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A Molecular Portrait of Arabidopsis Meiosis

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Key words: pairing, recombination, synapsis, crossover formation, interference, spindle assembly

Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; DSB, double strand break; DSBR, double strand break repair; SC, synaptonemal complex; TEM, transmission electron microscopy

Abstract

Meiosis is essential for eukaryotic sexual reproduction and important for genetic diversity among individuals. Efforts during the last decade in Arabidopsis have greatly expanded our understanding of the molecular basis of plant meiosis, which has traditionally provided much information about the cytological description of meiosis. Through both forward genetic analysis of mutants with reduced fertility and reverse genetic studies of homologs of known meiotic genes, we now have a basic knowledge about genes important for meiotic recombination and its relationship to pairing and synapsis, critical processes that ensure proper homolog segregation. In addition, several genes affecting meiotic progression, spindle assembly, chromosome separation, and meiotic cytokinesis have also been uncovered and characterized. It is worth noting that Arabidopsis molecular genetic studies are also revealing secrets of meiosis that have not yet been recognized elsewhere among eukaryotes, including gene functions that might be unique to plants and those that are potentially shared with animals and fungi. As we enter the post-genomics era of plant biology, there is no doubt that the next ten years will see an even greater number of discoveries in this important area of plant development and cell biology.

Introduction

Meiosis is a specialized cell division characteristic of eukaryotes and is essential for sexual reproduction. The function of meiosis is to generate cells that contain exactly half of the genetic materials of the parental cells and that develop into germ cells. Consequently, the fusion of germ cells can then restore to the offspring the ploidy and level of genetic complexity of the parent(s). In mitosis, DNA replication is followed by one round of nuclear division, which separate sister chromatids. In contrast, in meiosis, a single round of DNA replication is followed by two rounds of nuclear division, meiosis I and meiosis II. Meiosis I is unique and involves the segregation of homologous chromosomes (homologs); this division reduces ploidy by half, thus is also called reductional division. Meiosis II is very similar to mitosis in that it results in the segregation of sister chromatids; therefore, it is also called equational division. Both meiosis I and II are further divided into four phases: prophase, metaphase, anaphase, and telophase, roughly similar to those in mitosis.

Meiotic prophase I is a long and complex process and is further divided into five substages according to cytological features of the chromosomes: leptotene, zygotene, pachytene, diplotene, and diakinesis (Dawe, 1998; Zickler and Kleckner, 1999; Stack and Anderson, 2001; Armstrong and Jones, 2003). Very early in prophase I, chromosomes begin to condense, forming thin thread-like structures called the axial element; this substage is called **leptotene**. Because the purpose of meiosis I is to properly separate homologs, it is essential for the homologs to recognize each other during early prophase I. The recognition between homologs is achieved through homolog pairing. The term "pairing" has been used to describe various interactions or associations between homologs (Zickler and Kleckner, 1999). In this chapter, it is used with a narrow definition for the transient interaction at localized regions of homologs that occurs prior to synapsis (Zickler and Kleckner, 1999). In most organisms, pairing depends on DNA homology to verify that the interacting chromosomes are indeed homologous. Pairing then progresses to homolog juxtaposition, which is also homology-dependent and refers to the coming togeth-

er of homologs after the initial transient interaction (Zickler and Kleckner, 1999).

Synapsis is an extensive and stable interaction between chromosomes that involves the formation of a complex proteinacious structure called the synaptonemal complex (SC) (Roeder, 1990). When homologs are partial synapsed, parts of homologs can be visibly detected as "fused" together, at a substage called **zygotene**. Using electron microscopy, the SC can be seen as a tripartite proteinaceous structure (Zickler and Kleckner, 1999): two parallel filementous "lateral elements" are from the axial elements. A central element forms in between and parallel to the lateral elements and is connected to the lateral elements by the transverse elements (Zickler and Kleckner, 1999; Schwarzacher, 2003; Higgins et al., 2005). The central element consists of the heads of the proteins that form the transverse elements (Zickler and Kleckner, 1999; Higgins et al., 2005). When the homologs are fully synapsed with the completion of the SC, the stage is called the **pachytene** substage, with each pair of synapsed homologs appearing under a light microscope as a thick thread-like structure, although the SC proper requires electron microscopy to observe. During normal meiosis, SC is formed between homologs and is closely coupled with homolog pairing and juxtaposition; however, in some yeast and maize mutants, SC can also form between non-homologous chromosomes (Leu et al., 1998; Pawlowski et al., 2004) suggesting that synapsis per se does not require homology and can be uncoupled from pairing. After pachytene, the SC is disassembled and homologs partially separate, except at the chiasmata, which are cytological features corresponding to crossing over points between non-sister chromatids; this substage is recognized as **diplotene**. Subsequent further chromosome condensation results in highly compacted bivalents (attached pairs of homologs), reaching the **diakinesis** substage, the end of prophase I.

In addition to pairing, juxtaposition, and synapsis, homologs also undergo recombination during prophase I, resulting in crossovers (exchange of chromosomal regions) and gene conversion (copying of sequence from one homolog to the other). Under an electron microscope, electron dense bodies called meiotic nodules can be detected along the chromosome; they are also called recombination nodules because their number and distribution are correlated with those of recombination (Carpenter, 1975, 1987; Sherman et al., 1992; Sherman and Stack, 1995; Anderson et al., 2001). Recombinational crossover, along with sister chromatid cohesion, ensures the formation of chiasmata, which are the physical links between homologs in a bivalent at late prophase I through the metaphase I/anaphase I transition. In the absence of crossovers, homologs would separate following the disassembly of the SC, resulting in detached chromosomes called univalents, which cannot align properly at the metaphase plate and segregate correctly at anaphase I. Therefore, at the level of an individual organism, recombination is critical for the reproductive success, because it maintains homolog association until the metaphase I/anaphase I transition and allows correct segregation of homologs at anaphase I. At the population and species levels, meiotic recombination, along with independent assortment of chromosomes, facilitates the redistribution of genetic material between generations, and increases genetic diversity among individuals in a population. This obviously has great ecological and evolutionary implications, making meiosis and particularly meiotic recombination important genetic mechanisms that have likely contributed to the enormous success of eukaryotes.

In flowering plants, meiosis occurs in both the male organ anther, and the female structure called the ovule, which is housed within the ovary. Both male and female meioses produce haploid spores, which then develop further through mitoses and cell differentiation into gametophytes. Because of the ease with which one can obtain male meiocytes and observe chromosome behaviors in a number of flowering plants, male meiosis in these plants has been studied extensively using cytogenetics and genetics (Golubovskaya, 1979; Kaul and Murphy, 1985; Dawe et al., 1994; Neuffer et al., 1997; Dawe, 1998; Zickler and Kleckner, 1998, 1999; Hamant et al., 2006). Chromosomal behavior, including condensation, pairing, and synapsis have been described for a number of plants using light and electron microscopy. Traditionally, plants with many meiocytes and very large chromosomes, such as lily, tomato, and maize, have been favored (Moens, 1969; Stack, 1973; Anderson et al., 1985; Anderson et al., 1988; Sherman and Stack, 1995; Anderson et al., 1997; Peterson et al., 1999; Stack and Anderson, 2001). In particular, maize has been a favorite organism for decades and is an excellent system for genetic and cytological analyses (Golubovskaya, 1979; Anderson et al., 1988; Golubovskaya, 1989; Staiger and Cande, 1991, , 1992; Golubovskaya et al., 1993; Dawe et al., 1994; Dawe and Cande, 1996; Golubovskaya et al., 1997; Yu et al., 1997; Franklin et al., 1999; Golubovskaya et al., 2002; Anderson et al., 2003; Pawlowski et al., 2003; Anderson et al., 2004; Pawlowski et al., 2004).

In recent years, cytological procedures have been developed for *Arabidopsis thaliana* (Peirson et al., 1996; Ross et al., 1996; Klimyuk and Jones, 1997; Ross et al., 1997; Yang et al., 1999; Caryl et al., 2000; Armstrong and Jones, 2001; Grelon et al., 2001; Azumi et al., 2002; Chen et al., 2002; Caryl et al., 2003), even though it is well-known for its small chromosomes. These technical advances in combination with the molecular genetic properties of Arabidopsis (Meyerowitz, 1987, , 1989) and the availability of the complete genomic sequence and publicly available collections of insertional lines (Parinov et al., 1999; AGI,

2000; Alonso et al., 2003) have greatly facilitated the identification and characterization of meiotic genes using both forward and reverse genetics. This chapter will first briefly describe normal meiosis in Arabidopsis, and then present a series of discussions of genes that are important for meiosis (Table 1), roughly according to the progression of the meiotic process. For studies in others plants, the reader is referred to a number of excellent reviews (Dawe, 1998; Zickler and Kleckner, 1999; Schwarzacher, 2003; Harper et al., 2004; Pawlowski and Cande, 2005; Hamant et al., 2006). Much information about meiotic genes has been obtained from studies in the budding yeast *Saccharomyces cerevisiae*; unless otherwise indicated, in this chapter yeast refers to *S. cerevisiae*. Because meiosis is a conserved process common to eukaryotes, other reviews on meiosis in yeast and other non-plant systems also offer valuable references (Zickler and Kleckner, 1999; Haber, 2000; Page and Hawley, 2003; Bishop and Zickler, 2004; Page and Hawley, 2004).

Normal Arabidopsis Meiosis

In Arabidopsis, male meiosis occurs in the anther at anther stage 6 as define by Sanders et al. (1999), when the meiocytes are surrounded by four layers of somatic cells, including the tapetum (Ma, 2005). This is within stage 9 of flower development (Smyth et al., 1990). Each Arabidopsis flower has six anthers, each of which contains four lobes; within each lobe, there are approximately 30 meiocytes, giving rise to about 700 meiocytes per flower. To visualize meiotic chromosomes, a procedure called chromosome spreading is used to partially separate the chromosomes after limited digestion of the meiocytes to loosen the cell walls; this procedure has been widely used to study Arabidopsis meiotic mutants (Peirson et al., 1996; Ross et al., 1996; Klimyuk and Jones, 1997; Ross et al., 1997; Yang et al., 1999; Caryl et al., 2000; Siddiqi et al., 2000; Armstrong and Jones, 2001; Grelon et al., 2001; Sanchez Moran et al., 2001; Azumi et al., 2002; Chen et al., 2002; Caryl et al., 2003; Grelon et al., 2003; Yang et al., 2003a; Yang et al., 2003b; Bleuyard and White, 2004; Higgins et al., 2004; Li et al., 2004; Li et al., 2005).

Following spreading, the chromosome preparations are stained with DAPI (4',6-diamidino-2-phenylindole) before viewing under a fluorescence light microscope (Figure 1). At leptotene, partially condensed meiotic chromosomes are seen as thin lines (Figure 1A). From late leptotene to early zygotene (Figure 1B), homologs pair and become juxtaposed and then synapsed from zygotene through pachytene (Figure 1C). At diplotene (Figure 1D), the SCs are disassembled and the homologs separate in regions other than the chiasma(ta). At diakinesis (Figure 1E), further condensation results in very short chromosomal pairs, seen as five bivalents. The bivalents move to the center of the cell and are aligned at the equatorial plane at metaphase I (Figure 1F), due to the forces of the spindle microtubules. At the metaphase I/anaphase I transition, the removal of cohesins along chromosomal arms allows the separation of homologs, which are then pulled to the opposite poles of the spindle (not seen here) at anaphase I (Figure 1G). At telophase I (Figure 1H), five chromosomes decondense and form a cluster at each pole. The chromosomes re-condense during prophase II (Figure 1I), and the two groups are separated from each other by an organelle band. The two groups of chromosomes are aligned separately at two division planes at metaphase II (Figure 1J). Subsequently at anaphase II, separation and segregation of sister chromatids form four clusters of new chromosomes (Figure 1K). At telophase II, chromosome decondensation results in four haploid nuclei (Figure 1L), which are then packaged into four microspores by cytokinesis.

