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REVIEW

# Double-stranded DNA breaks and gene functions in recombination and meiosis

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Meiotic prophase I is a long and complex phase. Homologous recombination is an important process that occurs between homologous chromosomes during meiotic prophase I. Formation of chiasmata, which hold homologous chromosomes together until the metaphase I to anaphase I transition, is critical for proper chromosome segregation. Recent studies have suggested that the SPO11 proteins have conserved functions in a number of organisms in generating sites of double-stranded DNA breaks (DSBs) that are thought to be the starting points of homologous recombination. Processing of these sites of DSBs requires the function of RecA homologs, such as RAD51, DMC1, and others, as suggested by mutant studies; thus the failure to repair these meiotic DSBs results in abnormal chromosomal alternations, leading to disrupted meiosis. Recent discoveries on the functions of these RecA homologs have improved the understanding of the mechanisms underlying meiotic homologous recombination.

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Meiosis is a highly coordinated cell division, which generates four haploid reproductive cells required for sexual reproduction. Meiosis involves one round of DNA replication and two nuclear divisions, meiosis I and meiosis II. The first nuclear division leads to the segregation of homologous chromosomes (homologs), and is unique to meiosis. During the second division, sister chromatids are separated, resulting in the formation of four haploid nuclei and followed by the meiotic cytokinesis that forms four haploid cells. Similar to mitosis, both meiosis I and meiosis II can be divided into four stages: prophase, metaphase, anaphase, and telophase.

However, meiosis I differs from meiosis II as meiosis I involves homology search, pairing, interhomolog recombination, and synapsis of homologs, processes that are required for correct association and segregation of homologs. The prophase stage of meiosis I, or prophase I, has been the target of interest because of the occurrence of these major processes of chromosome interactions. During early prophase I, arms of sister chromosomes are closely associated by protein complexes called cohesins, which are removed during the metaphase I/anaphase I transition. However, the centromere regions remained associated until the segregation of sister chromatids during meiosis II. Homologous pairing is the result of interaction between homologous chromosomes relying on the homology of DNA sequences [1, 2], and is considered to be a transient and non-stable association between homologous chromosomes. Pairing between homologous chromosomes facilitates the process of recombination. The recombination process confers the interexchange of genetic information between non-sister chromatids, and generates chiasmata, which ensure proper association between homologous chromosomes prior to chromosome segregation at anaphase I. Synapsis, however, is a stable association by forming synaptonemal complexes between chromosomes. Synaptonemal complexes are tripartite proteinacious complexes formed between homologs and are to be removed at the end of meiotic prophase I.

In this review, we will discuss the mechanisms underlying meiotic recombination, based on the results from recent molecular and genetic studies, focusing on the formation

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and processing of double-stranded DNA breaks (DSBs) as well as genes involved in these processes in various organisms. Recently identified plant genes that are involved in meiotic recombination will also be discussed.

## Meiotic recombination between homologous chromosomes

Recombination refers to the exchange or transfer of DNA information between molecules [3]. Homologous recombination is a type of recombination based on the sequence homology between partners and is commonly formed in both mitotic and meiotic cells. Genetic and biochemical studies on recombination processes have generated a number of models, among which the double-stranded DNA break repair model (DSBR) is most supported (Figure 1).

The process of recombination is thought to occur at the sites of recombination nodules (RNs), which are small proteinacious structures associated with SCs. RNs were first identified under the electron microscopy in the female *Drosophila* [6]. Based on the timing of the appearance on meiotic chromosomes and other factors, including their frequency, shape, size, and staining properties, RNs were empirically identified as two types: early RNs and late RNs [7, 8]. Early RNs are abundant and they are associated with axial elements or SCs from leptotene to mid pachytene. From mid pachytene to late pachytene, late RNs appear on the central element of SCs. In most organisms, every pachytene chromosome has at least one late RN, and in a number of organisms, the number and position of late RNs are correlated with crossover formation [9, 10]. Since the RNs are correlated with sites of recombination and crossover formation, it is expected that the proteins involved in these processes are present in RNs. Indeed, RecA-like proteins have been found to be the components of early RNs in the lily plant [8], supporting the hypothesis that these RNs are sites of recombination.

