig. 6, D–F). At late zygotene, fewer OsGEN1 foci were observed (18.1, n = 10, range 13–24; Fig. 6, G–I) that continued to decline at pachytene ~7.18 (n = 11, range 5–13; Fig. 6, J–L) until diplotene (3.6, n = 8, range 3–5; Fig. 6, M–O).

OsGEN1 Does Not Colocalize with Recombination Proteins

To understand the relationship of OsGEN1 with other recombination proteins, we used dual-immunolocalization to investigate the colocalization of OsGEN1 with RPA2c, RAD51C, DMC1, and HEI10, respectively. At zygotene, the mean number of RPA2c foci per cell was 137.5 (n = 4, range 113–199) and OsGEN1 foci was 87 (n = 4, range 73–100), and two to

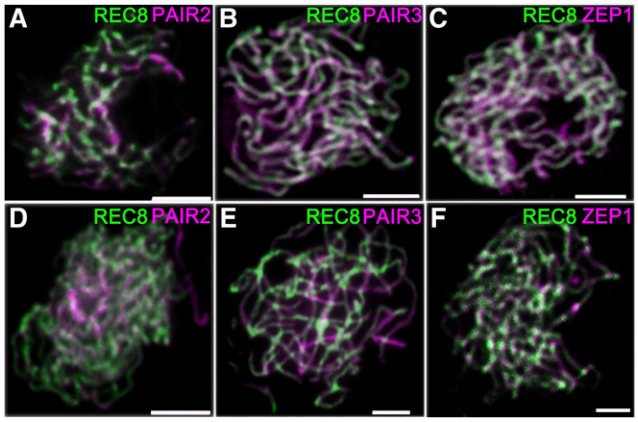

Figure 4. Dual immunolocalization of SC proteins OsZEP1 (magenta) and OsREC8 (green) in wild-type and *osgen1* PMCs. A to C, The wild type; D to F, *osgen1* mutant; A to F, pachytene. Bar = 2 μm.

three merged signals were found. At early pachytene, RPA2c foci per cell was 102 (n = 4, range 83–134) and OsGEN1 foci was 34.25 (n = 4, range 30–39), and two to three merged signals were observed. Later, both RPA2c and OsGEN1 foci decreased and were hardly detected. We also investigated the colocalization of OsGEN1 with DMC1 and RAD51C in PMCs. Different from MUS81 in Arabidopsis (Higgins et al., 2008), most of OsGEN1 foci do not colocalize with those of both proteins (Fig. 7, D–I). HEI10 is involved in the interference-sensitive CO pathway and marks these sites during diakinesis. To determine the relationship between HEI10 and OsGEN1, we counted the numbers of HEI10 and OsGEN1 foci in PMCs (Fig. 7, J–L). At zygotene, the mean number of HEI10 foci per cell was 170.25 (n = 4, range 141–201), and the mean number of OsGEN1 foci per cell was 116.25 (n = 4, range 88–160). At early pachytene, both HEI10 and OsGEN1 foci decreased, and the mean number of HEI10 foci per cell was 65.75 (n = 4, range 58–71), while the mean number of OsGEN1 foci per cell was 37.75 (n = 4, range 31–49). At these stages, only one to two foci colocalized, which may indicate that HEI10 and OsGEN1 are involved in different pathways during CO formation.

OsGEN1 Is Required for DSB Repair Postmeiosis

In Arabidopsis, AtMUS81 is the major resolvase involved in Class II CO formation. Mutation in *atMUS81* caused a 10% decrease in Class II CO formation but only a slight decrease in pollen viability and seed number per silique (Berchowitz et al., 2007; Higgins et al., 2008). Therefore, we suspected that the complete male sterility of *osgen1* is caused by developmental defects that occur in the microspore at later stages. To demonstrate this hypothesis, we observed the development of wild-type and *osgen1* microspores. Wild-type microspores undergo two rounds of mitosis after the meiotic cell division and produce a male gametophyte containing a vegetative nucleus and two generative nuclei to ensure double fertilization (Fig. 8, E–H). However, *osgen1* microspores arrest at mitosis I. In young *osgen1* microspores, chromosomes remain highly compacted (Fig. 8, I–L) and DNA fragmentation was observed (Fig. 8K). In plants, after meiosis the microspore undergoes two rounds of mitosis to generate the functional male gametophyte. In the microspore, nuclear DNA is replicated before two rounds of mitosis. In the eukaryotic mitotic cell cycle, DSBs are often generated during DNA replication, which leads to the stalling of replication forks (Burgoyne et al., 2007). Under these circumstances, DSBs are repaired by HR repair. These processes are carefully managed and cell division does not proceed until DSB repair is complete (O'Connell and Cimprich, 2005). The MRE11/Mre11 complex works together with exonucleases EXO1 and Dna2 to initiate DSB repair during replication fork stalling (D'Amours and Jackson, 2002; Symington, 2016). It has been reported that OsGEN1 also has 5′-flap endonuclease activity besides resolvase activity, implying that OsGEN1 may act in a similar way as FEN-1, which is involved in the removal of primer regions for Okazaki fragment maturation during lagging strand synthesis (Yang et al., 2012). To monitor OsGEN1

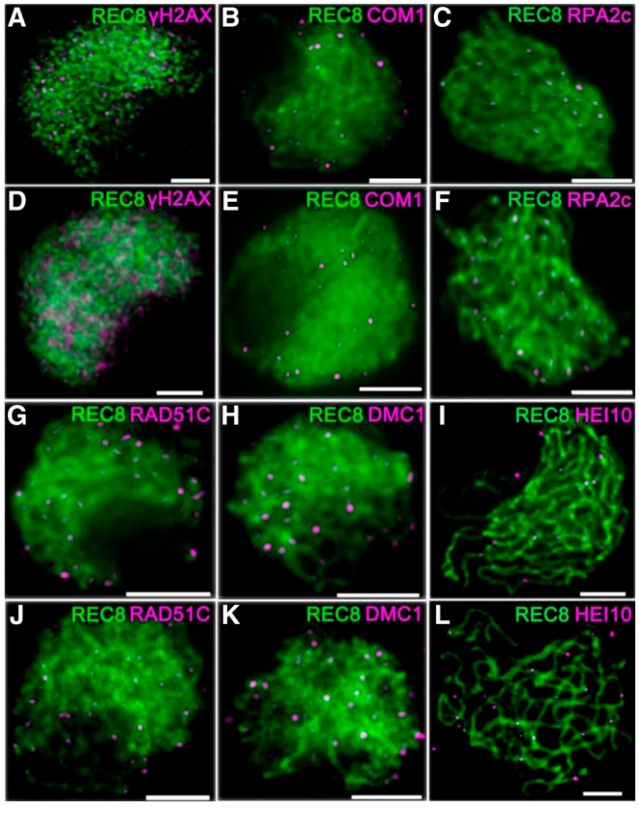

Figure 5. Dual immunolocalization of recombination proteins (magenta) and OsREC8 (green) in wild-type and *osgen1* PMCs. A to C and G to I, The wild type; D to F and J to L, *osgen1* mutant. A and D, Leptotene. B, E, G, H, J, and K, Zygotene. C, F, I, and L, Pachytene. Bar = 2 μm.