Unlike male meiosis, Arabidopsis female meiosis occurs in only about 50 cells per flower, one in each ovule. The relatively small number of female meiocytes and the fact that each female meiocyte is surrounded by somatic cells of the ovule make the analysis of female meiosis much harder than that of male meiosis. This is generally true for meiotic studies in other plants, such as maize. Much greater amounts of efforts are needed to obtain female meiotic images at various stages (Figure 2), which are generally very similar to the male counterparts. One important distinction is that female meiosis is asymmetric, resulting in one functional megaspore, which develops into the female gametophytes, and three others that degenerate. Unless specifically indicated, meiosis refers to male meiosis in this chapter.

In addition to DAPI-stained chromosome spreads, fluorescence in situ hybridization (FISH) methods have been applied to plant meiotic chromosomes, and are important for studying homolog pairing and juxtaposition (Zhong et al., 1996; Bass et al., 1997; Yu et al., 1997; Fransz et al., 1998; Peterson et al., 1999; Fransz et al., 2000; Armstrong et al., 2001). Furthermore, SCs and recombination nodules have been observed using transmission electron microscopy (TEM) (Armstrong et al., 2002; Schwarzacher, 2003; Higgins et al., 2004; Li et al., 2004; Li et al., 2005) (Figure 3). Using TEM, it seems that full-length axial elements are formed prior to homolog synapsis (Figure 3), unlike some organisms, in which SC formation is initiated before the axial element is completely formed (Zickler and Kleckner, 1999). The axial elements become the lateral elements of the SC following synapsis (Figure 3). The central element is formed from the heads of the transverse elements, which are connected to the lateral elements (Zickler and Kleckner, 1999; Schwarzacher, 2003). The light and electron microscopic methods provide the means to obtain a morphological basis for genetic and molecular analyses of Arabidopsis meiosis.

Table 1. Arabidopsis meiotic genes

Gene symbol [a]	ID number	Protein features	Function	References [b]
ASK1	At1g75950	SCF subunit	Homolog separation Inhibit recombination	Yang et al. 1999 Wang et al., 2005
ASK2	At5g42190	SCF subunit	Homolog separation (?)	Zhao et al., 2003
ASY1	At1g67370	HOP1 homolog	Homolog pairing	Caryl et al., 2000 Armstrong et al., 2002
ATK1	At4g21270	C-terminal kinesin	Spindle assembly	Chen et al, 2002
ATM	At3g48190	ATM homolog	Regulation of DNA repair	Garcia et al., 2000 Garcia et al., 2003
BRCA2a	At4g00020	BRCA2 homolog	Meiotic recombination	Siaud et al., 2004
BRCA2b	At5g01630	BRCA2 homolog	Meiotic recombination	Siaud et al., 2004
CAP-E1=TTN3	At5g62410	SMC2 homolog	Condensation	Siddiqui et al., 2003
CAP-E2	At3g47460	SMC2 homolog	Condensation	Siddiqui et al., 2003
CDC45	At3g25100	CDC45 homolog	Meiotic progression	Stevens et al., 2004
DIF1=SYN1	At5g05490	REC8 homolog	Sister chromatid cohesion	Blatt et al., 1999
DMC1	At3g22880	DMC1	Meiotic recombination	Doutriaux et al., 1998 Couteau et al., 1999
DUET=MMD1	At1g66170	PHD finger	Meiotic progression	Reddy et al., 2003
DYAD=SWI1	A5g51330	Novel	Sister chromatid cohesion	Siddiqi et al., 2000 Agashe et al., 2002
MEI1	t1g77320	BRCT motifs	Meiotic DNA repair	Grelon et al., 2003
MER3=RCK	At3g27730	MER3 homolog	Crossover formation	Mercier et al., 2005
MMD1=DUET	At1g66170	PHD finger	Meiotic progression	Yang et al., 2003b
MRE11	At5g54260	MRE11 homolog	Meiotic DNA repair	Hartung and Puchta, 1999 Puizina et al., 2004
MS5=TDM	At4g20900	Novel	Meiotic progression	Glover et al., 1998
MSH2	At3g18524	MSH2 homolog	Inhibit recombination	Emmanuel et al., 2006
MSH4	At4g17380	MSH4 homolog	Crossover formation	Higgins et al., 2004
PTD	At1g12790	Similar to ERCC1	Crossover formation	Wijeratne et al., 2006
RAD50	At2g31970	RAD50 homolog	Homolog pairing, synapsis, recombination	Gallego et al., 2001 Bleuyard et al., 2004b
RAD51	At5g20850	RAD51 homolog	Homolog pairing, synapsis, recombination	Doutriaux et al., 1998 Li et al., 2005
RAD51C	At2g45280	RAD51C	homolog Homolog pairing, synapsis, recombination	Abe et al., 2005 Bleuyard et al., 2005 Li et al., 2005
RCK=MER3	At3g27730	MER3 homolog	Crossover formation, synapsis	Chen et al., 2005
SCC3	At2g47980	SCC3 homolog	Sister chromatid cohesion	Chelysheva et al., 2005
SDS	At1g14750	Novel cyclin	Homolog pairing, synapsis, recombination	Azumi et al., 2002 Wang et al., 2004a
SMC1=TTN8	At3g54670	SMC1 homolog	Highly expressed in floral buds	Lam et al., 2005b
SMC3=TTN7	At2g27170	SMC3 homolog	Localized to chromosomes and the spindle	Lam et al., 2005b
SPO11-1	At3g13170	SPO11 homolog	Homolog pairing, synapsis, recombination	Grelon et al., 2001

Table 1 (*continued*). Arabidopsis meiotic genes

Gene symbol [a]	ID number	Protein features	Function	References [b]
STD=TES	At3g43210	Kinesin	Meiotic cytokinesis	Hulskamp et al., 1997
SWI1=DYAD	At5g51330	Novel	Sister chromatid cohesion	Mercier et al., 2001, 2003
SYN1=DIF1	At5g05490	Rec8 homolog	Sister chromatid cohesion	Bai et al., 1999 Cai et al., 2003
SYN2	At5g40840	Rec8 homolog	Highly expressed in dividing cells	Dong et al., 2001
SYN3	At3g59550	Rec8 homolog	Highly expressed in dividing cells	Dong et al., 2001
TAM=CycA1,2	At1g77390	Cyclin A	Meiotic progression	Magnard et al., 2001 Wang et al., 2004b
TDM=MS5	At4g20900	Novel	Meiotic progression	Ross et al., 1997
TES=STD	At3g43210	Kinesin	Meiotic cytokinesis	Spielman et al., 1997 Yang et al., 2003a
XRCC3	At5g57450	XRCC3 homolog	Homolog pairing, synapsis, recombination	Bleuyard and White, 2004 Bleuyard et al., 2004a
ZYP1a	At1g22260	Similar to ZIP1	Synapsis, meiotic progression	Higgins et al., 2005
ZYP1b	At1g22275	Similar to ZIP1	Synapsis, meiotic progression	Higgins et al., 2005

[a]The "At" in gene names for *Arabidopsis thaliana* is not shown here.
[b]For additional references, please see text.

Chromosome Condensation and Sister Chromatid Cohesion

In both mitosis and meiosis, chromosome condensation from long interphase fibers to highly compacted units is essential for successful segregation. In meiosis, chromosomes condense twice, one for each of the meiotic divisions. Analyses in animals and yeast have identified a group of highly conserved proteins, called Structural Maintenance of Chromosomes (SMCs) (Hirano, 2000; Hirano, 2002). In eukaryotes, there are six distinct types of SMC proteins, SMC1-6; among these, SMC2 and SMC4 form a heterodimer that is specifically required for condensation, whereas others have different functions (Hirano, 2002). Because condensation is a common feature of both mitosis and meiosis, genes required for condensation are likely important for mitotic development, such as gametophyte development and embryogenesis. At the same time, due to the highly duplicated Arabidopsis genome, often a specific function is fulfilled by two or more highly related genes. Indeed, a recent report showed that Arabidopsis possesses two highly similar *SMC2* genes, called *AtCAP-E1* and *AtCAP-E2* (for Chromosome Associated Protein subunit E) (Siddiqui et al., 2003). *AtCAP-E1* is expressed at a level approximately six times that of *AtCAP-E2*. In addition, T-DNA insertional mutants in either gene are viable and fertile, although the *atcap-E1* mutant (called *titan3* for enlarged embryos) exhibits mild embryo development defects (Liu and Meinke, 1998). In contrast, the *atcap-E1 atcap-E2* double homozygous plants are embryonically lethal; even the presence of one copy of *AtCAP-E2* is not sufficient for embryo viability. Therefore, possible meiotic chromosome condensation defects of the double homozygous plants could not be assessed. Nevertheless, the authors reported that one copy of the *AtCAP-E1* gene was sufficient for viability and the male meiocytes in the mutant plants exhibited abnormal chromosomal morphology at metaphase I and anaphase I, consistent with a defect in chromosome condensation. It is not known whether chromosome condensation during early prophase I is abnormal in these mutant meiocytes.