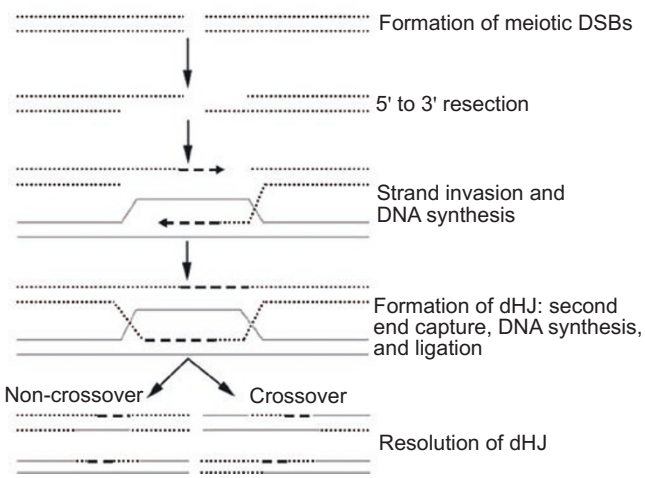


**Figure 1** A diagram of the double-strand break repair (DSBR) model. According to this model, recombination is initiated by the generation of a double-stranded DNA break (DSB) on one of the recombining molecules, followed by the resection of the 5' strand ends to generate two 3' single-stranded DNA overhangs. One of these 3'overhangs, then invades the partner chromatid, and forms a D-loop. DNA synthesis from the 3' ends using the partner as a template and subsequent ligation form a double Holliday junction (dHJ). The pattern of resolution of this double-Holliday junction determines whether the recombination product is a crossover or a non-crossover [3-5]. The crossover corresponds to the chiasma, an important chromosome structure holding two homologs together from late prophase I to metaphase I- anaphase I transition.

## Genes involved in DSB formation: SPO11 and others

The mechanisms for pairing can be divided into two groups: DSB-independent mechanisms and DSB-dependent mechanisms [11, 12]. DSB-dependent mechanism involves the formation of DSBs and the interaction of DNA sequences for homology search. Recombination in budding yeast is initiated by the formation of DSBs, a process which may be highly conserved among other organisms [13, 14]. Depending on the size of genome, one meiotic nucleus may contain numerous DSBs [15] and recombinational repair of the DSBs occurs at the sites of initiation of synapsis, providing a way for pairing between homologous chromosomes [2, 16, 17].

In budding yeast, the formation of DSBs requires a number of proteins (SPO11, RAD50, MRE11, XRS2, MER1, MER2, MEI4, MRE2, REC102, REC104, REC114) [18]. SPO11 catalyzes the formation of DSBs and is linked covalently to the 5 ends of unprocessed DSBs [19]. The SPO11 sequence suggests that it belongs to a novel family with similarity to the *topo6A* gene product from the archaea *Sulfolobus shibatae* [20]. In a topoisomerase-catalyzed cleavage reaction, a tyrosine residue attacks a phosphodiester bond, and generates DSBs via a transesterase mechanism with the assistance of a set of other gene products. This tyrosine residue is conserved in the SPO11 protein and is critical for its function [20]. Moreover, SPO11 homologs have been found in a wide range of organisms, including *Arabidopsis thaliana*, *Caenorhabditis elegans*, *D. melanogaster*, *Coprinus cinereus,* and mammals, indicating that this SPO11-catlayzed DSB generation is a conserved process among organisms [21-28]. Although the biochemical activity of SPO11 has not yet been determined, mutations in genes required for meiotic DSB repair result in chromosome fragmentation. However, this fragmentation can be

eliminated in a *spo11* background in different organisms, suggesting that SPO11 is responsible for generating DSBs during meiosis [29-35].