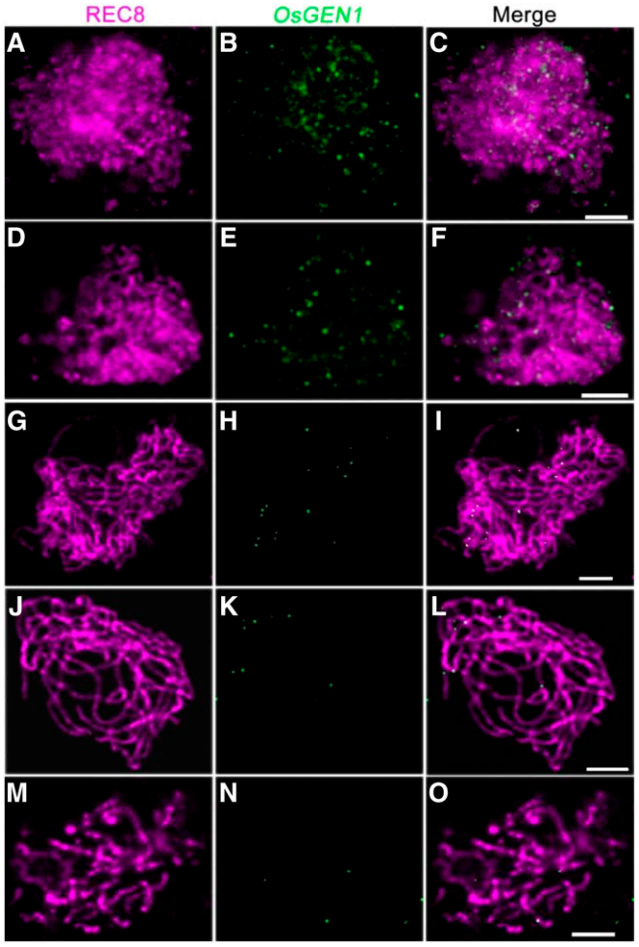

Figure 6. Dual immunolocalization of OsGEN1 (green) and OsREC8 (magenta) in wild-type PMCs. A to C, Leptotene; D to F, early zygotene; G to I, late zygotene; J to L, pachytene; M to O, diakinesis. Bar = 2 μm.

function after meiosis, we detected OsGEN1 localization and found that OsGEN1 relocalized on chromosomes after the microspore released from the tetrad. OsGEN1 foci reached a peak when the microspore nucleus became loose (Fig. 8B) and released from chromosomes when the nucleus becomes condensed again (Fig. 8, C and D), indicating that OsGEN1 has an important function in DNA replication before the two rounds of mitosis.

To reveal the effect of *osgen1* on DSB occurrence in *osgen1* and wild-type microspores, we used γH2AX as a marker. In the wild type, γH2AX foci were rarely found in microspore nuclei, while in the *osgen1* mutant γH2AX foci were up to 53.39 (n = 7, range 37–56; Fig. 9, A–F). In addition, a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay detected a strong programmed cell death signal in the nucleus of *osgen1* microspores (Fig. 9, G–L), suggesting that accumulation of DSBs results in cell death of the mutant microspore. Furthermore, disturbance of the cytoskeleton was found in *osgen1* microspores. Tubulin and actin did not form grid structures and only showed fuzzy signals, confirming cell death of *osgen1* microspores (Smertenko and Franklin-Tong, 2011; Supplemental Fig. S9). Taken together, these results suggest that OsGEN1 has an important role in DNA replication or DNA repair before two rounds of mitosis during early microspore development. The unrepaired DSBs induce cell death and abortion of the microspore.

OsSEND1 Is Not Essential for Rice Development

Two homologs of GEN1/Yen1 that are designated GEN1 and SEND1 exist across the plant kingdom (Olivier et al., 2016). It was reported that in Arabidopsis, AtSEND1 but not AtGEN1 acts as functional GEN1/Yen1 homolog. AtSEND1 plays important roles in repairing toxic replication intermediates and maintaining telomere homeostasis, but is not required for meiotic recombination (Olivier et al., 2016). *OsSEND1* is induced by UV and DNA damage reagents, and consistently OsSEND1 plays a role in DNA repair in somatic cells (Furukawa et al., 2003). We used qRT-PCR to investigate the spatio-temporal expression pattern of the *OsSEND1* and found that *OsSEND1* is expressed at a lower level than that of *OsGEN1* in the anther and in vegetative tissues (Supplemental Fig. S5). To understand the biological function of OsSEND1, we created

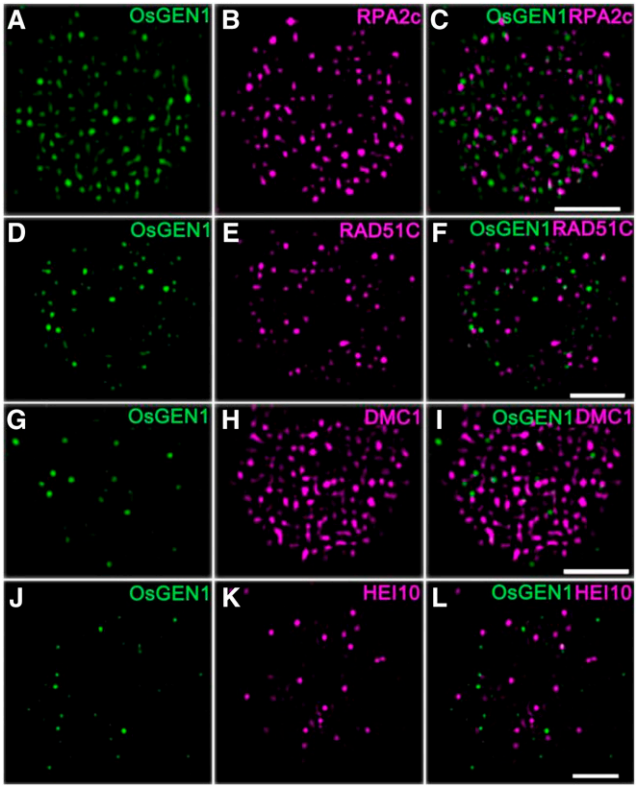

Figure 7. Dual immunolocalization of OsGEN1 (green) with recombination proteins (magenta). A to C, Early zygotene; D to L, zygotene. Bar = 2 μm.

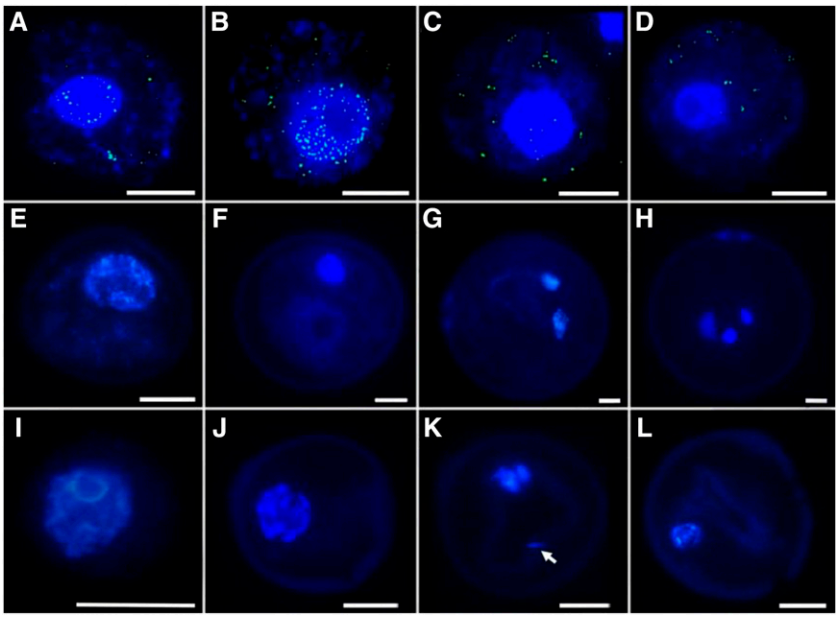

Figure 8. OsGEN1 is essential for microspore development. A to D, OsGEN1 localization on microspore chromosomes; OsGEN1 foci are shown in green. E to L, Microspore development in the wild type (E–H) and *osgen1* (I–L) after meiosis. The arrow in K shows DNA fragmentation. Bar = 2 μm.

two mutant alleles using the CRISPR/Cas9 system. Both alleles were created with a 1 bp insertion at 143 bp downstream of ATG (A in *ossend1-1*, T in *ossend1-2*), leading to a frame shift and premature translation termination. All of the homozygous *ossend1-1* and *ossend1-2* plants showed normal growth and fertility (Supplemental Fig. S10). We used DAPI staining to monitor *ossend1* meiotic progression. The mutants showed no obvious abnormality when compared with the wild type (Fig. 10, A–H) and the *ossend1 osgen1* double mutant is comparable to the *osgen1* single mutant (Fig. 10, I–P). A small number of DAPI bodies were found (6.25%, $n = 96$) at diakinesis, metaphase I (9.32%, $n = 236$), anaphase I (10.53%, $n = 114$), telophase I (6.41%, $n = 156$), prophase II (10.18%, $n = 226$), metaphase II (11.36%, $n = 88$), anaphase II (7.4%, $n = 54$), and tetrad (2.72%, $n = 147$). These results suggest that OsSEND1 is not essential for rice development and meiotic progression even though it shows a similar expression pattern with OsGEN1.