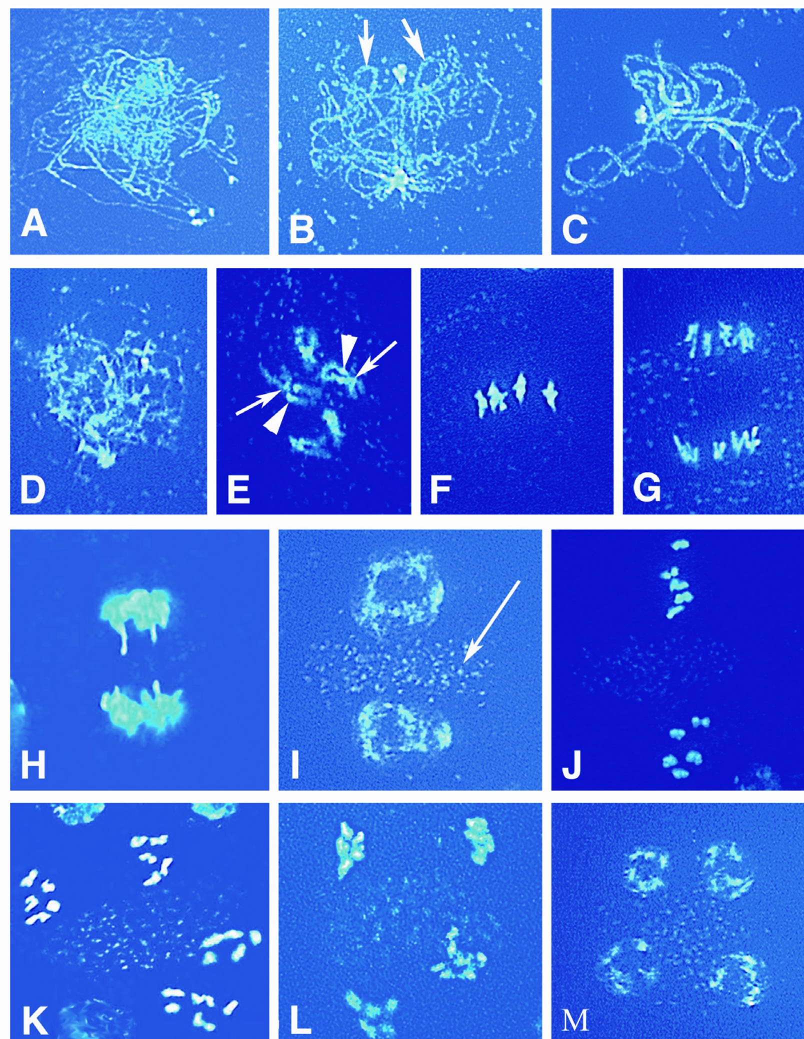

Figure 1. Arabidopsis male meiosis. A, leptotene. B, zygotene; arrows indicate regions of pairing between homologs. C, pachytene. Fully synapsed chromosomes appear as thick threads in light micrographs. D, diplotene. E, diakinesis; arrows point to the position of chiasmata and arrowheads indicate the centromeric regions. F, metaphase I. G, anaphase I. H, telophase I. I, prophase II; the arrow points to the organelle band between the two groups of chromosomes. J, metaphase II. K, anaphase II. L, telophase II, M. Four newly formed nuclei. The images were obtained from DAPI-stained chromosome spreads following the fixation of floral buds. These images were provided by W. Li and were previously published in Ma (2005).

Following DNA replication, the sister chromatids are closely associated with each other via sister chromatid cohesion (Michaelis et al., 1997; Nasmyth, 1999). In mitosis, sister chromatid cohesion is the primary means of chromosomal association until the metaphase/anaphase transition, when sister chromatid cohesion is removed to allow sister separation. As mentioned in the introduction, in meiosis I, sister chromatid cohesion, along with a crossover, is important for maintaining the chiasmata, which hold homologs together prior to the metaphase I/anaphase I transition. Removal of meiotic cohesion along the arm then allows the homologs to separate (Klein et al., 1999; Orr-Weaver, 1999; Watanabe and Nurse, 1999; van Heemst and Heyting, 2000). Therefore, sister chromatid cohesion and its regulated removal is critical for meiosis. Cohesion depends on a multiprotein complex called cohesin, which has at least four proteins; two of the four proteins, SMC1 and SMC3 are related to the SMC2 and SMC4 condensins, whereas the other two, SCC1, and SCC3 are specific subunits of cohesins (Losada et al., 1998; Nasmyth, 1999; Orr-Weaver, 1999). In addition, there is a meiosis-specific SCC1-like protein REC8; both SCC3 and REC8 in yeast are required for meiotic sister chromatid cohesion (Klein et al., 1999; Orr-Weaver, 1999; Parisi et al.,

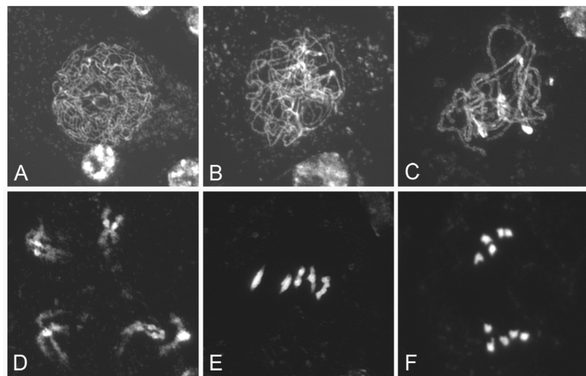

Figure 2. Arabidopsis female meiosis. A. Leptotene. B. Zygotene. C. Pachytene. D. Diakinesis. E. Metaphase I. F. Anaphase I. These images were provided by W. Li.

1999; StoopMyer and Amon, 1999; Watanabe and Nurse, 1999).

In Arabidopsis, a gene named *SYN1* or *DIF1* has been found to be a homolog of REC8 and mutant analysis indicates it is essential for normal meiosis (Peirson et al., 1996; Peirson et al., 1997; Bai et al., 1999; Bhatt et al., 1999). In *syn1/dif1* mutant meiocytes, sister chromatid cohesion is abnormal; in addition, chromosome condensation is also affected and mutant meiocytes produce chromosomal fragments. Recent immunolocalization experiments (Cai et al., 2003) using antibodies against the SYN1 protein indicate that the SYN1 protein is localized to the meiotic chromosomes from interphase to pachytene. At diplotene and diakinesis, the amount of SYN1 signal on the chromosomes reduces dramatically and the signal seems to localize to discrete regions of the chromosomes. Concurrent with the reduction of the chromosomal SYN1 signals, there is an increase in nucleoplasm SYN1 staining, suggesting that SYN1 is released from the chromosome to the nucleoplasm. This is consistent with the idea that initially cohesins are cleaved rather than completely degraded. At metaphase I, the SYN1 signal is further reduced and limited to the chromosomes, only detectable at a few loci on the highly condensed chromosomes; at anaphase I, SYN1 signals on chromosomes disappear. These results indicate that SYN1 is localized to the arms, as is the case for Rec8 protein. Although SYN1 signal is not detected at the centromere during prophase I or metaphase I, it should still be present at very low levels before anaphase I, as supported by the loss of centromere cohesion at anaphase I in *syn1* meiocytes (Cai et al., 2003).

From anaphase I to metaphase II, the sister chromatids remain associated at the centromeres. Therefore, other cohesins are likely responsible for sister chromatid cohesion at the centromere responsible for sister association until anaphase II. Arabidopsis has at least two other REC8 homologs, SYN2 and SYN3 (Dong et al., 2001). SYN2 and SYN3 are distantly related to each other (18% amino acid sequence identity) and to SYN1 (16% and 19% aa sequence identity, respectively), with greater similarity near the N- and C-terminal regions than the central region. SYN2 and SYN3 are widely expressed, with elevated levels in tissues with dividing cells, such as root and shoot meristems, suggesting a role in mitosis. It is possible that SYN2/3 may also be involved in sister cohesion during meioses I and/or II, especially considering that SYN1 does not seem to be localized to the centromere of meiotic chromosomes.

Arabidopsis also has homologs for both *SMC1* and *SMC3*, *AtSMC1* and *AtSMC3*, respectively, which are expressed widely, consistent with a role in both mitosis and meiosis (Lam et al., 2005b). Both *AtSMC1* and

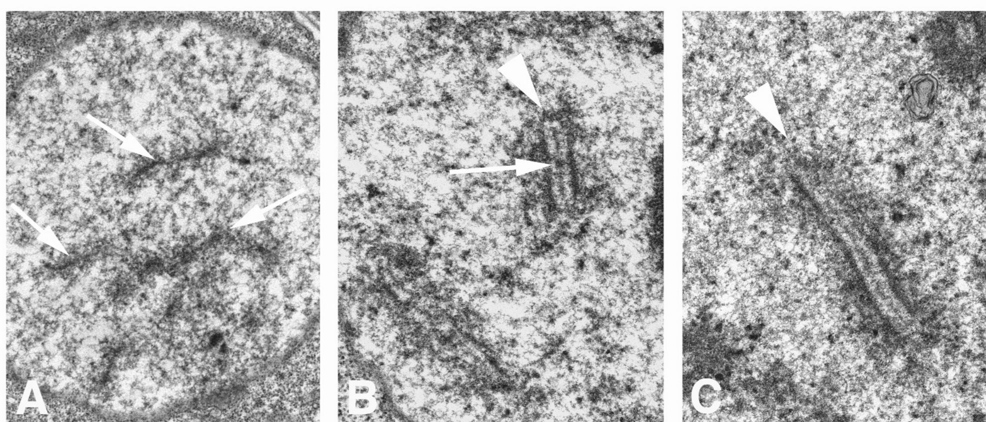

Figure 3. Transmission electron microscopy of Arabidopsis male meiotic prophase I. A. A portion of a leptotene nucleus showing axial elements (arrow). B. A portion of a zygotene nucleus shown a short SC (arrowhead) with a recombination nodule (arrow). C. A portion of a pachytene nucleus showing a long SC (arrowhead). Bar = 500 nm. These images were provided by L. Timofejeva and were previously published in Ma (2005).

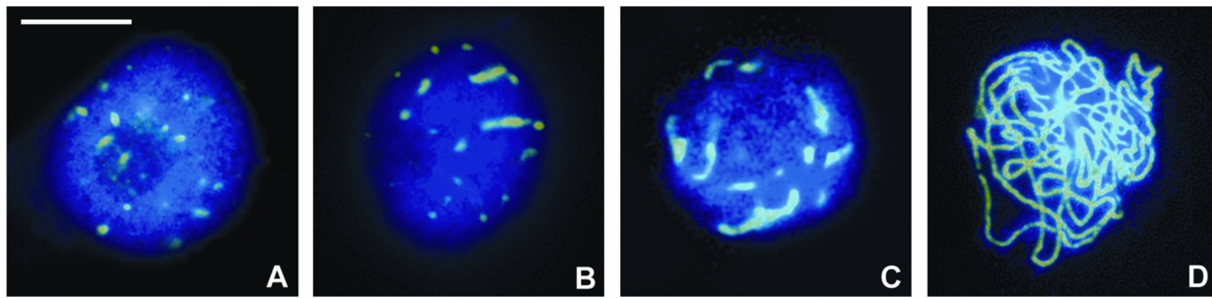

Figure 4. Immunolocalization of ZYP1 protein (bright light blue) in wild-type male meiocytes. A, leptotene, showing a small number of foci. B, early zygotene, with some expansion to larger chromosome regions. C, mid-zygotene, showing further expansion of ZYP1 regions. D, pachytene, with ZYP1 distribution to all chromosomes. DNA was stained with DAPI (blue). Bar = 10 μm. This figure was modified from Figure 2 of Higgins et al. (2005), with permission from Genes and Development.

AtSMC3 are expressed more highly in floral buds than other tissues, with *AtSMC1* at a higher level, and more non-specifically, than *AtSMC3*. Both AtSMC1 and AtSMC3 are about 140 kD in size and have predicted domains similar to other SMC1 and SMC3 proteins: an N-terminal ATP-binding domain, two long coiled-coil regions flanking a flexible hinge region, and a C-terminal DA domain (Hirano, 2002). Protein localization studies using cell fractionation and antibodies indicate that AtSMC3 is found in both the cytoplasm and the nucleus (Lam et al., 2005b). In particular, from prophase and anaphase, AtSMC3 is associated with chromosomes in both mitotic and meiotic cells, supporting the idea that AtSMC3 is involved in sister chromatid cohesion, as its homologs do in other organisms. This hypothesis is further supported by the observation that *syn1* mutant meiocytes exhibit abnormal AtSMC3 localization, indicating that SYN1 is required for normal chromosomal localization of AtSMC3 in meiocytes. Surprisingly, AtSMC3 is also associated with the spindle from metaphase to telophase in a SYN1-independent manner, suggesting a function previously unknown from studies in other systems (Lam et al., 2005b). Mutations in the *AtSMC1* and *AtSMC3* genes were previously isolated as *titan8* and *titan7* mutants, respectively; these mutants have enlarged embryos and arrested embryo and endosperm development (Liu et al., 2002a), indicating that these genes are critical for mitotic development.