Furthermore, mutant studies have been performed with some of the identified *SPO11* genes. In the budding yeast, loss of *SPO11* function eliminates meiotic recombination and the formation of synaptonemal complexes but does not affect mitotic recombination and the mitotic cell cycle [36-38]. In addition, the *spo11* mutant exhibits reduced condensation and pairing; however, there is still residual pairing, suggesting that either SPO11 is not essential in DSB-dependent pairing or there might be DSB-independent pairing mechanisms [39]. This is consistent with the report that somatic pairing has been found in the budding yeast [39, 40]. A recent study assigns an early function of SPO11 in regulating meiotic S-phase progression, suggesting that SPO11 might be required for the formation of certain chromosome structures required for cell cycle progression [41]. In *S. pombe*, mutations in the *SPO11* homolog *rec12* cause reduced recombination, and *rec12* may have additional functions after the completion of meiotic prophase I, as the Rec12 protein persists until anaphase II [42, 43]. Similarly, a mouse *spo11* mutant is infertile and mutant spermatocytes exhibit little or no synapsis [44]. Mutation in the *Arabidopsis AtSPO11-1* gene shows abnormalities similar to those of the yeast *spo11* mutants, including greatly reduced recombination and synapsis. However, the presence of residual bivalents indicates that there might be SPO11-independent pairing, synapsis, and/or recombination mechanisms [45].

Therefore, *spo11* mutations in yeast and plants affect both recombination and synapsis, supporting the hypothesis that these two processes are closely associated in these organisms. However, in *Drosophila* and *C. elegans spo11* mutants, although meiotic recombination is defective, formation of synaptonemal complexes were observed independent of SPO11 function [21, 22]. Interestingly, the *Drosophila SPO11* homolog, *MEI-W68,* has a function in mitotic recombination. Careful phylogenetic analysis of known SPO11 proteins and other types of topoisomerases and functional analysis of SPO11 in other organisms would help to explain whether this is a new function for MEI-W68, or a function that appears to have been lost in yeast and plants.

Proteins other than SPO11 may also function in chromosome pairing and modeling to facilitate DSB formation, in regulating SPO11 activity, or in removing SPO11 from the sites after DSB formation [18]. In budding yeast RAD50, a large 153 kDa protein with N-terminal ATP-binding domain exhibits ATP-dependent binding to double-stranded DNA, and is required for both meiotic recombination and synapsis [46, 47]. It is also necessary for repairing mitotic DSBs. RAD50 may serve in chromatin remodeling and is required for removing SPO11 from the 5' end after DSB is made [19, 48, 49]. MRE11 and XRS2 function together with RAD50 as a complex [50-52]. This complex plays a role in a set of processes, including homologous recombination, non-homologous end joining, telomere maintenance, and removal of SPO11 from 5' end of newly formed DSBs [52-55].

In the *Arabidopsis atrad50* mutant, chromosomes fail to synapse and the processing of meiotic DSBs is defective, resulting in the fragmentation of meiotic chromosomes that are visible starting at premetaphase I [56, 57]. Abnormalities in mitotic development is not obvious under normal growth conditions; however, mutant plants exhibit an increased sensitivity to the DNA-damaging agent methylmethane sulfonate [57]. An *Arabidopsis atmre11* mutant meiocytes lack chromosome synapsis, and both mitotic and meiotic mutant cells contain AtSPO11-1-dependent chromosome fragments, suggesting that this gene functions downstream of AtSPO11-1; this is consistent with the function of RAD50-MRE11-XRS2 complex in budding yeast [35]. Moreover, the meiotic breaks in *atmre11* are not completely suppressed by *atspo11-1* mutation, indicating that other pathways might be involved in generating DSBs.