DISCUSSION

OsGEN1 Is Irreplaceable for Male Reproduction in Rice

The resolution of JMs such as HJs arising from the HR process is critical for the assurance of genome stability and proper chromosome segregation. Enzymes capable of resolving HJs have been isolated from a wide variety of eukaryotic organisms including yeasts, worms, flies, mammals, and plants. It has been shown that three structure-specific endonucleases or enzyme complexes, Mus81-Mms4/MUS81-EME1, Slx1-Slx4/SLX1-SLX4, and Yen1/GEN1, function as HJ resolvases in eukaryotes. Previous studies indicate that Mus81-Mms4/MUS81-EME1 play a leading role in HJ resolution in most eukaryotes. By contrast, Yen1 has been reported to serve as a backup pathway of Mus81-Mms4 in yeast to deal with the processing of residue JMs that escape the activity of Mus81-Mms4 (Blanco et al., 2010; Ho et al., 2010). While in animals, GEN1 homologs define a distinct recombination intermediate resolution pathway to remove detrimental JMs together with MUS81-EME1 and SLX1-SLX4 pathways. Different from other eukaryotes, most plants possess two Yen1/GEN1 homologs, named GEN1 and SEND1, but only one Yen1/GEN1 homolog exists in the genome of more ancient mosses, implying that a gene duplication occurred during the early development of plants after separation from mosses (Bauknecht and Kobbe, 2014; Supplemental Fig. S2C). A very recent report showed that in Arabidopsis, SEND1 but not GEN1 acts as the functional Yen1/GEN1 homolog and plays an important role in toxic replication intermediate repair and telomere homeostasis in the absence of MUS81 (Olivier et al., 2016). In our study, we demonstrated that OsGEN1 but not OsSEND1 has indispensable roles in resolving meiotic and DNA replication intermediates in rice male reproductive cells.

In Arabidopsis, the single and double mutants of *GEN1* and *SEND1* show no observable developmental defects nor enhanced sensitivity to DNA damaging reagents. The activity of SEND1 is only required in plants lacking MUS81, suggesting that it provides a backup resolution pathway. However, disruption of *GEN1* has no impact on the function of both *SEND1* and *MUS81*. On the contrary, the loss of function of *OsGEN1* in rice leads to complete male sterility. The abortion of mutant pollen appears to be caused by programmed cell death from the accumulation of deleterious recombination intermediates during DNA replication in young microspores. Although deficiency of OsGEN1 does not interfere with meiotic progression, a moderate decrease in CO frequency was observed in *osgen1*

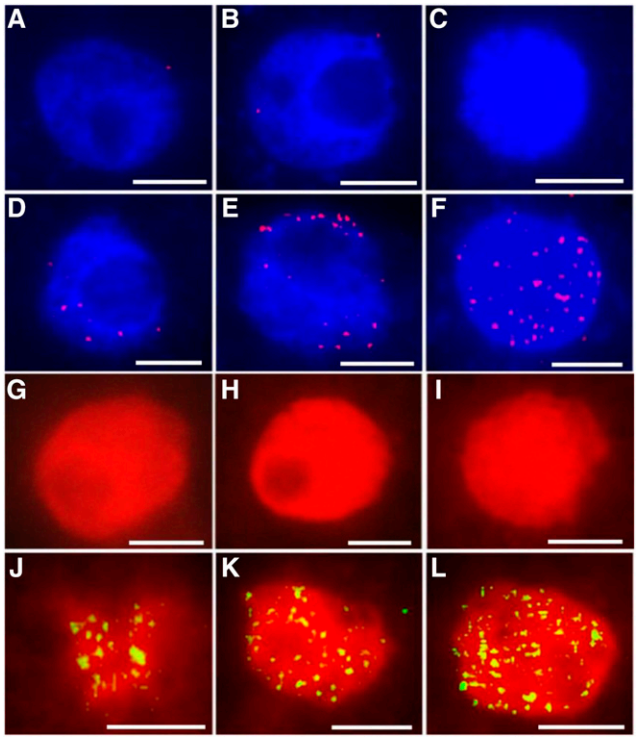

Figure 9. Accumulation of DNA damage causes cell death of *osgen1* PMCs. A to C and G to I, The wild type; D to F and J to L, *osgen1*. A to F, Distribution of γH2Ax foci; G to L, TUNEL assays in wild-type and *osgen1* microspores. Bar = 1 μm.

pollen mother cells, indicating that OsGEN1 is also involved in the meiotic JMs resolution. Furthermore, the *osgen1 ossend1* double mutant shows no difference from the *osgen1* single mutant. These results suggest that the two paralogs have acquired nonredundant biological functions after the gene duplication. Specifically, OsGEN1 has obtained essential roles in resolving recombinant intermediates in rice male germ cells. The biochemical activity and in vivo roles of OsSEND1 and the rice MUS81-MMS4 homologs remain to be determined. Previous studies showed that *OsSEND1* is preferentially expressed in meristematic tissues and induced by DNA damaging agents (Furukawa et al., 2003). *OsGEN1* is also highly expressed in vegetative tissues (Supplemental Fig. S5). Therefore, it could not be excluded that OsSEND1 and/or OsGEN1 have redundant functions with OsMUS81 in mitotic cells, especially under stress conditions.

Both OsGEN1 and OsSEND1 contain an XPG type amino-terminal domain, a central XPG nuclease domain, and an HhH DNA binding domain. The functional differences between OsGEN1 and OsSEND1 may be attributed to the highly polymorphic carboxyl-terminal region. Comparative analysis found that Arabidopsis SEND1 possesses a carboxyl-terminal chromodomain-like motif involved in DNA and histone recognition, which is not present either in GEN1 or in two rice paralogs. In our study, we found that a deletion of the carboxyl-terminal of OsGEN1 severely compromises the function of the protein. We used CRISPR technology to knock out *OsGEN1*. The CRISPR lines with truncated OsGEN1 lacking C terminus, HhH, and/or XPGI domains show identical phenotypes with *osgen1* mutant (Supplemental Fig. S11), which highlights the importance of the C terminus of OsGEN1 for its function. The activities of yeast Yen1 and human GEN1 are controlled by master cell cycle regulators and are regulated by phosphorylation or dephosphorylation. The phosphorylation sites reside in the C terminus of Yen1/GEN1 (Blanco et al., 2014; Chan and West, 2014; Eissler et al., 2014). We hypothesize that the function of OsGEN1 may be controlled by regulators through interaction with its C terminus.