Recently, the Arabidopsis homolog of *SCC3, AtSCC3,* has been characterized (Chelysheva et al., 2005). The AtSCC3 protein was detected in mitotic and meiotic cells; during meiosis, AtSCC3 was localized to the chromosome from leptotene to anaphase I. In addition, *atscc3-2*, a null mutation, causes embryonic lethality and a weak allele, *atscc3-1*, also results in defects during mitotic development, indicating that *AtSCC3* is important for the mitotic cell cycle. Furthermore, *AtSCC3* is required for maintenance of sister chromatid cohesion at centromeres at anaphase I, similar to *SYN1* (Cai et al., 2003; Chelysheva et al., 2005). In normal meiosis, the kinetochores of the sister chromatids are oriented in the same direction to allow attachment to microtubules from the same pole of the spindle during the reductional meiosis I. Analysis of the *atscc3* mutant meiocytes indicates that this monopolar orientation of the meiosis I kinetochores requires *AtSCC3* function.

In addition to cohesin subunits, the Arabidopsis *SWI1/DYAD* gene is also required for normal sister chromatid cohesion during male and female meiosis (Mercier et al., 2001; Agashe et al., 2002). In mutant male meiocytes, sister chromatid cohesion is lost, resulting in 20 separated chromatids at late prophase I to metaphase I, in contrast to the five bivalents at wild-type diakinesis. Detailed examination of the male meiotic chromosomes in the *swi1-2* mutant indicates that formation of the axial element is affected, suggesting that *SWI1* is also important for condensation (Mercier et al., 2003). Furthermore, the *swi1-2* mutation can suppress the chromosome fragmentation phenotype of *dif1-1* (allelic to *syn1*), suggesting that *SWI1* controls an early step in meiosis, as supported by the detection of the SWI1 protein in meiotic G1 and S phases. In the *swi1* mutant, the absence of sister chromatid cohesion does not result in chromosome breakage, unlike the *syn1* mutant chromosomes. In female meiosis of the *swi1* mutant, a mitosis-like division takes place, suggesting that the centromeres behave like mitotic centromeres, although it is not clear how this might have occurred.

The predicted SWI1 protein is a novel protein with 639 amino acids; Arabidopsis has another gene (At5G23610) with a predicted protein of 360 amino acids that shares 38% identity and 52% similarity with SWI1 over the N-terminal 205 aa regions of both proteins. In addition, there is a rice gene (accession number: AK064367) encoding a protein with 33% identity and 48% similarity to SWI1 over nearly the entire protein. Therefore, SWI1 might represent a conserved plant gene family. Although the SWI1 protein lacks clear homologs among proteins with known func-

tions, it has limited sequence similarity to predicted proteins in *Schizosaccharomyces pombe* and human.

Homolog Pairing/Juxtaposition and the Synaptonemal Complex

As mentioned earlier, homolog pairing/juxtaposition, synapsis, and recombination promote correct recognition and association of homologs, such that they can properly segregate subsequently. It is thought that telomeres play an important role in chromosome pairing (Dawe, 1998; Scherthan, 2001). In early prophase I of several organisms, including plants (such as maize) and yeasts, dispersed telomeres move and attach to the nuclear envelope, forming a configuration called bouquet with telomeres clustered on one face of the nucleus. Bouquet formation may be important for initiating homolog pairing (Dawe et al., 1994; Scherthan et al., 1996; Bass et al., 1997). In Arabidopsis, the telomeres seem to cluster during interphase to the nucleolus rather than on the nuclear envelope, then pair before synapsis (Armstrong et al., 2001). The paired telomeres then dissociate from the nucleolus and are dispersed during leptotene, suggesting that telomere clustering might also facilitate homolog pairing in Arabidopsis (Armstrong et al., 2001).

Following pairing and juxtaposition, homologs form the synaptonemal complex (SC) between the two aligned chromosomes. In light microscopy, juxtaposed chromosomes can be visualized as thick lines (Figure 1C), which represent synapsed homologs in normal Arabidopsis meiotic cells. The SC consists of three parallel filamentous elements (Figure 3): the two lateral elements and a central element. The lateral elements correspond to the two aligned homologs, with regions of the chromatin in complex with SC proteins and large loops of chromatin emanating away from the SC. The central element is seen in TEM as a thin filament in between the lateral elements; it is known to be composed of the overlapping heads of the transverse elements, which are very fine filaments that are perpendicular to and connecting the lateral elements.

Because pairing, juxtaposition and synapsis are highly coordinated processes, mutants defective in one often are also abnormal in another, and have generally been called synaptic mutants. These can be classified as either asynaptic mutants, which fail to establish synapsis, or desynaptic mutants, which cannot maintain homolog association after forming SC. At diakinesis, both asynaptic and desynaptic mutants have univalents rather than bivalents (Ross et al., 1997; Caryl et al., 2000; Cai and Makaroff, 2001; Armstrong et al., 2002; Bass et al., 2003; Schommer et al., 2003; Nonomura et al., 2004). In Arabidopsis, the *asy1* mutant is asynaptic, unable to form SC in both male and female meiocytes (Ross et al., 1997; Caryl et al., 2000). The ASY1 protein is localized to the axial/lateral elements, suggesting that it plays critical role in SC formation (Armstrong et al., 2002). The ASY1 protein share sequence similarity in its N-terminal domain with the yeast HOP1 protein (Hollingsworth et al., 1990; Caryl et al., 2000), which is known to be important in SC assembly and is localized on axial elements (Hollingsworth et al., 1990). In rice there is an *ASY1* homolog called *PAIR2*; a mutation in *PAIR2* causes defects in homolog alignment at pachytene and the formation of univalents instead of bivalents at diakinesis (Nonomura et al., 2004). Therefore, *ASY1, PAIR2,* and *HOP1* are functionally conserved genes in plants and yeast. Another Arabidopsis mutant, *ahp2*, is defective in pairing and bivalent formation (Schommer et al., 2003). The AHP2 protein is similar to the *S. cerevisiae* HOP2, *S. pombe* Meu13p, and human TBPIP (Tat-binding protein 1-interacting protein) proteins. Both HOP2 and Meu13p are important for homolog pairing during meiosis.

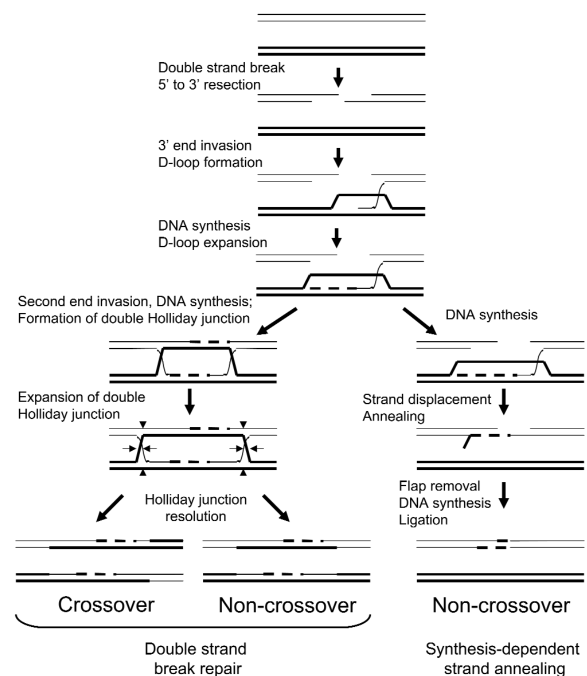

Figure 5. Models for homologous recombination. The double stranded break repair model (left) and the synthesis-dependent strand anneal model (right). Both start with a DSB, 5' to 3' resection to 3' single strands, the invasion of one end into the intact recombination partner to form a D-loop, and some DNA synthesis. In the traditional DSBR model, the second end invades, leading to the formation of the double Holliday junction, which expand and then can be resolved to either crossover or noncrossover. Recently studies in yeast indicate that double Holliday junctions usually resolve into crossovers. In addition, a number of results support the synthesis-dependent strand annealing model, which branch off following D-loop formation. This alternative model can generate noncrossovers, but not crossovers. This figure was adopted from Figure 1 of Maloisel et al. (2004).

In addition, *TBPIP* is expressed in the testis. Therefore, *AHP2* is a likely functional homolog of HOP2/Meu13p in Arabidopsis.

Although SC is structurally similar among eukaryotes under TEM, SC proteins from yeast and animals do not share any obvious sequence similarity. Nevertheless, analogous protein components do show similarity in secondary structures. For example, in yeast, the ZIP1 protein is a subunit of the transverse element; its counterparts in animals are SCP1 from mammals, SYP1 from *C. elegans*, and C(3)G from *Drosophila* (Zickler and Kleckner, 1999; Page and Hawley, 2004). They lack amino acid sequence similarities, but share secondary structural properties, each with globular heads and a long coiled-coil central region. The lack of strong sequence similarity has made it difficult to identify Arabidopsis genes encoding SC proteins. However, recently, Higgens et al. (Higgins et al., 2005) have used a combination of various bioinformatics tools to identify an adjacent pair of divergently transcribed genes, *ZYP1a* and *ZYP1b*. They encode highly similar (90% similarity) proteins with structural features of ZIP1 and other related proteins.

Immunolocalization experiments indicate that the ZYP1 proteins are located at 20-25 foci on leptotene chromosomes, and expand during zygotene to cover the entire length of the pachytene chromosomes, consistent with the hypothesis that ZYP1 is a SC component (Higgins et al., 2005) (Figure 4). The appearance of ZYP1 foci is later than the appearance of the ASY1 foci, which are found on the axial element at leptotene. At pachytene, ASY1 signals flank both sides of the ZYP1 signal, both along the synapsed homologs, suggesting that ZYP1 forms part of the central region of the SC. Single mutants in either *ZYP1a* or *ZYP1b* genes have reduced fertility, slight reduction in the number of chiasmata and a delay in prophase I. Because these two genes are adjacent, it was not possible to generate the double mutant. Localization analysis in the single mutants indicates that the distributions of the ZYP1a and ZYP1b proteins are indistinguishable from each other and from that in the wild-type. Several RNAi lines in single mutant backgrounds were obtained that had no detectable ZYP1 proteins; these lines produced meiocytes that usually did not have aligned chromosomes but occasionally did have fully aligned chromosomes, although these aligned chromosomes are not synapsed fully. The great increase of the number of cells with unaligned chromosomes suggested a severe delay of meiotic progression. This is further supported by an increase in the period between DNA replication and the completion of meiosis I from ~30 hours in normal meiocytes to ~50 hours in cells of the *ZYP1* RNAi lines.

The ZYP1 RNAi lines also display evidence of abnormality in recombination (Higgins et al., 2005). The affected meiocytes have a slightly reduced number of MLH1 foci (7.3), which likely mark crossover sites, in comparison with 10 found in the wild-type cells. In addition, the average number of chiasmata was estimated to between 7 and 8, rather the normal ~10. These results suggest that recombination is slightly affected. Nevertheless, the fact that MLH1 foci and chiasmata are present in substantial numbers indicates that recombination was able to progress to a relatively late stage. Strikingly, fluorescence in situ hybridization experiments indicate that nonhomologous chromosomes are closely associated, suggesting that pairing might have been affected in the ZYP1 RNAi lines. The results from the analysis of ZYP1 RNAi lines support the idea that pairing, synapsis and recombination are tightly coupled. Clearly, a severe reduction of the ZYP1 protein has a significant effect on pairing and recombination. It is possible that the RNAi lines still have some residual ZYP1 function, which may allow recombination at a level greater than possible if the ZYP1 function were completely absent.