## DSB processing and function of RecA proteins

After the generation of DSBs by SPO11 and the resection of the DSBs by the complex of RAD50, MRE11, and XRS2 to produce 3' single-stranded DNA ends, the invasion of one of the 3' ssDNA ends is catalyzed by a group of proteins, including DMC1, RAD51, RAD52, RAD54, RAD55, RAD57, and TID1 [58, 59]. Among these proteins, DMC1 is a meiosis-specific protein. Phylogenetic analysis indicates that DMC1 is a RecA-like protein [60]. DMC1 homologs have been identified in a wide range of organisms, including budding and fission yeast, mammals, plants, and fungi [61-70]. In the budding yeast, the severity of *dmc1* mutants varies among different strains. The severe *dmc1* mutants of an SK1 strain show defective recombination, accumulation of unprocessed DSBs, and formation of abnormal SCs [71]. Mutations in the fission yeast *DMC1* cause reduced recombination [72]. Mouse mutants in *DMC1* are both male and female sterile, with gametogenesis arrested at meiotic prophase I and meiotic chromosomes failing to synapse [73]. The *Arabidopsis atdmc1* mutant fails to undergo meiotic synapsis; however, chromosomes remain intact [74]. Although normal SC is not formed, yeast and mouse *dmc1* mutants show signs of axial element formation, suggesting that DMC1 is required for the establishment of a connection linking two axial elements.

In addition to DMC1, the budding yeast possesses another RecA homolog, RAD51. Different than DMC1, RAD51 functions in both meiotic and mitotic homologous recombinations [75]. In meiosis, *rad51* mutants accumulate DSBs and exhibits reduced recombination [75]. The yeast RAD51 interacts directly with RAD54, a member of SWI2/MOT1 family [76]. Detailed analysis of the *dmc1* and *rad51* mutants suggests that these two genes perform both overlapping and distinct functions in meiotic recombination [77]. Interestingly, these two proteins may share the same or similar recombination functions, because they can substitute each other under certain circumstances. Consistently, RAD51 may act in a complex with DMC1 and function in recombination as these proteins might form nuclear complexes prior to chromosome synapsis [78]. However, synapsis appears not to be severely affected in the *rad51* and *dmc1* mutants, although the association of axial elements is substantially delayed [79], suggesting that the RAD51 and DMC1 may modulate synapsis or function later than the formation of synaptonemal complex.

RAD51 homologs have been isolated and characterized in a wide range of organisms [61, 70, 80-84]. The fission yeast *RAD51* homolog *RHP51* plays roles similar to those of the budding yeast *RAD51*, with roles that are overlapping with and distinct from those of the fission yeast *DMC1* gene in DSBs repair and meiotic recombination [72, 81]. The RAD51 homolog in *Aspergillus nidulans* shows a 67.4% sequence identity to budding yeast RAD51 [80]. While a deletion of two amino acid residues in the *A. nidulans RAD51* homolog (*uvsC*) causes arrest at meiotic prophase I, a null mutation causes the failure of meiotic cells to enter meiosis, indicating that this gene has a function prior to entering meiosis [80]. In *Drosophila* females, *RAD51* plays essential roles in repairing meiotic DSBs and also in meiotic chromosome pairing [85, 86]. In *C. elegans*, *rad51* RNAi lines show defective chromosome morphology at diakinesis and accumulation of meiotic DSBs in oocytes [84, 87]. In addition, *rad51* mutations in mice cause early embryo lethality, indicating that the mouse *RAD51* gene has an essential function in mitotic development [88, 89]. In *Arabidopsis*, a null mutation in *RAD51* causes defective meiotic recombination and synapsis, and an accumulation of unprocessed meiotic DSBs [33]. Different than the function of *RAD51* in budding and fission yeast, *RAD51* homologs in *A. nidulans*, *Drosophila*, *C. elegans*, and *Arabidopsis* seem to be dispensable for processing mitotic DSBs under normal growth conditions. Nevertheless, hypersensitivity to DNA damaging reagents have been revealed in these *rad51* mutants, suggesting that there is a RAD51-dependent pathway active in response to an increased number of mitotic DSBs caused by damage.