OsGEN1 Is Required for CO Formation in Rice

During meiotic prophase I, crossover formation via homologous recombination is required to establish physical connections between chromosomes to ensure correct segregation. Meiotic crossovers are generated via two pathways (Higgins et al., 2004). In most eukaryotes, most COs are formed by the interference-sensitive pathway (Class I COs) in which one CO reduces the probability of a second CO nearby. The Class II COs are insensitive to interference and produce randomly distributed COs (Osman et al., 2011). In budding yeast, mammals, and Arabidopsis, Class I COs depend on the activity of ZMM proteins (Zip1-4, Mer3, Msh4, and Msh5; Börner et al., 2004; Bishop and Zickler, 2004;

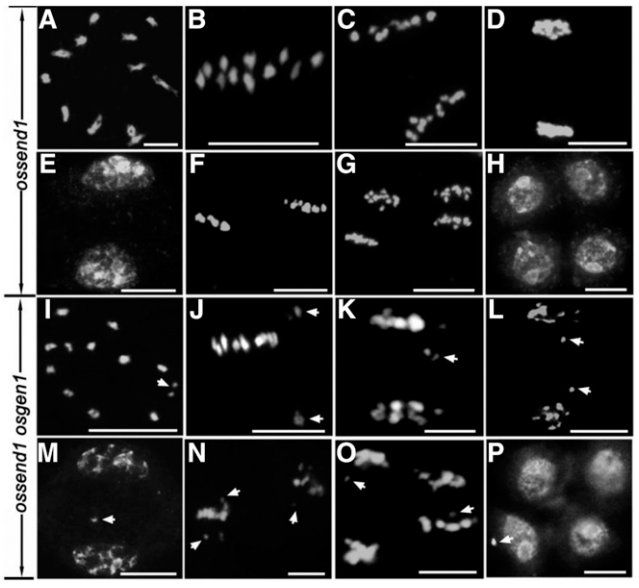

Figure 10. Meiosis in *ossend1* and *ossend1 osgen1* PMCs. A to H, *ossend1*; I to P, *ossend1 osgen1*; A and I, late diakinesis. B and J, Metaphase I; C and K, anaphase I; D and L, telophase I; E and M, prophase II; F and N, metaphase II; G and O, anaphase II; H and P, tetrad. Bar = 5 μm.

Lynn et al., 2007; Guiraldelli et al., 2013) and Class II COs depend on HJ resolvase Mus81-Mms4 (de los Santos et al., 2003; Osman et al., 2003; Hollingsworth and Brill, 2004; Higgins et al., 2008). SLX4 has also been reported to play an important role in Class II CO formation in mice (Holloway et al., 2011).

It has been shown that there are two kinds of COs in rice: one appears to be sensitive to interference, and the other one is not (Wang et al., 2009). Rice homologs of ZMM components, including MER3, ZIP4, HEI10, OsMSH4, and OsMSH5, were reported to have conserved functions in Class I CO formation (Wang et al., 2009, 2012, 2015; Shen et al., 2012; Luo et al., 2013; Zhang et al., 2014c). It remains unknown which resolvase is required for Class II CO formation in rice. In Arabidopsis, Class II pathway accounts for only a small fraction of CO formation (Higgins et al., 2004, 2008; Berchowitz et al., 2007). In rice, disruption of Class I CO components cause 60 to 90% reduction in the CO number, suggesting that the Class II pathway accounts for no more than 10% CO formation. In our study, we showed that depletion of OsGEN1 leads to an ~6% decrease in chiasmata compared to the wild type, which resembled the small fraction of CO decrease observed in Class II mutants in Arabidopsis (Higgins et al., 2008). Furthermore, distribution of remaining chiasmata was also affected by the OsGEN1 mutation. Besides interference, having at least one CO per homologous chromosome pairs is another basic rule of meiotic CO formation. The Class I CO pathway is essential for obligatory CO formation. Disruption of the Class I CO pathway leads to the loss of most obligatory COs and the production of a large number of univalents. In *hei10*, the proportion of chromosome pairs with at least one CO was substantially decreased (Fig. 3C). Previous studies and recent modeling analyses suggested that interference is an outcome of obligatory CO formation (Jones and Franklin, 2006; Zhang et al., 2014b). In *osgen1*, only a very small fraction of chromosome pairs (0.28%) lacked a crossover, suggesting that OsGEN1 has minor effects on obligatory CO formation and the interference pathway. On the contrary, OsGEN1 deficiency has a significant effect on non-obligatory CO formation. In the wild type, the proportions of chromosome pairs with two or three COs were 58.20% and 6.48% in the wild type (63 cells were counted), while in the *osgen1* mutant it decreased to 51.91% and 2.82%, respectively (59 cells were counted). In contrast, the proportion of chromosome pairs having one CO increased from 35.32% in the wild type to 44.99% in the *osgen1* mutant (Fig. 3C). On the other hand, chromosome pairing and synapsis are not affected by the dysfunction of OsGEN1 (Fig. 4). In addition, OsGEN1 proteins localize to meiotic chromosomes from leptotene to pachytene but do not colocalize with ZMM proteins (Figs. 6 and 7). Collectively, these results suggest that OsGEN1 is very likely responsible for the generation of Class II COs. OsGEN1 might be occasionally involved in resolving Class I double HJs and in its absence, could produce a low number of univalents.

In Arabidopsis, MUS81 has been shown to be the mediator of the interference-insensitive pathway. Mutation of MUS81 causes a reduction of ~10% of meiotic CO (Berchowitz et al., 2007; Higgins et al., 2008). However, the single mutants or the double mutant of *gen1* and *send1* do not show any meiotic defects. Although 10% of telophase I and anaphase II nuclei in *mus81 send1* meiocytes exhibit segregation defects, it is difficult to conclude that SEND1 is required for meiotic CO formation due to the severe mitotic defects that occurred at earlier stage (Olivier et al., 2016). But it could not be ruled out that SEND1 may also have a minor role in chiasmata formation in Arabidopsis.

Previous studies indicate that interference-sensitive COs arise from double HJs processed by ZMM complex (Börner et al., 2004; Lynn et al., 2007). The MSH4 and MSH5 heterodimers play an earlier role in stabilizing CO-specific recombination intermediates to ensure double HJs form and resolve (Shinohara et al., 2008). In contrast, interference-insensitive COs are generated by the action of MUS81/MMS4 on aberrant joint molecules, such as single or nicked HJs, that cannot be resolved by the ZMM proteins. Biochemical and genetic analysis in *S. cerevisiae* indicates that Mus81-Eme1 act late in meiotic recombination (Oh et al., 2008). In vitro, OsGEN1 has the ability to cleave ligated or nicked double HJs. However, which of these structures are targeted by OsGEN1 in vivo is still unclear, but the observation that synapsis is completed and the localization of ZMM proteins is not affected in *osgen1* (Figs. 4 and 5), suggests that OsGEN1 may act at a late stage to process JMs that are not resolved by the MSH4-MSH5 pathway. The exact substrates of OsGEN1 during meiosis and its interaction with other meiotic CO formation pathways need further investigation.

OsGEN1 Functions in DNA Repair in Germ Cells

In Arabidopsis, a 10% decrease in chiasmata in the *atmus81* mutant leads to only a small reduction in pollen viability and seed set (Berchowitz et al., 2007; Higgins et al., 2008), indicating that the presence of a small subset of unresolved recombination intermediates during meiosis in *osgen1* mutant is not enough to cause total male sterility. We found that after release from tetrad, OsGEN1 relocalized in microspore nuclei (Fig. 8, A–D). *osgen1* was completely sterile and the *OsGEN1*+/− pollen were all fertile, suggesting that OsGEN1 proteins were synthesized in the male meiocytes and allocated to each microspore in the tetrad. *osgen1* microspores do not undergo mitosis (Fig. 8, I–L) and accumulate DSBs that lead to programmed cell death and pollen abortion at a later stage (Fig. 9). This indicates that OsGEN1 also functions in pollen mitosis, which is a specific process in angiosperms where microspores freed from the tetrad undergo an asymmetric mitosis division to form bicellular pollen (Mascarenhas, 1989; McCormick, 1993).