Meiotic Recombination: Initial Events

In addition to pairing/juxtaposition and synapsis, homologous recombination occurs during prophase I between homologs. It is initiated around late leptotene to early zygotene and is completed at late pachytene. Using TEM, electron dense bodies called meiotic nodules can be found associated with the chromosome; they are also called recombination nodules because they are thought to be related to recombination (Carpenter, 1975, 1987; Sherman et al., 1992; Sherman and Stack, 1995; Anderson et al., 2001). Late recombination nodules are very well correlated with recombination crossovers (Anderson et al., 2004), which are observed cytologically as the chiasmata. The maintenance of chiasmata also requires sister chromatid cohesion.

A well-known model for recombination called the double-strand break repair (DSBR) model was proposed primarily on the basis on biochemical studies in yeast and animal cells (Szostak et al., 1983). According to this model (Figure 5, left), recombination is initiated by a double-strand break (DSB) in one of two participating chromosomes, usually a non-sister chromatid during meiosis; 5' resection of the DSB produces 3' single-stranded DNAs, one of which then forms a D loop with the intact partner chromatid. DNA synthesis ensues primed from the invading strand using the intact molecular as the template. The invasion of the second broken end and further DNA synthesis yield the double-Holliday junction, which can be

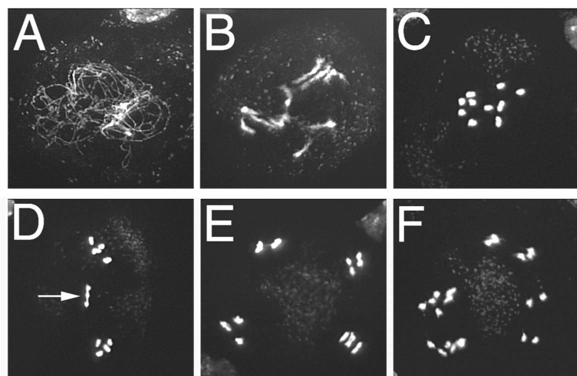

Figure 6. Male meiosis in the *atspo11-1* mutant. A, leptotene. B, early diakinesis, showing many univalents. C, prometaphase I, with 10 univalents. D, metaphase I, with one bivalent (arrow) and 8 univalents. E, metaphase II to anaphase II, showing abnormal distribution. F, anaphase II. This figure was modified from Figure 8 of Li et al. (2005).

resolved following cutting at alternative strands, effecting either crossovers or non-crossovers.

Recent studies have yielded evidence supporting the idea that the decision for crossover/non-crossover is made very early during recombination, prior to the formation of the double-Holliday junction, prompting a revision of the DSBR model (Bishop and Zickler, 2004; Borner et al., 2004; Haber et al., 2004). This revised model (Figure 5, right) posits that the recombination pathway branches following the formation of the D loop. One branch leads to the formation of the double-Holliday junction, which then progresses to form a crossover, but usually not a non-crossover. In the second branch of the pathway, the invading single strand, with its newly synthesized DNA, dissociates from the homolog and re-anneals with the other broken end; DNA synthesis and ligation then restore the chromosome, resulting in a non-crossover.

Many genes important for meiotic recombination have been identified by studies in the budding yeast *Sacchromyces cerevisiae*, as well as in the fission yeast *Schizosaccharomyces pombe*, *C. elegans, Drosophila,* and others (Zickler and Kleckner, 1999). The relatively ease with which to perform reverse genetics and the available sequence information have allowed the testing of the function of Arabidopsis homologs of these known meiotic genes. Because of the intimate relationship between recombination, pairing and synapsis, studies of genes important for meiotic recombination have also yielded insights into their roles in pairing and synapsis. In budding yeast, DSBs are generated by SPO11 (Keeney, 2001; Lichten, 2001), which was discovered a number of year ago as a mutant defective in sporulation (Esposito and Esposito, 1969), the formation of haploid spores from a diploid cell through meiosis. SPO11 encodes a member of a novel family of type II topoisomerase and has a transesterase activity that creates DSBs along with several other proteins (Bergerat et al., 1997; Keeney, 2001). SPO11 homologs have been identified in many organisms including *S. pombe, Drosophila, C. elegans*, mammals, the mushroom *Coprinus cinereus*, and Arabidopsis (Dernburg et al., 1998; McKim et al., 1998; Keeney et al., 1999; Romanienko and Camerini-Otero, 1999; Celerin et al., 2000; Grelon et al., 2001), suggesting a highly conserved function in eukaryotes.

Genetic studies using mutations in the *SPO11* gene in various organisms have revealed differences in the relationship between recombination initiation in the form of DSBs and synapsis. In both budding and fission yeasts, as well as mouse, *spo11* mutants are defective in SC formation, indicating a requirement of DSBs for homolog synapsis (Cervantes et al., 2000; Romanienko and Camerini-Otero, 2000; Zenvirth and Simchen, 2000). In contrast, SPO11-dependent DSBs are not required for homolog synapsis in *C. elegans* and *Drosophila* (Dernburg et al., 1998; McKim et al., 1998). In Arabidopsis, there are three SPO11 homologs (Hartung and Puchta, 2000, 2001). A mutant in one of these *SPO11* homologs, *AtSPO11-1*, was shown to be defective in bivalent formation in prophase I (Figure 6) and has a sharply reduced recombination rate between homologous chromosomes, indicating that *AtSPO11-1* is important for recombination (Grelon et al., 2001). The *atspo11-1* mutant phenotypes also suggest that AtSPO11-1 is required for homolog pairing, juxtaposition, and/or synapsis. Therefore, meiotic recombination and its relationship to pairing and synapsis may be more similar between yeast, mammals and plants, then between these organisms and *C. elegans* or *Drosophila* (see below for more discussion). The other two SPO11 homologs, *AtSPO11-2* and *AtSPO11-3*, are not known to be involved in meiosis.

After the formation of SPO11-dependent DBSs in yeast, then a complex of RAD50, MRE11, and NBS1 (XRS2) proteins carries out the resection step to generate single-stranded ends (Connelly and Leach, 2002). RAD50 and MRE11 are similar in sequence to the bacterial sbcC and sbcD subunits of a nuclease complex; in particular, the MRE11 and sbcD proteins contain conserved phosphoesterase motifs (Sharples and Leach, 1995). The Arabidopsis homologs of *RAD50* and *MRE11* are known, but a NBS1 homolog has not yet been identified in Arabidopsis (Hartung and Puchta, 1999; Gallego et al., 2001). An *atrad50* mutant is completely sterile and is hypersensitive to DNA-damaging radiation and chemicals (Gallego et al., 2001). Further studies indicate *atrad50* meiotic cells have numerous chromosome fragments in prophase I, suggesting that the repair of DSBs is defective (Bleuyard et al., 2004b). This is consistent with a role for *AtRAD50* just downstream of *AtSPO11-1*. The predicted Arabidopsis MRE11 protein has 720 amino acids and

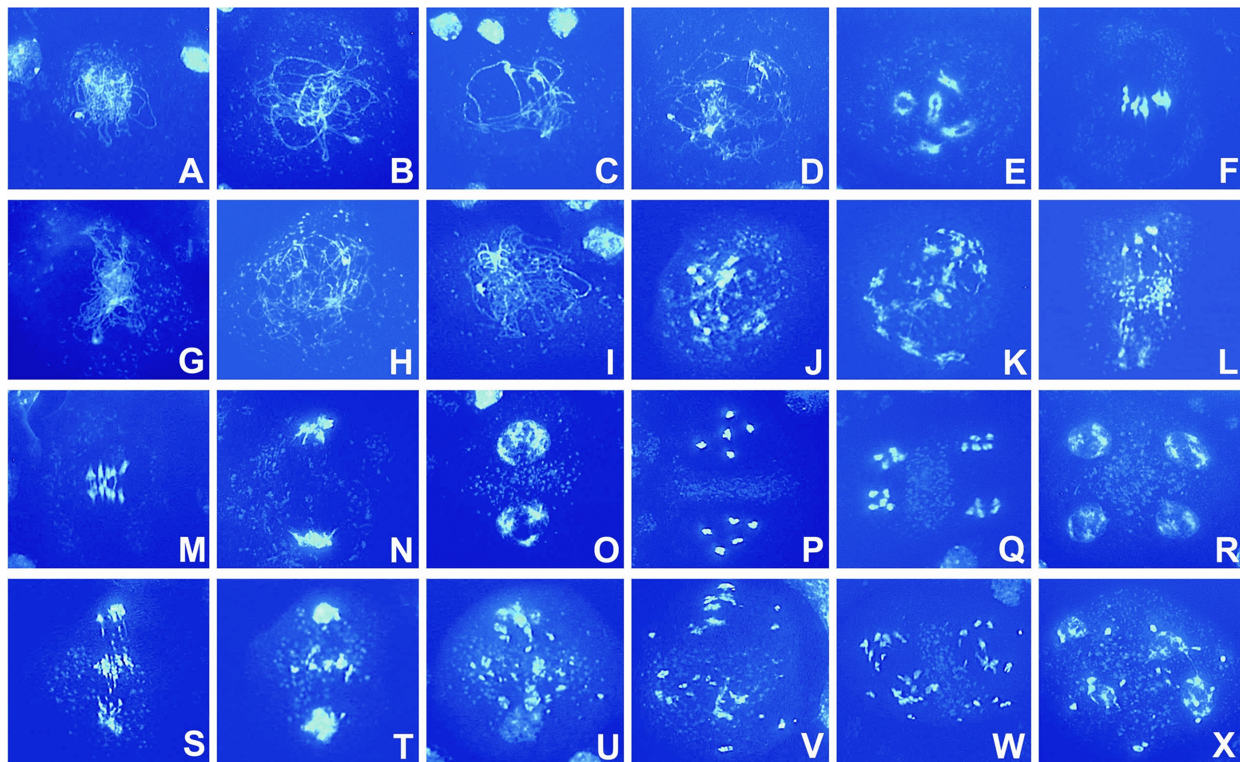

Figure 7. Male meiosis in wild-type (A-F, M-R) and *atrad51-1* (G-L, S-X) plants. A and G, leptotene. B and H, zygotene. C and I, pachytene. D and J, diplotene. E and K, diakinesis. F and L, metaphase I. M and S, anaphase I. N and T, telophase I. O and U, prophase II. P and V, metaphase II. O and W, anaphase II. R and X, telophase II. Chromosome fragmentation is evident starting at diakinesis and through telophase II. In addition, pairing and juxtaposition is apparently defective at zygotene and pachytene. This figure was modified from Figure 4 of Li et al. (2004).

shares strong sequence similarity with yeast and human MRE11 proteins in the N-terminal two thirds, which contains four phosphoesterase motifs (Hartung and Puchta, 1999; Bundock and Hooykaas, 2002). A T-DNA insertional *mre11* mutant was found to have small and abnormally shaped leaves and enlarged shoot apical meristem (Puizina et al., 2004). In addition, the Arabidopsis *mre11* mutant is sterile, has meiotic chromosome fragmentation, is defective in homolog pairing and bivalent formation, and exhibits abnormal chromosome segregation (Puizina et al., 2004). The chromosome fragmentation phenotype is due to a failure to process SPO11-induced DBSs because *spo11-1 mre11* double mutant meiocytes do not show chromosome fragmentation. Therefore, similar to *RAD50* and *MRE11* in yeast, the Arabidopsis *AtRAD50* and *MRE11* genes are required for normal meiotic recombination. In addition to its meiotic function, *AtRAD50* and *MRE11* are also needed for normal mitotic growth. In yeast, RAD50 and MRE11 are also important for repair of DSBs by non-homologous end joining (Haber, 1998); therefore, at least some of the somatic phenotypes in the *Arabidopsis atrad50* and *mre11* mutants could be due defects in this pathway, rather than that in the homologous recombination pathway.