Another protein, BRCA2, has recently been shown to be involved in the processing of meiotic DSBs, possibly by interacting with RAD51 and/or DMC1. In animals, BRCA2 has been demonstrated to function in maintaining genome stability and a mutation in mouse *BRCA2* results in early embryo lethality that might be caused by aberrant chromosome rearrangements and fragmentations [90, 91]. The *brca2* mutant of *Ustilago* and a partial *brca2* mutant of mouse exhibit disrupted meiosis, and the presence of abnormal chromosomal alterations indicates a role of *BRCA2* in maintaining genome stability in meiosis [92, 93]. Notably, it has been found that in animals, *BRCA2* might be required for correct positioning of RAD51 foci in both mitotic and meiotic cells [91, 92], consistent with the similar abnormalities of the *brca2* to the *rad51* knockout mice. In *Arabidopsis*, silencing of the *BRCA2* genes induces formation of fragmented chromosomes, possibly the consequence of failure to process meiotic DSBs [34]. In *Ustilago*, BRCA2 was found to interact with RAD51 [93]; similarly, the *Arabidopsis* BRCA2 interacts with RAD51 and DMC1 in yeast two-hybrid experiments [34], indicating the BRCA2 may have conserved functions in the processing of meiotic DSBs by properly loading RAD51 and DMC1 to the sites of breaks.

In addition to mutant studies, biochemical results including patterns of protein localization and interaction also suggest functions of RAD51 and DMC1 in chromosome paring, synapsis and recombination. Moreover, these studies together with the protein characterization results support the idea that these two proteins have both distinct and overlapping functions. RAD51 foci exhibit a change in numbers during meiotic prophase I, and studies suggest that these foci are correlated with the sites of DSBs and the formation of early RNs [8, 94]. In the budding yeast, both proteins colocalize to meiotic chromosomes during meiotic prophase I and are thought to be components of early RNs [78]. In lily, however, although these two proteins colocalize on or adjacent to chromosomes from leptotene on to zygotene, the DMC1 foci disappear but the RAD51 foci retain at pachytene, indicating that RAD51 might have a late function after crossover formation [95]. Furthermore, in the budding yeast, normal loading of DMC1 onto chromosomes seems to be dependent on the presence of RAD51, as DMC1 foci are detectable but reduced in *rad51* mutants, but RAD51 foci are normal in *dmc1* mutants [77, 78].

Although DMC1 and RAD51 proteins share a high degree of sequence similarity, recent studies suggest that their structural properties might be remarkably different. The human RAD51, like RecA protein in *E. coli*, forms typical helical nucleoprotein filaments that promote strand invasion and formation of heteroduplex DNA. However, the human DMC1, showing binding preference to ssDNA and promoting formation of strand exchange between

homologous DNAs, forms octameric ring structures that may be stacked to form short filaments [96]. Further studies identified hydrogen bond interaction at the monomer-monomer interfaces of DMC1 that is required for forming octamer ring structure [97]. In fission yeast, RAD51 also forms helical nucleoprotein filaments; however, different than that in human, fission yeast DMC1 protein can form both helical filaments and stacked rings [98]. This study may stimulate further research in understanding the functions of DMC1 in fission yeast and its relationship to RAD51 function.

Biochemical studies indicate that both DMC1 and RAD51 exhibit DNA-stimulated ATPase activity, prefer to binding ssDNA, and catalyze ATP-dependent strand exchange reactions with homologous duplex DNA. However, double mutant studies of *red1* with *dmc1* and *rad51* provide evidences supporting the conclusion that the functions of these two proteins are not identical [77]. RED1 is a phosphoprotein that serves as a structural component of axial element, and is important for synapsis and the establishment of an interhomolog-specific recombination pathway by promoting DSB formation [99-101]. Although both RAD51 and DMC1 function in promoting interhomolog recombination, in a *red1 dmc1* double mutant, interhomolog recombination is almost completely absent (Interhomolog: intersister < 0.1), whereas a *red rad51* double mutant has a reduced but relatively high level of residual interhomolog recombination (Interhomolog: intersister ratio is 0:35) [99]. Therefore, DMC1 may play a more critical role in directing the recombination into interhomolog pathway.