Before mitosis, the microspore genome is replicated to ensure that the haploid state is maintained until mature pollen formation. During DNA replication,

HR-mediated repair is critical to remove DNA lesions that will lead to the failure of replication fork progression. It has been reported that Mus81-Mms4/MUS81-EME1, Yen1/GEN1, and Slx1-Slx4/SLX1-SLX4 can process failing RFs and/or the resolution of intermediates of HR-dependent RF recovery (Rass, 2013). The failure to repair damaged RFs may cause DSB accumulation in microspores. It has been reported that DSBs are extremely hazardous lesions and that cell division does not occur when DSBs are present (Burgoyne et al., 2007). Evidence suggests that the mitotic cell cycle is managed by a checkpoint mechanism that acts to delay cell division when DSBs are present at the end of G2. Previous studies indicate that GEN-1 has dual roles in both HR-mediated DNA repair and DNA damage signaling in *C. elegans* (Saito et al., 2012, 2013). OsGEN1 may not only participate in DNA repair, but also act as a cell-cycle controlling signal during the meiosis to mitosis cell-cycle transition.

OsGEN1 mutation may also result in unrepaired recombination intermediates during meiosis and lead to accumulation of DNA damage. However, compared with mutants of genes involved in DSB repair, such as *oscom1*, *osrad51c*, and *osmre11* (Ji et al., 2012, 2013; Tang et al., 2014), only a low number of abnormal DAPI bodies appears in 8% meiocytes. In addition, we did not detect obvious DNA damage by TUNEL assay at tetrad stage. Therefore, we propose that there is no or only very limited effect of *OsGEN1* single mutation on meiotic DSB repair, which is unlikely to be causing strong DNA damage at later stage. The low number of abnormal DAPI bodies in the male meiocytes could suggest that there is a redundant pathway that can repair most of the recombination intermediates in the absence of OsGEN1, but not all. This redundant pathway would therefore not be present post-male meiosis.

CONCLUSION

In summary, we have determined that *OsGEN1* has essential roles in processing recombination intermediates during male reproduction in rice. This is distinct from *AtSEND1* and *AtGEN1* that have essential overlapping roles with AtMUS81 in JM resolution in Arabidopsis, suggesting functional variations and divergence of the two Yen1/GEN1 paralogs in plant evolution. Further studies of mutants lacking a combination of osmus81/osgen1/ossend1 should provide additional insights into the mechanisms of recombination intermediate resolution during meiosis and mitosis in rice.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Molecular Cloning of *OsGEN1*

Rice (*Oryza sativa*) plants from the 9522 background (*O. sativa* ssp. *japonica*) were grown in the paddy field of Shanghai Jiao Tong University. F2 progenies for mapping were generated from a cross between cultivar 9311 (*O. sativa* ssp. *indica*) and the *osgen1* mutant. One-hundred-forty-six male-sterile plants in the F2 population were selected for mapping, and bulked segregated analysis (Liu et al., 2005) was used to isolate *OsGEN1*.

Complementation of *osgen1*

For functional complementation, a 5,599-bp genomic sequence of *OsGEN1* including the entire *OsGEN1* coding region (3,623 bp) and 1,976-bp upstream sequence was amplified from wild-type rice genomic DNA and cloned into the binary vector pCAMBIA1301. We also exchanged the pCAMBIA1301 GUS reporter gene with eGFP, thus generating two marker constructs. Calli induced from young panicles of the homozygous *osgen1* plants were used for transformation with *Agrobacterium tumefaciens* (EHA105), which carries the pCAMBIA1301-OsGEN1-GUS, pCAMBIA1301-OsGEN1-eGFP plasmid, or the control plasmid pCAMBIA1301. For transgenic plants, at least 10 independent lines were obtained from each construct and identified by PCR using the primers listed in Supplemental Table S1.

Mutant Phenotypic Analyses

Plant materials were photographed with a digital camera (Nikon; catalog no. E995) and a dissecting microscope (Motic; catalog no. K400). Transverse section observation was performed as described by Li et al. (2006). DAPI staining of microspores was performed as reported in Cheng (2013). Chromosomes were stained with DAPI (Vector Laboratories) and photographed using the Eclipse Ni-E microscope (Nikon).

Antibody Production

For preparation of OsGEN1 polyclonal antibody, a 627-bp DNA fragment encoding a 209-amino acid peptide of OsGEN1 (residues 284–492) was amplified from rice anther cDNA and cloned into pGEX-4T-1 (GE) with *Bam*HI-*Xho*I. The recombinant protein was expressed in *Escherichia coli* DE3 (BL21; Novagen) and purified to produce mouse polyclonal antibodies (prepared by Abclonal) specific to OsGEN1 (Supplemental Fig. S12). A rabbit DMC1 polyclonal antibody was prepared using the peptide MAPSKQYDEGGQLQLMDA (prepared by Abclonal). The polyclonal antibodies OsREC8, PAIR2, PAIR3, ZEP1, γH2AX, OsCOM1, RAD51C, and HEI10 used in this study were prepared according to Wang et al. (2009, 2010, 2011, 2012), Ji et al. (2012), Miao et al. (2013), and Tang et al. (2014). The antibody against RPA2c was described in Li et al. (2013). Anti-β-tubulin antibody was obtained from Sigma-Aldrich (catalog no. T4026).

Immunolocalization Assays

Immunolocalization assays were performed according to Cheng (2013). Fresh young panicles at suitable phase were fixed in 4% (w/v) paraformaldehyde for 20 min at room temperature and wished thrice with PBS. Anthers in the proper stages were squashed on a slide with PBS solution and soaked in liquid N and removed from the coverslip quickly with a blade. The slides were dehydrated through an ethanol series (70, 90, and 100%), then incubated in a humid chamber at 37°C for 4 h with different antibody combinations diluted 1:500 in TNB buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, and 0.5% blocking reagent). After three rounds of washing in PBS, Alex 555-conjugated goat anti-rabbit antibody (Life Technologies) and DyLight 488-conjugated goat anti-mouse antibody (Abbkine, 1:1,000; Abcam) were added to the slides. Finally, after incubation in the humidity chamber at 37°C for 1 h, the slides were counterstained with DAPI. The slides were photographed using the Eclipse Ni-E microscope (Nikon), and analysis was done using NIS-Elements Advanced Research software.

TUNEL Assay

Microsporocytes at appropriate stages were collected and added onto a slide. Anthers were dissected from the spikelet and broken using forceps that released the microsporocytes into 10 mL of enzyme digestion mixture (includes 0.1 g cytohelicase, 0.0375 g Suc, and 0.25 g polyvinyl pyrrolidone, MW 40,000; all Sigma-Aldrich) to hydrolyze 20 min at room temperature. Five microliters 0.1% Triton X-100 was added to slides with 10 μL 4% (w/v) paraformaldehyde and allowed to air dry. Slides were washed with 1× PBS for 5 min followed by use of the TUNEL detection kit (Dead End Fluorometric TUNEL system; Promega).

Fluorescence Microscopy Visualization of Actin Filaments

According to Zhang et al. (2011c), fresh free microspores were incubated in PEM buffer (100 mM PIPES, 10 mM EGTA, 5 mM $MgSO_4$, and 0.3 M mannitol, pH 6.9) that contains 2% (w/v) glycerol (Sigma-Aldrich) and 6.6 mM Alexa Fluor 488-phalloidin staining (Invitrogen). After 35 min incubation, pollen was observed using a TCS SP5 confocal laser scanning microscope (Leica).