Meiotic Recombination: The *RAD51* Gene Family and DSB Repair

Another yeast gene important for meiotic recombination is *RAD51*, which encodes a eukaryotic homolog of the *E. coli* RecA recombinase critical for DSB repair in homologous recombination (Smith et al., 1987; Smith and Wang, 1989; Cox, 1999). RecA is a 38-KDa protein that has activities in single-stranded DNA (ssDNA) binding, ATP binding, and ATP hydrolysis (McEntee et al., 1980; Weinstock et al., 1981). The yeast RAD51 and *E. coli* RecA proteins are 30% identical (54% conserved) over a ~210-amino acid region. In addition, the meiosis-specific yeast protein DMC1 is another RecA homolog and is highly similar to RAD51 (Bishop et al., 1992). The *RAD51* gene is required for both meiotic and mitotic recombination, whereas *DMC1* is specifically required for meiotic progression, synaptone-

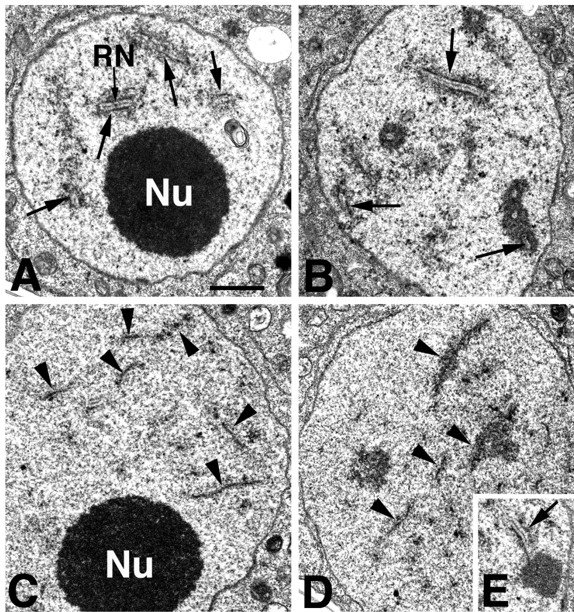

Figure 8. Transmission electron micrographs of male meiocyte nuclei in wild type and *atrad51*. A and B, wild type nuclei with SC (arrows) at zygotene (A) and pachytene (B). C and D, *atrad51-1* nuclei, with axial elements (arrowheads), which are unpaired in nuclei at zygotene (C) and pachytene (D) stages. E, an occasional fragmentary SC in the *atrad51-1* mutant. Nu: nucleolus. Bar = 1 μm. This figure is Figure 5 of Li et al. (2004).

mal complex formation, and homologous recombination (Bishop et al., 1992; Shinohara et al., 1992). In *C. elegans* and *Drosophila*, the RAD51 homologs are required for normal meiosis (Rinaldo et al., 1998; Staeva-Vieira et al., 2003). It is worth noting that *Drosophila* and *C. elegans* do not seem to possess a *DMC1* gene. In mouse, *RAD51* has been shown to interact with *DMC1* (Masson et al., 1999; Tarsounas et al., 1999), but its function in meiosis has not been shown genetically because the *rad51* mutant is embryonic lethal. The mouse *dmc1* mutant is defective in meiosis (Habu et al., 1996; Pittman et al., 1998; Yoshida et al., 1998).

Homologs of *RAD51/DMC1* have been identified in several plants, including Arabidopsis and maize (Sato et al., 1995; Anderson et al., 1997; Klimyuk and Jones, 1997; Doutriaux et al., 1998; Franklin et al., 1999; Ding et al., 2001; Shimazu et al., 2001). Protein localization studies in maize and Arabidopsis using antibodies indicate that the RAD51 protein is located on the chromosome at a large number of foci at the zygotene stage (Franklin et al., 1999; Mercier et al., 2003). The number of early RAD51 foci is in the hundreds, easily allowing more than a few sites per pair of homologs. In addition, it was observed that pairs of RAD51 sites are associated with pairing homologs and that meiocytes of maize pairing defective mutants have few or no RAD51 foci (Franklin et al., 1999; Franklin et al., 2003; Pawlowski et al., 2003). These results led to the proposal that RAD51 is important for homology search during pairing (Franklin et al., 1999; Franklin et al., 2003; Pawlowski et al., 2003).

The hypothesis that *RAD51* is important for pairing/juxtaposition is supported by analysis of a T-DNA insertional mutant in the Arabidopsis *RAD51* ortholog (*AtRAD51*) (Li et al., 2004). The *atrad51* mutant is male and female sterile; using both light microscopy of DAPI-stain chromosome spreads and TEM, it was found that mutant meiocytes are defective in homolog juxtaposition, lacking the typical "thin threads" seen at pachytene (Figures 7). Analysis using TEM revealed that mutant meiocytes were able to form axial elements, but they did not progress to form SC (Figure 8). Moreover, consistent with a predicted role in D-loop formation according to the DSBR model of recombination, *atrad51* mutant meiotic cells have multiple chromosomal fragments detectable beginning at diakinesis and continuing through anaphase II, suggesting a severe defect in repair of SPO11-induced DBSs. Indeed, meiocytes of the *atspo11-1 atrad51* double mutant lack chromosome fragments, indicating the fragmentation in the *atrad51* mutant requires the *SPO11-1* function (Li et al.,

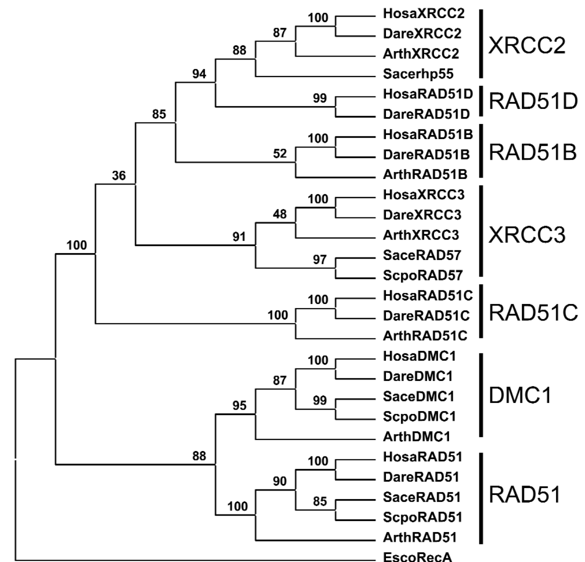

Figure 9. A Neighbor-Joining phylogenetic tree of the *RecA/RAD51* gene family. Species designation: Arth: *Arabidopsis thaliana*; Dare: *Danio rario*; Esco: *Escherichia coli*; Hosa: *Homo sapien*; Sace: *Saccharomyces cerevisiae*; Scpo: *Schizosaccharomyces pombe*. Eukaryotic recA/RAD51 gene family members form 7 clades; Arabidopsis genes are present in 6 of these clades, indicating an orthologous relationship between each Arabidopsis gene and animal genes. Bootstrap values are indicated near the stem of each clade. The tree in this figure was generated by Z. Lin.

2004). Therefore, *AtRAD51* participates in the *SPO11-1* dependent meiotic recombination, and the *RAD51* meiotic function is similar between yeast and plants.

Protein localization studies also indicate that at the pachytene stage, the number of RAD51 foci is greatly reduced (Franklin et al., 1999; Mercier et al., 2003), consistent with the number of crossovers per cell (Copenhaver et al., 1997). This suggests that RAD51 is also needed for the branch of the recombination pathway that led to crossover formation. In addition, studies of a T-DNA knockout mutant of *AtDMC1* indicate that the mutant meiocytes have largely univalents instead of bivalents at late prophase I (Couteau et al., 1999). Because crossovers are needed for maintaining bivalents at diakinesis, the *atdmc1* phenotypes indicate that AtDMC1 is crucial for Arabidopsis meiotic crossover formation. Indeed, the recombination frequency is greatly reduced in the *atdmc1* mutant (Couteau et al., 1999). Although both AtRAD51 and AtDMC1 are RecA homologs and are required for normal meiosis, particularly meiotic recombination, the meiotic phenotypes of the corresponding mutants are very different. Specifically, *atdmc1* mutant meiocytes do not have detectable chromosome fragmentation. It is possible that *atdmc1* is primarily functioning to promote crossover formation, and is not required for the non-crossover branch of homologous recombination.

DSBs in chromosomes can occur due to radiation and chemical mutagens, even during DNA replication in a normal cell cycle. These DSBs must be repaired to maintain chromosome stability and cell viability because a single chromosomal break can cause cell death. One of the major pathways of DSB repair is homologous recombination (Masson and West, 2001). As mentioned above, the yeast RAD51 is important for the repair of DSBs caused by DNA-damaging agents, but is not required for mitotic cell viability (Shinohara et al., 1992; Game, 1993). Similarly, in *C. elegans* and *Drosophila*, the *RAD51* gene is not essential for mitotic cell viability, unless the animals are exposed to DNA-damaging agents (Rinaldo et al., 2002; Staeva-Vieira et al., 2003). On the other hand, the mouse *RAD51* gene is essential during embryogenesis (Lim and Hasty, 1996; Tsuzuki et al., 1996). Consistent with this, *RAD51* is important for DNA repair and chromosome stability during the mitotic cell cycle in mammalian and chicken cell cultures (Li and Maizels, 1997; Sonoda et al., 1998; Kim et al., 2001; Lambert and Lopez, 2002; Lundin et al., 2003). Reduction or loss of *RAD51* function in these cells cause hypersensitivity to ionizing radiation and methyl methanesulfonate (MMS) and a deficiency in DSB repair and cell death (Taki et al., 1996; Sonoda et al., 1998; Collis et al., 2001). In Arabidopsis, the *atrad51* mutant plants appear healthy, identical to the wild type, when grown under normal conditions (Li et al., 2004). The size and number of *atrad51* leaves and floral organs all seem normal. In addition, mitosis in the *atrad51* mutant appears to proceed normally, unlike the mammalian *rad51* knockout, which is embryo lethal. Therefore, the Arabidopsis *AtRAD51* gene is not critical for DNA repair during vegetative and floral development.