## RAD51 paralogs in animals and plants: RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3

In addition to *RAD51* and *DMC1*, mammals possess other five RAD51 paralogs: *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2*, and *XRCC3* [102-106]. These genes exhibit various levels of sequence similarity to the *RAD51* gene.

The human *RAD51B* gene encodes a 305 amino-acid protein and is expressed widely, with the highest levels in tissues that are active in recombination [106]. Mutant analysis of *RAD51B* in a chicken B lymphocyte DT40 cell line indicates that *RAD51B* is important for maintaining genome integrity and for homologous recombinational repair [107, 108]. However, the *rad51b* mutant cell line is viable; although about 20% cells die after each division as a result of spontaneous chromosomal aberrations, indicating that *RAD51B* is required for repairing DNA breaks or damages under normal growth conditions. Moreover, the *rad51b* cells exhibit increased sensitivity to DNA cross-linking agents. Interestingly, *rad51b* cells exhibit a reduction of damage-induced RAD51 foci formation, suggesting that RAD51B might function in proper loading of RAD51 upon DNA damages. *Arabidopsis* has a *RAD51B* homolog, named *AtRAD51B*. The *atrad51b* mutant is fertile, undergoes normal meiosis, and normal vegetative growth under standard growth conditions [109, 110]. The *atrad51b* mutant exhibits moderate sensitivity to DNA-damaging agents, including gamma-radiation and cisplatin, indicating that the function of *RAD51B* is less important in plant mitosis, possibly because homologous recombination is less active in plants.

*RAD51C* in human encodes a 376 AA protein showing 18-26% identity to other human RAD51 homologs [103]. The *RAD51C* gene is expressed widely, with the highest level in the testis. Mutation in *RAD51C* results in DNA damage-sensitivity in a hamster cell line, and an increase in spontaneous chromosomal aberrations such as a lack of sister chromatid exchanges and increased isochromatid breaks [111, 112]. Moreover, mutant cell lines also exhibit reduced RAD51 foci formation upon DNA damage treatments, supporting the notion that RAD51C is a key component in RAD51-dependent processes. *Drosophila* possesses a *RAD51C* homolog that encodes a 271 AA protein and mutation in this gene results in abnormal chromosomal behaviors, including a high percentage of oblong and fragmented chromosomes (77%) [113]. However, *Drosophila RAD51C* seems to be unnecessary for mitotic DNA repair or other mitotic processes as the mutant grows normally and does not show an increased sensitivity to either MMS or gamma rays. The authors proposed that because *Drosophila* lacks a detectable homolog of *DMC1*, it is possible that *Drosophila RAD51C* and *XRCC3* (see below) function together in a pathway specific for meiosis as a replacement of the *DMC1* function. In *Arabidopsis*, which has a *DMC1* homolog, *RAD51C* is critical in meiosis and a mutation in this gene causes striking fragmentation of meiotic chromosomes visible starting at the end of prophase I, suggesting that *Arabidopsis RAD51C* is critical for processing meiotic DNA breaks [31, 109]. Furthermore, homologous chromosomes in *atrad51c* fail to undergo synapsis and homolog juxtaposition [31].