CRISPR Knockout OsSEND1, HEI10, and OsGEN1

The sgRNA-Cas9 plant expression vectors were supplied by Professor Jiankang Zhu and constructed as previously described in Feng et al. (2013), Mao et al. (2013), and Zhang et al. (2014a). The primers for constructing the sgRNA vectors for *OsSEND1* and HEI10 are listed in Supplemental Experimental Procedures. The constructed *OsSEND1* sgRNA-Cas9 and *HEI10* sgRNA-Cas9 plasmids were separately transformed into *A. tumefaciens* (EHA105) and then used to infect wild-type rice calli separately. The *OsGEN1* CRISPR transgenic plants were produced by an outside company (Biogle). The transgenic plants identified by PCR using the primers for amplifying targeted regions of *OsSEND1*, *HEI10*, and *OsGEN1* are listed in Supplemental Experimental Procedures and were sequenced directly. The third generation of the homozygous lines showing the same genotyping was used to observe the phenotype.

qRT-PCR Assay

Total RNA from the wild type was isolated using TRIZOL reagent (Sigma-Aldrich) from rice root, shoot, leaf, lemma/palea, and anthers. Rice anthers from different developmental stages as defined by Zhang et al. (2011a). Roots, shoots, and leaves were collected from 20-d-old seedlings. An amount of 0.2 mg of RNA per sample was reverse transcribed to synthesize cDNA using Primescript RT reagent kit with genomic DNA eraser (Takara). qRT-PCR was performed as described by Fu et al. (2014).

Phylogenetic and Structure Analysis

The protein sequences were aligned using ClustalX (version 2.0; Larkin et al., 2007), and then adjusted manually using GeneDoc software (version 2.6.002; http://www.psc.edu/biomed/genedoc/). Neighbor-joining trees were constructed using the MEGA (version 4.0) software (Tamura et al., 2007) with the following parameters: Poisson correction, pairwise deletion, and bootstrap (1,000 replicates; random seed). The protein domain predictions were from InterProScan (http://www.ebi.ac.uk/interpro/search/sequence-search).

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: *OsGEN1* (Os09g0521900), *OsSEND1* (Os08g0101600), *OsSDS* (Os03g0225200), *OsREC8* (Os05g0580500), *PAIR2* (Os09g0506800), *PAIR3* (Os10g0405500), *ZEP1* (Os04g0452500), *OsCOM1* (Os06g0613400), *RPA2c* (Os06g0693300), *DMC1* (Os11g0146800, Os12g0143800), *RAD51C* (Os01g0578000), *HEI10* (Os02g0232100), and *H2AX* (Os03g0721900).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Transverse section observation of the anther development in the wild type and *osgen1*.

Supplemental Figure S2. Map-based cloning of *OsGEN1* and phylogenetic analysis of RAD2/XPG family members.

Supplemental Figure S3. Structural similarity and biochemical properties of Yen1/GEN1 homologs.

Supplemental Figure S4. Complementation of *osgen1*.

Supplemental Figure S5. Spatio-temporal analysis of *OsGEN1* and *OsSEND1* transcription by qRT-PCR.

Supplemental Figure S6. Phenotypes of *HEI10* CRISPR lines.

Supplemental Figure S7. Dual immunolocalization of SC proteins OsZEP1 (magenta) and OsREC8 (green) in wild-type and *osgen1* PMCs pachytene.

Supplemental Figure S8. Dual immunolocalization of recombination proteins (magenta) and OsREC8 (green) in wild-type and *osgen1* PMCs.

Supplemental Figure S9. Tubulin arrays (red) and actin filaments (green) in wild-type and *osgen1* PMCs.

Supplemental Figure S10. Phenotypes of *OsSEND1* CRISPR lines.

Supplemental Figure S11. Phenotypes of *OsGEN1* CRISPR mutation lines.

Supplemental Figure S12. Dual immunolocalization of OsREC8 (magenta) and OsGEN1 (green) in wild-type and *osgen1* PMCs at zygotene.

Supplemental Table S1. Primers used in this study.

ACKNOWLEDGMENTS

We thank Mingjiao Chen and Zhijing Luo for mutant screening, generation of F2 population for mapping, and rice crosses; Zhenzhen Fu who prepared the OsREC8 antibody; Changyin Wu for providing the RPA2c antibody; RGRC for providing the cDNA clone; and Jiankang Zhu for providing the sgRNA-Cas9 vector.

Received November 30, 2016; accepted December 29, 2016; published January 3, 2017.

LITERATURE CITED

Andersen SL, Kuo HK, Savukoski D, Brodsky MH, Sekelsky J (2011) Three structure-selective endonucleases are essential in the absence of BLM helicase in *Drosophila*. PLoS Genet **7:** e1002315

Andersen SL, Sekelsky J (2010) Meiotic versus mitotic recombination: two different routes for double-strand break repair: the different functions of meiotic versus mitotic DSB repair are reflected in different pathway usage and different outcomes. BioEssays **32:** 1058–1066

Bailly AP, Freeman A, Hall J, Déclais AC, Alpi A, Lilley DM, Ahmed S, Gartner A (2010) The *Caenorhabditis elegans* homolog of Gen1/Yen1 resolvases links DNA damage signaling to DNA double-strand break repair. PLoS Genet **6:** e1001025

Bastin-Shanower SA, Fricke WM, Mullen JR, Brill SJ (2003) The mechanism of Mus81-Mms4 cleavage site selection distinguishes it from the homologous endonuclease Rad1-Rad10. Mol Cell Biol **23:** 3487–3496

Bauknecht M, Kobbe D (2014) AtGEN1 and AtSEND1, two paralogs in Arabidopsis, possess Holliday junction resolvase activity. Plant Physiol **166:** 202–216

Berchowitz LE, Francis KE, Bey AL, Copenhaver GP (2007) The role of AtMUS81 in interference-insensitive crossovers in *A. thaliana*. PLoS Genet **3:** e132

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

Blanco MG, Matos J, Rass U, Ip SC, West SC (2010) Functional overlap between the structure-specific nucleases Yen1 and Mus81-Mms4 for DNA-damage repair in *S. cerevisiae*. DNA Repair (Amst) **9:** 394–402

Blanco MG, Matos J, West SC (2014) Dual control of Yen1 nuclease activity and cellular localization by Cdk and Cdc14 prevents genome instability. Mol Cell **54:** 94–106

Boddy MN, Gaillard PH, McDonald WH, Shanahan P, Yates III JR, Russell P (2001) Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell **107:** 537–548

Börner 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

Burgoyne PS, Mahadevaiah SK, Turner JM (2007) The management of DNA double-strand breaks in mitotic G2, and in mammalian meiosis viewed from a mitotic G2 perspective. BioEssays **29:** 974–986

Bzymek M, Thayer NH, Oh SD, Kleckner N, Hunter N (2010) Double Holliday junctions are intermediates of DNA break repair. Nature **464:** 937–941

Castor D, Nair N, Déclais AC, Lachaud C, Toth R, Macartney TJ, Lilley DM, Arthur JS, Rouse J (2013) Cooperative control of Holliday junction resolution and DNA repair by the SLX1 and MUS81-EME1 nucleases. Mol Cell **52:** 221–233

Chan YW, West SC (2014) Spatial control of the GEN1 Holliday junction resolvase ensures genome stability. Nat Commun **5:** 4844

Chen J, Aström SU (2012) A catalytic and non-catalytic role for the Yen1 nuclease in maintaining genome integrity in *Kluyveromyces lactis*. DNA Repair (Amst) **11:** 833–843

Chen L, Chu HW, Yuan Z, Pan AH, Liang WQ, Huang H, Shen MS, Zhang DB (2006) Isolation and genetic analysis for rice mutants treated with ^{60}Co γ-ray. J. Xiamen Univ. Nat. Sci. **45:** 81–85

Che L, Tang D, Wang K, Wang M, Zhu K, Yu H, Gu M, Cheng Z (2011) OsAM1 is required for leptotene-zygotene transition in rice. Cell Res **21:** 654–665