In addition to *RAD51* and *DMC1*, yeast also has two other distant homologs of *recA*: *RAD55* and *RAD57,* which were identified as mutants hypersensitive to radiation (Kans and Mortimer, 1991; Lovett, 1994). Several lines of evidence support the hypothesis that RAD57 interacts with RAD51 to promote recombination. It was found that *rad57* mutants (Kans and Mortimer, 1991; Game, 1993) can be partially rescued by the overexpression of *RAD51* (Hays et al., 1995). In addition, RAD57 promotes RAD51-mediated homologous pairing and strand exchange in vitro (Sung, 1997). Amino acid substitutions in RAD51 that increase its DNA-binding affinity can suppress *rad57* mutations (Fortin and Symington, 2002). These observations are consistent with the idea that RAD57 facilitates the binding RAD51 to DNA. In *S. pombe*, hypersensitivity to DNA damaging agent MMS and gamma-rays were used to identify mutations in the *RHP57* gene, which is the putative ortholog of the *S. cerevisiae RAD57* gene (Tsutsui et al., 2000).

Vertebrate animals also possess five other *RAD51* paralogs: *RAD51B, RAD51C, RAD51D, XRCC2,* and *XRCC3* (Albala et al., 1997; Cartwright et al., 1998; Dosanjh et al., 1998; Liu et al., 1998b; Pittman et al., 1998; Braybrooke et al., 2000; Havre et al., 2000) (Figure 9). *RAD51B, RAD51C*, and *RAD51D* were isolated as cDNAs with similarity to *RAD51*, and *XRCC2* and *XRCC3* genes were identified by their ability to counter the sensitivity of hamster mutant cell lines, irs1 and irs1SF, to DNA-damaging radiation and chemicals (Liu et al., 1998b). Phylogenetic analysis suggests that *XRCC3* is a putative ortholog of the budding yeast *RAD57* and fission yeast *RHP57* (Tsutsui et al., 2000). Similar to RAD57, the XRCC3 protein can also associate with RAD51 in a yeast two-hybrid assay (Schild et al., 2000). The human XRCC3 is required for the assembly of RAD51 complexes in vitro (Bishop et al., 1998) and for homology-directed repair of DNA DSBs in cell lines (Pierce et al., 1999; Brenneman et al., 2000). In addition, biochemical studies have detected two protein complexes involving these proteins: one containing RAD51B, RAD51C, RAD51D, and XRCC2 and called the BCDX2 complex; the other containing XRCC3 and RAD51C (Masson et al., 2001; Liu et al., 2002b). The BCDX2 complex binds to nicks in duplex DNA, and may play a role in the recognition of nicks during repair of DSBs (Masson et al., 2001). In the XRCC3-RAD51C complex, RAD51C facilitates homolog pairing, and XRCC3 contributes to preferential binding to ssDNAs (Kurumizaka et al., 2001).

Disruption of one of mouse *RAD51, RAD51B, RAD51D,* or *XRCC2* genes causes early embryo lethality (Lim and Hasty, 1996; Tsuzuki et al., 1996; Shu et al., 1999; Deans

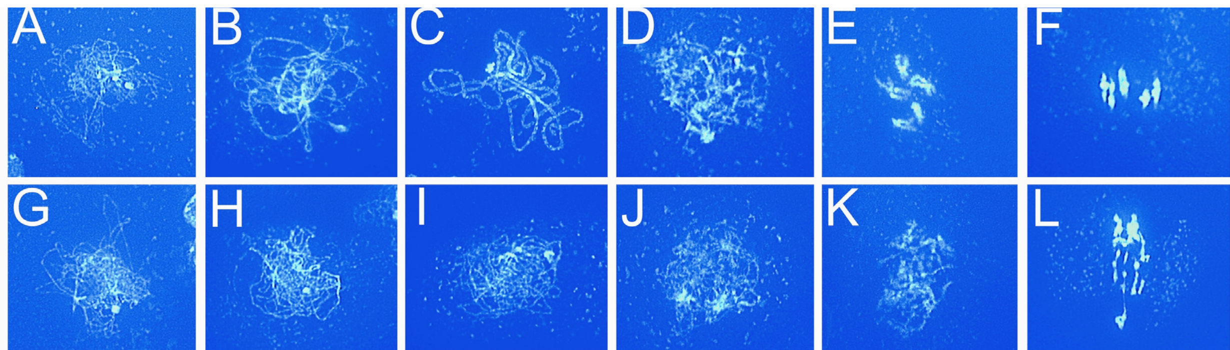

Figure 10. Male meiosis in wild-type (A-F) and *atrad51c-1* (G-L) plants. A and G, leptotene. B and H, zygotene. C and I, pachytene. D and J, diplotene. E and K, diakinesis. F and L, metaphase I. In *atrad51c-1*, multiple chromosome fragments were detected at diakinesis and metaphase I. This figure is modified from Figure 4 of Li et al. (2005).

et al., 2000; Pittman and Schimenti, 2000), indicating non-redundant essential roles in embryogenesis, consistent with their having important functions in DSB repair in the mitotic cell cycle. Furthermore, the early lethality of *rad51* and *rad51b* mutant embryos can be delayed due to partial suppression by a mutation in *p53*, a checkpoint control gene, suggesting that the loss of the *p53* function can allow cell cycle progression even with a defect in DNA repair. However, the *rad51 p53* double mutant embryos eventually also die (Lim and Hasty, 1996; Shu et al., 1999). Therefore, the *RAD51*-dependent function is linked to mitotic DNA damage checkpoint control. Indeed, *RAD51* has been found to interact genetically and physically with tumor suppressor genes *p53, ATM, BRCA1, BRCA2,* and others (Scully et al., 1997; Sharan et al., 1997; Wong et al., 1997; Marmorstein et al., 1998; Chen et al., 1999), suggesting that it is involved in the normal cellular process that maintains chromosome integrity and stability.

In Arabidopsis, molecular cloning and genomic sequence analysis indicate that there are at least six Arabidopsis *RAD51* homologs. In addition to *AtRAD51* and *AtDMC1*, Arabidopsis also has putative orthologs of

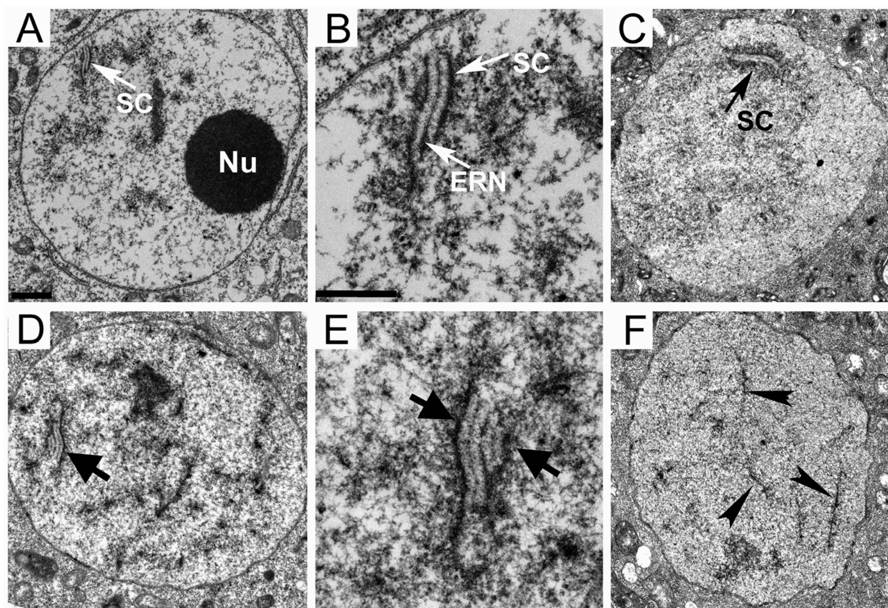

Figure 11. Male meiotic prophase I images from TEM. A-C, wild-type; D-F, *atrad51c-1*. A and B, zygotene; C, pachytene. Synaptonemal complexes (SC, arrows) can be seen in these images. In *atrad51c-1*, at zygotene (D) or pachytene (F), the majority of the axial elements (arrowheads) remained unpaired; sometimes, an abnormal SC (thick arrows) was also observed (D and E). Nu: nucleolus; ERN: early recombination nodule. Bar in A equals to 100 nm (same scale for C, D, and F); bar in B equals to 500 nm (same scale for E). This figure is Figure 7 of Li et al. (2005).

RAD51B, RAD51C, XRCC2, and *XRCC3: AtRAD51B, AtRAD51C, AtXRCC2,* and *AtXRCC3*, respectively (Doutriaux et al., 1998; Couteau et al., 1999; Osakabe et al., 2002; Bleuyard and White, 2004; Li et al., 2004; Bleuyard et al., 2005; Li et al., 2005), as supported by phylogenetic analysis (Figure 9). *AtRAD51C* is expressed at low levels in somatic cells (Li et al., 2005). *RAD51C* is also expressed in meiocytes, but at a lower level than that of *RAD51* (Li et al., 2004; Li et al., 2005). Similar to the *atrad51* mutant, an *atrad51c* T-DNA insertional mutant is also completely sterile and defective in meiosis, with fragmented chromosomes (Abe et al., 2005; Bleuyard et al., 2005; Li et al., 2005) (Figure 10). Moreover, the *rad51c* male and female meiocytes display pairing/juxtaposition defects, as indicated by fluorescence in situ hybridization experiments (Li et al., 2005). Furthermore, TEM experiments indicate that *atrad51c* meiocytes have abnormal partial SCs, sometimes with more than two chromosomes, suggesting that non-homologous chromosomes might have partially synapsed (Figure 11). At late pachytene, no SC was detected; therefore, the partial SCs at earlier stages could not progress to complete SCs. Therefore, *RAD51C* also is required for normal homolog juxtaposition, synapsis, and recombination, following the formation of DSBs during prophase I. Similar to the *atrad51 atspo11-1* double mutant, the *atrad51c atspo11-1* double mutant also lacks chromosome fragments, indicating that AtRAD51C also acts downstream of AtSPO11-1 to repair DSBs. In addition to the meiotic defectives, the *atrad51c* mutant is hypersensitive to gamma radiation and a DNA crosslinking agent and is reduced in somatic recombination frequency (Abe et al., 2005; Bleuyard et al., 2005).

The function of the Arabidopsis *XRCC3* homolog, *AtXRCC3*, was revealed by the analysis of a T-DNA insertional mutant, which showed sterility and fragmented meiotic chromosomes (Bleuyard and White, 2004), similar to the phenotypes of the *atrad51* mutant. In addition, the appearance of chromosome fragments depends on the function of *AtSPO11-1*, indicating that the fragmentation is due to failure to repair SPO11-induced DSBs (Bleuyard et al., 2004a; Bleuyard and White, 2004). When grown under normal conditions, the *atxrcc3* mutant can develop normally; in contrast, mutant cells in culture are sensitive to DNA cross-linking agents but not to DSB-inducing chemicals. Therefore, *AtXRCC3* is not required for normal mitotic development, unlike the mammalian *XRCC3* gene. The role of *AtXRCC3* in DNA repair also seems to be different from that of the *XRCC3* gene, which is involved in DSB repair. Insertions in the *AtRAD51B* and *AtXRCC2* genes have no visible developmental defects, but cause DNA repair defects in somatic cells (Abe et al., 2005; Bleuyard et al., 2005; Osakabe et al., 2005). Therefore, AtRAD51, AtRAD51C, and AtXRCC3 are required for normal processing of DSBs induced by AtSPO11-1 during meiosis; in addition, AtRAD51B, AtRAD51C, AtXRCC2 and AtXRCC3 are involved in repair of damaged DNA during the mitotic cell cycle.