*RAD51D* in mouse encodes a 319 AA protein with significant sequence similarity to other RAD51 proteins [105]. The mouse *RAD51D* gene is expressed widely and different sizes of transcripts are detected, suggesting the different mRNA splicing might be involved in regulating the *RAD51D* function [105]. In human, the splicing variants of *RAD51D* are also present, suggesting that this might be a conserved mechanism in mammals [114, 115]. Genetic studies suggest that *RAD51D* also plays an important role in homologous recombination. In mouse, *rad51d* knockout mutants exhibit embryo lethality and fibroblast cell lines undergo cell lethality, suggesting that *RAD51D* plays an im-

portant role in cell proliferation [116]. In addition, *rad51d* mutant cells exhibit increased centrosome fragmentation and RAD51 foci formation [117]. Therefore, RAD51D may bind, possibly indirectly, to RAD51 and may be required for maintaining genome stability by repairing DNA damage, in conjunction with RAD51 [116]. The death of *rad51d* mouse embryo may also be a result of p53 activation, as a *p53* deletion extends the life span of *rad51d* embryos and rescues the cell lethality [117]. A recent study reports that RAD51D is also involved in telomere maintenance [118]. RAD51D is shown to localize to telomere regions in both mitotic and meiotic cells, and the mouse *rad51d trp53* mutant exhibits telomeric DNA repeat shortening and increased telomere end-to-end joining. A RAD51D homolog has also been discovered in *Arabidopsis*; however, functional analysis has not yet been reported.

X-ray repair complementing defective repair in Chinese hamster cells 2 (*XRCC2*) was identified in human cells based on isolating cDNA that can complement X-ray-sensitive mutant cell lines. XRCC2 from human and mouse exhibits high similarity and in mouse, *XRCC2* is expressed widely, with an elevated level in the testis [119]. The mutant cell line, *irs1*, shows extreme sensitivity to DNA cross-linking agents and genome instability [119, 120]. The mouse *XRCC2*-deficient mutant exhibits embryo lethality and dies before or at birth. Embryonic cells exhibit genome instability as shown by the presence of high level of chromosomal aberrations and sensitivity to gamma rays. In *irs1* mutant cells, homologous recombinational repair of DNA DSBs is greatly reduced, although a normal level of non-homologous end-joining is present [121]. Therefore, *XRCC2* might play a critical role specific for homologous repair of mitotic DSBs [121, 122]. Detailed analysis of the human XRCC2 protein suggests that its putative ATP-binding domain is critical for its function in homologous recombination repair as site-specific mutations in the putative ATP-binding region fail to restore the *irs1* phenotype [123]. Similar to RAD51C and RAD51D, XRCC2 is also required for proper loading of RAD51 onto chromosomes as determined by the lack of RAD51 foci formation in *irs1* mutant cells [123].

Human *XRCC3* was isolated as a cDNA that partially restores MMC resistance to an *irs1SF* cell line [104]. *irs1SF* mutant cells exhibit reduced plating efficiency and growth rate, severe chromosome instability, and sensitivity to cisplatin and gamma rays. Further analysis using a novel fluorescence-based assay of the XRCC3-deficient irs1SF cell line revealed a 25-fold reduction in homologous recombinational repair of DSBs, which suggests that *XRCC3* is involved in homologous recombination [124-126]. In addition, RAD51 foci do not occur in the *irs1SF* mutant cells, indicating that a possible function of *XRCC3* in homologous recombinational repair is to establish or to stabilize the RAD51 foci upon DNA damages [127]. Transfection of the mutant cell line with the *XRCC3* cDNA increases the frequencies of homologous recombinational repair [125]. In addition to early function including initiation of homologous recombination, XRCC3 may also function at late stages in resolving HR intermediates [128]. In *Arabidopsis*, *AtXRCC3* was identified as a homolog of mammalian *XRCC3* [129]. *AtXRCC3* is expressed widely but with the highest level in floral buds [129]. Mutation in *AtXRCC3* results in defective male and female meioses with severe fragmentation of meiotic chromosomes after pachytene [130]. This chromosome fragmentation can be largely suppressed by the *atspo11-1* mutation, indicating that *AtXRCC3* is required for repairing the meiotic DSBs generated by AtSPO11-1 [32]. Although the *atxrcc3* mutant plant has normal vegetative development, mutant cells and plants exhibit increased sensitivity to DNA-damaging treatments with MMC and bleomycin [130].