Chen XB, Melchionna R, Denis CM, Gaillard PH, Blasina A, Van de Weyer I, Boddy MN, Russell P, Vialard J, McGowan CH (2001) Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol Cell **8:** 1117–1127

Cheng Z (2013) Analyzing meiotic chromosomes in rice. Methods Mol Biol **990:** 125–134

Chu H-W, Liu H-S, Li H, Wang H-M, Wei J-L, Li N, Ding S-Y, Huang H, Ma H, Huang C-F (2005) Genetic analysis and mapping of the rice leafy-hull mutant Oslh. J Plant Phys Mol Biol **31:** 594–598

Ciccia A, Constantinou A, West SC (2003) Identification and characterization of the human mus81-eme1 endonuclease. J Biol Chem **278:** 25172–25178

D'Amours D, Jackson SP (2002) The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling. Nat Rev Mol Cell Biol **3:** 317–327

de los Santos T, Hunter N, Lee C, Larkin B, Loidl J, Hollingsworth NM (2003) The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics **164:** 81–94

Dunin-Horkawicz S, Feder M, Bujnicki JM (2006) Phylogenomic analysis of the GIY-YIG nuclease superfamily. BMC Genomics **7:** 98

Eissler CL, Mazón G, Powers BL, Savinov SN, Symington LS, Hall MC (2014) The Cdk/cDc14 module controls activation of the Yen1 Holliday junction resolvase to promote genome stability. Mol Cell **54:** 80–93

Fekairi S, Scaglione S, Chahwan C, Taylor ER, Tissier A, Coulon S, Dong MQ, Ruse C, Yates III JR, Russell P, et al (2009) Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. Cell **138:** 78–89

Feng Z, Zhang B, Ding W, Liu X, Yang DL, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK (2013) Efficient genome editing in plants using a CRISPR/Cas system. Cell Res **23:** 1229–1232

Fricke WM, Brill SJ (2003) Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes Dev **17:** 1768–1778

Fu Z, Yu J, Cheng X, Zong X, Xu J, Chen M, Li Z, Zhang D, Liang W (2014) The rice basic helix-loop-helix transcription factor TDR INTERACTING PROTEIN2 is a central switch in early anther development. Plant Cell **26:** 1512–1524

Furukawa T, Kimura S, Ishibashi T, Mori Y, Hashimoto J, Sakaguchi K (2003) OsSEND-1: a new RAD2 nuclease family member in higher plants. Plant Mol Biol **51:** 59–70

Gao M, Rendtlew Danielsen J, Wei LZ, Zhou DP, Xu Q, Li MM, Wang ZQ, Tong WM, Yang YG (2012) A novel role of human Holliday junction resolvase GEN1 in the maintenance of centrosome integrity. PLoS One **7:** e49687

Garner E, Kim Y, Lach FP, Kottemann MC, Smogorzewska A (2013) Human GEN1 and the SLX4-associated nucleases MUS81 and SLX1 are essential for the resolution of replication-induced Holliday junctions. Cell Reports **5:** 207–215

Guiraldelli MF, Eyster C, Wilkerson JL, Dresser ME, Pezza RJ (2013) Mouse HFM1/Mer3 is required for crossover formation and complete synapsis of homologous chromosomes during meiosis. PLoS Genet **9:** e1003383

Higgins JD, Armstrong SJ, Franklin FC, Jones GH (2004) The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in Arabidopsis. Genes Dev **18:** 2557–2570

Higgins, JD, Buckling, EF, Franklin, FC Jones, GH (2008) Expression and functional analysis of AtMUS81 in *Arabidopsis* meiosis reveals a role in the second pathway of crossing-over. J Plant Phys Mol Biol **54:** 152–162

Ho CK, Mazón G, Lam AF, Symington LS (2010) Mus81 and Yen1 promote reciprocal exchange during mitotic recombination to maintain genome integrity in budding yeast. Mol Cell **40:** 988–1000

Hollingsworth NM, Brill SJ (2004) The Mus81 solution to resolution: generating meiotic crossovers without Holliday junctions. Genes Dev **18:** 117–125

Holloway JK, Mohan S, Balmus G, Sun X, Modzelewski A, Borst PL, Freire R, Weiss RS, Cohen PE (2011) Mammalian BTBD12 (SLX4) protects against genomic instability during mammalian spermatogenesis. PLoS Genet **7:** e1002094

Ip SC, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC (2008) Identification of Holliday junction resolvases from humans and yeast. Nature **456:** 357–361

Ji J, Tang D, Wang K, Wang M, Che L, Li M Cheng Z (2012) The role of OsCOM1 in homologous chromosome synapsis and recombination in rice meiosis. J Plant Phys Mol Biol **72:** 18–30

Ji J, Tang D, Wang M, Li Y, Zhang L, Wang K, Li M, Cheng Z (2013) MRE11 is required for homologous synapsis and DSB processing in rice meiosis. Chromosoma **122:** 363–376

Jones GH, Franklin FC (2006) Meiotic crossing-over: obligation and interference. Cell **126:** 246–248

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics **23:** 2947–2948

Lee SH, Princz LN, Klügel MF, Habermann B, Pfander B, Biertümpfel C (2015) Human Holliday junction resolvase GEN1 uses a chromodomain for efficient DNA recognition and cleavage. eLife **4:** 4

Li N, Zhang DS, Liu HS, Yin CS, Li XX, Liang WQ, Yuan Z, Xu B, Chu HW, Wang J, et al (2006) The rice tapetum degeneration retardation gene is required for tapetum degradation and anther development. Plant Cell **18:** 2999–3014

Li X, Chang Y, Xin X, Zhu C, Li X, Higgins JD, Wu C (2013) Replication protein A2c coupled with replication protein A1c regulates crossover formation during meiosis in rice. Plant Cell **25:** 3885–3899

Liu H, Chu H, Li H, Wang H, Wei J, Li N, Ding S, Huang H, Ma H, Huang C (2005) Genetic analysis and mapping of rice (*Oryza sativa* L.) male-sterile (OsMS-L) mutant. Chin Sci Bull **50:** 122–125

Luo Q, Tang D, Wang M, Luo W, Zhang L, Qin B, Shen Y, Wang K, Li Y, Cheng Z (2013) The role of OsMSH5 in crossover formation during rice meiosis. Mol Plant **6:** 729–742

Lynn, A, Soucek, R Borner, GV (2007) ZMM proteins during meiosis: crossover artists at work. Chromosome Res **15:** 591–605

Mahadevaiah SK, Turner JM, Baudat F, Rogakou EP, de Boer P, Blanco-Rodríguez J, Jasin M, Keeney S, Bonner WM, Burgoyne PS (2001) Recombinational DNA double-strand breaks in mice precede synapsis. Nat Genet **27:** 271–276

Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu JK (2013) Application of the CRISPR-Cas system for efficient genome engineering in plants. Mol Plant **6:** 2008–2011

Mascarenhas JP (1989) The male gametophyte of flowering plants. Plant Cell **1:** 657–664

McCormick S (1993) Male gametophyte development. Plant Cell **5:** 1265–1275

Miao C, Tang D, Zhang H, Wang M, Li Y, Tang S, Yu H, Gu M, Cheng Z (2013) Central region component1, a novel synaptonemal complex component, is essential for meiotic recombination initiation in rice. Plant Cell **25:** 2998–3009

Mimitou EP, Symington LS (2009) Nucleases and helicases take center stage in homologous recombination. Trends Biochem Sci **34:** 264–272

Moritoh S, Miki D, Akiyama M, Kawahara M, Izawa T, Maki H, Shimamoto K (2005) RNAi-mediated silencing of OsGEN-L (OsGEN-like), a new member of the RAD2/XPG nuclease family, causes male sterility by defect of microspore development in rice. Plant Cell Physiol **46:** 699–715