In mammals, biochemical analyses have shown that these mammalian RAD51 paralogs function in the form of complexes. Yeast two-hybrid tests confirmed that human RAD51D interacts with XRCC2 and RAD51C; in addition, yeast three-hybrid system indicates that a number of different pairs of interactions between these human RAD51 paralogs may occur simultaneously [131]. Later experiments with isotope-tagged proteins suggest that RAD51B is also involved in the above complex, making a four-component complex including RAD51B, RAD51C, RAD51D, and XRCC2 (defined as BCDX2) [132]. The BCDX2 complex binds to nicks of duplex DNA and exhibits ATPase activity *in vitro*. These results together with the observations that RAD51B [107, 108], RAD51C [111], RAD51D [117], and XRCC2 [123] are required for proper formation of RAD51 foci, indicate that these proteins may function as a complex both in assembling the RAD51 complex and in the initial processing of the single-stranded DNA at the damaged sites. Another complex, with RAD51C and XRCC3, was originally detected with a yeast two-hybrid system and then with interaction of proteins expressed in baculovirus-infected insect cells [132-134], and then confirmed with human cells lines that express isotope-tagged XRCC3 or RAD51C proteins [135]. In addition, XRCC3 is shown to be required for stabilizing RAD51C possibly by forming dimers, and this function requires the ATPase activity, suggesting that XRCC3 may regulate the dimerization through its ATP binding and hydrolysis activity [136]. Consistently, the *XRCC3* cDNA with a point mutation at a conservative amino acid of the ATPase domain fails to complement the *irs1SF* mutant cells, indicating that the ATPase activity is critical for the proper function of XRCC3 [136]. In *Arabidopsis*, yeast two-hybrid experiments have identified an

interaction between AtXRCC3 and AtRAD51C [129] and an interaction between AtRAD51B and AtRAD51C [110], suggesting that the mammalian patterns of the RAD51 paralog functions via forming complexes are also present in plants.

## Other plant genes involved in meiotic recombination

In addition to studies using reverse genetics described above, plants also provide excellent systems in characterizing meiotic genes using forward genetics. In maize, this approach has identified a large number of mutants defective in meiosis [137], although mutated genes for most of them have not been isolated. Maize has served as an excellent model plant as it has a partially polyploidy genome, and some mutations allow pairing and synapsis between non-homologous chromosomes, possibly interfering with normal recombination between homologous chromosomes. Mutants defective in specifying homolog recognition include *desynaptic1*, *desynaptic2*, and *phs1* [137-139]. In addition, it is notable that in *phs1*, the number of RAD51 foci on meiotic chromosomes is greatly reduced, although the RAD51 protein is present at normal level [139]. These results suggest that in addition to specifying paring and synapsis between homologous chromosomes, PHS1 may also function in the proper loading of RAD51 onto meiotic chromosomes. Furthermore, DSB can be formed and repaired in *phs1* even though this repair might be delayed, suggesting that maize may have a DSB-repairing system independent of RAD51.

In *Arabidopsis,* a mutant called *solo dancers (sds)* was isolated because of its greatly reduced fertility from a transposon insertional population [140]. The *sds* mutant exhibits a severe defect in homolog pairing, recombination, and bivalent formation. These phenotypes are very similar to those of *atspo11-1* and *atdmc1* mutants, suggesting that SDS may be involved in the SPO11-dependent recombination pathway. *SDS* is specifically expressed in male and female meiotic cells and encodes a putative novel cyclin [140, 141]. Cyclins are known to regulate multiple processes during the mitotic cell cycle by activating cyclin-dependent kinases, suggesting that SDS may coordinate pairing, synapsis, and recombination by regulating protein activities via phosphorylation. In addition, proteolytic events might also be involved in the normal progression of meiotic recombination [142]. These results suggest that synapsis and recombination may be achieved by multiple pathways and be performed by multiple components. Further analysis of these and possibly additional mutants, the interplay between these genes, and the interplay between synapsis and recombination would help dissecting the mechanisms underlying associations between meiotic homologous chromosomes.

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