Mullen JR, Kaliraman V, Ibrahim SS, Brill SJ (2001) Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. Genetics **157:** 103–118

Neale MJ, Keeney S (2006) Clarifying the mechanics of DNA strand exchange in meiotic recombination. Nature **442:** 153–158

Nonomura K, Nakano M, Eiguchi M, Suzuki T, Kurata N (2006) PAIR2 is essential for homologous chromosome synapsis in rice meiosis I. J Cell Sci **119:** 217–225

O'Connell MJ, Cimprich KA (2005) G2 damage checkpoints: what is the turn-on? J Cell Sci **118:** 1–6

Oğrünç M, Sancar A (2003) Identification and characterization of human MUS81-MMS4 structure-specific endonuclease. J Biol Chem **278:** 21715–21720

Oh SD, Lao JP, Taylor AF, Smith GR, Hunter N (2008) RecQ helicase, Sgs1, and XPF family endonuclease, Mus81-Mms4, resolve aberrant joint molecules during meiotic recombination. Mol Cell **31:** 324–336

Olivier M, Da Ines O, Amiard S, Serra H, Goubely C, White CI, Gallego ME (2016) The structure-specific endonucleases MUS81 and SEND1 are essential for telomere stability in Arabidopsis. Plant Cell **28:** 74–86

Osman F, Dixon J, Doe CL, Whitby MC (2003) Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol Cell **12:** 761–774

Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FC (2011) Pathways to meiotic recombination in *Arabidopsis thaliana*. New Phytol **190:** 523–544

Rass U (2013) Resolving branched DNA intermediates with structure-specific nucleases during replication in eukaryotes. Chromosoma **122:** 499–515

Rass U, Compton SA, Matos J, Singleton MR, Ip SC, Blanco MG, Griffith JD, West SC (2010) Mechanism of Holliday junction resolution by the human GEN1 protein. Genes Dev **24:** 1559–1569

Rodrigue A, Coulombe Y, Jacquet K, Gagné JP, Roques C, Gobeil S, Poirier G, Masson JY (2013) The RAD51 paralogs ensure cellular protection against mitotic defects and aneuploidy. J Cell Sci **126:** 348–359

Saito TT, Lui DY, Kim HM, Meyer K, Colaiácovo MP (2013) Interplay between structure-specific endonucleases for crossover control during *Caenorhabditis elegans* meiosis. PLoS Genet **9:** e1003586

Saito TT, Mohideen F, Meyer K, Harper JW, Colaiácovo MP (2012) SLX-1 is required for maintaining genomic integrity and promoting meiotic noncrossovers in the *Caenorhabditis elegans* germline. PLoS Genet **8:** e1002888

Sanchez Moran, E, Armstrong, SJ, Santos, JL, Franklin, FC Jones, GH (2001) Chiasma formation in *Arabidopsis thaliana* accession Wassileskija and in two meiotic mutants. Chromosome Res **9:** 121–128

Sarbajna S, Davies D, West SC (2014) Roles of SLX1-SLX4, MUS81-EME1, and GEN1 in avoiding genome instability and mitotic catastrophe. Genes Dev **28:** 1124–1136

Schwartz EK, Heyer WD (2011) Processing of joint molecule intermediates by structure-selective endonucleases during homologous recombination in eukaryotes. Chromosoma **120:** 109–127

Shao T, Tang D, Wang K, Wang M, Che L, Qin B, Yu H, Li M, Gu M, Cheng Z (2011) OsREC8 is essential for chromatid cohesion and metaphase I monopolar orientation in rice meiosis. Plant Physiol **156:** 1386–1396

Shen Y, Tang D, Wang K, Wang M, Huang J, Luo W, Luo Q, Hong L, Li M, Cheng Z (2012) ZIP4 in homologous chromosome synapsis and crossover formation in rice meiosis. J Cell Sci **125:** 2581–2591

Shinohara M, Oh SD, Hunter N, Shinohara A (2008) Crossover assurance and crossover interference are distinctly regulated by the ZMM proteins during yeast meiosis. Nat Genet **40:** 299–309

Smertenko A, Franklin-Tong VE (2011) Organisation and regulation of the cytoskeleton in plant programmed cell death. Cell Death Differ **18:** 1263–1270

Svendsen JM, Smogorzewska A, Sowa ME, O'Connell BC, Gygi SP, Elledge SJ, Harper JW (2009) Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. Cell **138:** 63–77

Symington LS (2016) Mechanism and regulation of DNA end resection in eukaryotes. Crit Rev Biochem Mol Biol **51:** 195–212

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol **24:** 1596–1599

Tang D, Miao C, Li Y, Wang H, Liu X, Yu H, Cheng Z (2014) OsRAD51C is essential for double-strand break repair in rice meiosis. Front Plant Sci **5:** 167

Taylor ER, McGowan CH (2008) Cleavage mechanism of human Mus81-Eme1 acting on Holliday-junction structures. Proc Natl Acad Sci USA **105:** 3757–3762

Wang C, Wang Y, Cheng Z, Zhao Z, Chen J, Sheng P, Yu Y, Ma W, Duan E, Wu F, et al (2015) The role of OsMSH4 in male and female gamete development in rice meiosis. J Exp Bot **67:** 1447–1459

Wang K, Tang D, Wang M, Lu J, Yu H, Liu J, Qian B, Gong Z, Wang X, Chen J, Gu M, Cheng Z (2009) MER3 is required for normal meiotic crossover formation, but not for presynaptic alignment in rice. J Cell Sci **122:** 2055–2063

Wang K, Wang M, Tang D, Shen Y, Miao C, Hu Q, Lu T, Cheng Z (2012) The role of rice HEI10 in the formation of meiotic crossovers. PLoS Genet **8:** e1002809

Wang K, Wang M, Tang D, Shen Y, Qin B, Li M, Cheng Z (2011) PAIR3, an axis-associated protein, is essential for the recruitment of recombination elements onto meiotic chromosomes in rice. Mol Biol Cell **22:** 12–19

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

Wechsler T, Newman S, West SC (2011) Aberrant chromosome morphology in human cells defective for Holliday junction resolution. Nature **471:** 642–646

Whitby MC, Osman F, Dixon J (2003) Cleavage of model replication forks by fission yeast Mus81-Eme1 and budding yeast Mus81-Mms4. J Biol Chem **278:** 6928–6935

Wu L, Hickson ID (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature **426:** 870–874

Wyatt HD, Sarbajna S, Matos J, West SC (2013) Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. Mol Cell **52:** 234–247

Yang Y, Ishino S, Yamagami T, Kumamaru T, Satoh H, Ishino Y (2012) The OsGEN-L protein from *Oryza sativa* possesses Holliday junction resolvase activity as well as 5′-flap endonuclease activity. J Biochem **151:** 317–327

Zhang, D, Luo, X Zhu, L (2011a) Cytological analysis and genetic control of rice anther development. J Gen Genom **38:** 379–390

Zhang H, Zhang J, Wei P, Zhang B, Gou F, Feng Z, Mao Y, Yang L, Zhang H, Xu N, Zhu JK (2014a) The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. Plant Biotechnol J **12:** 797–807

Zhang L, Liang Z, Hutchinson J, Kleckner N (2014b) Crossover patterning by the beam-film model: analysis and implications. PLoS Genet **10:** e1004042

Zhang L, Tang D, Luo Q, Chen X, Wang H, Li Y, Cheng Z (2014c) Crossover formation during rice meiosis relies on interaction of OsMSH4 and OsMSH5. Genetics **198:** 1447–1456

Zhang Z, Zhang Y, Tan H, Wang Y, Li G, Liang W, Yuan Z, Hu J, Ren H, Zhang D (2011b) RICE MORPHOLOGY DETERMINANT encodes the type II formin FH5 and regulates rice morphogenesis. Plant Cell **23:** 681–700