Mutant phenotypes from DAPI analysis suggest that *atrad51c* and *atxrcc3* meiocytes might have fewer chromosome fragments than that in the *atrad51* mutant, although the number of fragments is difficult to quantify and the possible meiotic phenotypic difference between these three mutants needs to be further investigated. If *atrad51c* and *atxrcc3* meiocytes do have weaker phenotypes than does *atrad51*, there might be a functional difference between these genes. EM results also support the idea that these genes are not functionally identical, *atrad51* cells had almost no SC (Figure 8), except very occasional abnormal ones (Li et al., 2004), additional to axial elements, whereas *atrad51c* meiocytes had occasional polycomplexes (Figure 11), and *xrcc3* cells had abnormal SC, at a frequency greater than those in the *atrad51* cells (L. Timofejeva, W. Li, and H. Ma, unpublished data). The fact that each of the three single mutants has such severe meiotic defects indicates that these genes are not genetically redundant. In addition, the fact that separate *RAD51, RAD51C,* and *XRCC3* evolutionary lineages have been maintained and conserved in both animals and plants supports the hypothesis that *RAD51, RAD51C*, and *XRCC3* have distinct essential functions (Figure 9).

Immunolocalization studies indicate that there are numerous (more than 200) AtRAD51 foci in early Arabidopsis prophase I cells (leptotene to zygotene) and this number is reduced to fewer than 20 at pachytene (Mercier et al., 2003). From DAPI-stained images, the numbers of chromosome fragments in the *atrad51, atrad51c,* and *atxrcc3* seem to be far fewer than the number of early AtRAD51 foci (Bleuyard and White, 2004; Li et al., 2004; Abe et al., 2005; Bleuyard et al., 2005; Li et al., 2005). It is possible that each of these proteins is present at a subset of the DSBs. This is consistent with the observation that RAD51 labeled 44% of early meiotic nodules and 79% of late nodules in lily (Anderson et al., 1997). It is possible that AtRAD51, AtRAD51C, and AtXRCC3 are localized to non-identical but possibly overlapping subsets of DSBs, so that some AtRAD51 foci also have AtRAD51C and/or AtXRCC3 proteins. It is also possible that AtRAD51C and AtXRCC3 are localized to fewer foci than AtRAD51.

RAD51 and *XRCC3* are essential for mitotic growth in mammals and birds, but their putative orthologs are not required for cellular viability in yeast, *Drosophila*, or Arabidopsis. Clearly, *AtRAD51, AtRAD51C,* and *AtXRCC3* are each required for meiosis and fertility, indicating that they cannot replace each other during meiosis. As mentioned above, *AtRAD51C* and *AtXRCC3* are also important for DNA repair when the plant is exposed to DNA-damaging chemicals, as are *AtRAD51B* and *AtXRCC2*. Therefore, even though these genes are members of the same fami-

ly, they are not redundant genetically. It is possible that these genes are needed when the numbers of DSBs are large, as in meiosis or when caused by mutagens. The fact these genes are highly conserved suggests that they are also important for meiosis in animals, even though the embryo lethality has thus far prevented a direct test of this hypothesis. The fact that *atrad51, atrad51c*, and *atrxcc3* mutants all appear normal during mitotic development under standard growth conditions suggests two different possibilities. First, DSB repair is not important for normal mitotic cell cycle, although this is not likely because Arabidopsis *rad50* and *mre11* mutants have vegetative phenotypes. Second, DSBs might be repaired by other pathways, such as those involving other *RAD51* paralogs, or the non-homologous end joining pathways.

In vitro studies of mammalian RAD51 indicate that it binds to ssDNA and catalyzes the formation of a D loop by promoting the invasion of the ssDNA into a double stranded DNA (Petukhova et al., 2000). In addition, mammalian XRCC3 and RAD51C form a complex that promotes the loading of RAD51 onto DNA (Masson et al., 2001; Sigurdsson et al., 2001; Wiese et al., 2002; Lio et al., 2003). Also, *xrcc3* and *rad51c* mutant animal cell lines have reduced number of RAD51 foci; furthermore, RAD51C, but not RAD51, is needed for the formation of XRCC3 foci on chromosome (Yoshioka et al., 2003; Lio et al., 2004). Because the Arabidopsis AtRAD51, AtRAD51C, and AtXRCC3 proteins are orthologous to their mammalian counterparts, the Arabidopsis proteins might have similar biochemical properties, suggesting the following model for how these genes function in meiosis. During meiosis, AtRAD51C and AtXRCC3 form a complex that promotes the formation of AtRAD51 foci. It is possible that formation of AtRAD51 foci does not absolutely require AtRAD51C or AtXRCC3; when AtRAD51C or AtXRCC3 are eliminated by mutations, the number of AtRAD51 foci is reduced. This model can explain the residual homolog interaction in the *atrad51c-1* mutant. In addition, the observation of a smaller number of chromosome fragments in *atrad51c-1* meiocytes than in *atrad51-1* meiocytes supports the idea that some DSBs are repaired in *atrad51c-1* cells.

Besides the RAD51 homologs, the BRCA1, BRCA2 and ATM tumor suppressor proteins have been linked with DSB repair in animals and interact with each other (Khanna and Jackson, 2001; Thompson and Schild, 2002; Henning and Sturzbecher, 2003). *ATM* is the gene that is defective in the human disease called ataxia telangiectasia (AT) and encodes a very large protein of over 3000 amino acids with a C-terminal protein kinase domain that is similar to phosphatidyl inositol-3 kinase (Savitsky et al., 1995; Rotman and Shiloh, 1998). Mutations in *ATM* cause meiotic defects associated with the inability to repair DSBs (Barlow et al., 1998; Ashley et al., 2004). Mutations in the *BRCA1* and *BRCA2* genes causes risk in breast cancer and are also associated with other cancers (Khanna and Jackson, 2001). ATM has been shown to phosphorylate BRCA1, which interacts with BRCA2; BRCA2 can interact with RAD51, which is regulated via phosphorylation by c-Abl, another target of ATM (Khanna and Jackson, 2001). Mice deficient in *BRCA1* usually die during embryo development, but a small number of *brca1 p53* double homozygous mice survive; these mice exhibit multiple abnormalities including growth retardation, DNA repair defects and failure to complete meiosis (Cressman et al., 1999). The *brca2* knockout mutation causes embryonic lethality in mouse and mutant cells have chromosome breaks and rearrangement (Yu et al., 2000). In humans, the BRCA2 protein is localized to meiotic chromosomes, suggesting that it plays a role in meiosis (Chen et al., 1998), although this has not been demonstrated genetically in part because of the embryo lethality of the mouse mutant.

The Arabidopsis *ATM* homolog, *AtATM*, is widely expressed and encodes a protein of 3856 amino acids (Garcia et al., 2000). The predicted AtATM protein has a 350-aa C-terminal phosphatidyl inositol-3 kinase like domain (58% identity and 67% similarity to the same domain in ATM), as well as a RAD3 domain just upstream of the C-terminal domain, similar to other ATM homologs. Two T-DNA insertional mutations in *AtATM* have been identified and found to cause reduced fertility (Garcia et al., 2003). Meiosis is abnormal in *atatm* mutants, with chromosome fragmentation and chromosome bridges, although pairing and synapsis seem to be normal. In addition, *atatm* mutants are partially fertile, but gametophytes often arrest in development. Therefore, *AtATM* promotes normal meiosis, possibly facilitate DNA repair, but it is not essential for meiosis, as supported by the normal meiotic recombination frequency in the mutant (Garcia et al., 2003). The *atatm* mutants are hypersensitive to gamma radiation and unable to induce elevated expression of DNA repair genes, such as *AtRAD51*, in response to DNA damage (Garcia et al., 2003), indicating it plays an important role in regulating DNA damage response.

A true BRCA1 homolog has not yet been found in Arabidopsis; nevertheless, the Arabidopsis MEI1 protein bears some resemblance to BRCA1. The Arabidopsis *MEI1* gene was discovered originally for its mutant meiotic defects and has been recently shown to encode a protein with five BRCT motifs (He and Mascarenhas, 1998; Yang and McCormick, 2002; Grelon et al., 2003). BRCT motifs are found in BRCA1 and other proteins involved in DNA repair (Koonin et al., 1996; Grelon et al., 2003); the presence of BRCT motifs in MEI1 suggests that it may have related protein activities. Detailed analysis of *mei1* mutants revealed that mutant meiocytes exhibit chromosome breakage during meiosis I (Grelon et al., 2003). Unlike the *atrad51, atrad51c*, and *atxrcc3* cells, *mei1* mutant meiocytes exhibit variable phenotypes in terms of

chromosome fragmentation (Grelon et al., 2003). In about 75% of *mei1* male meiocytes, chromosome breakage is severe, results in multiple fragmentation, whereas in about 20% of the *mei1* cells, fragmentation is infrequent yet detectable. In about 5% of the cells, the chromosomal morphology appeared normal (Grelon et al., 2003). In the *mei1 spo11-1* double mutant, most meiocytes have a phenotype similar to the severe class of *mei1* single mutant cells, indicating that *spo11-1* could not rescue the *mei1* mutant phenotype and the DNA breaks in the *mei1* mutant are not SPO11-1 dependent. A small number of the double mutant cells had a phenotype similar to *spo11-1* single mutant, suggesting that these cells did not have DNA breaks, similar to the small number of *mei1* cells with normal chromosomes. The *mei1* somatic cells were indistinguishable from wild-type cells in terms of sensitivity to gamma and UV-C radiations (Grelon et al., 2003).

There are two highly similar Arabidopsis homologs of *BRCA2* (97% nucleotide sequence identity) (Siaud et al., 2004). Using a meiosis-specific RNAi construct, it was found that reduction of *AtBRCA2* expression causes the formation univalents instead of bivalents and mis-segregation of chromosome at anaphase I, similar to those of the *atdmc1* mutant cells. An interaction with the DNA repair process was further supported by the observation that the AtBRCA2 protein can interact with AtRAD51 and AtDMC1 in a yeast two-hybrid assays. These results suggest that AtBRCA2 is involved in meiotic recombination. Furthermore, the lack of chromosome fragmentation in the *AtBRCA2* RNAi transgenic plants further suggests that *AtBRCA2* is similar to *AtDMC1*, not needed for the repair of most DSBs, unlike the mutants defective in *AtRAD51*, *AtRAD51C*, or *AtXRCC3*. The analysis of Arabidopsis *AtATM, MEI1,* and *AtBRCA2* genes suggests that they play a role in the regulation of the repair of meiotic DSBs breaks and may be functionally conserved compared with their animal counterparts.

Meiotic Recombination: Crossover Formation

As mentioned before, crossing over is required to maintain homolog association after disassembly of the SC and the recombinational exchange of sequences flanking a crossover is a major mechanism of re-distribution of genetic information among meiotic products. Crossing over in many organisms exhibits interference, which refers to the phenomenon that the presence of a crossover alters the probability of a nearby second crossover as expected from random distribution. In the budding yeast *S. cerevisiae,* several genes are required for normal crossing over, including *MSH4, MSH5,* and *MER3* (Hollingsworth et al., 1995; Nakagawa and Ogawa, 1999; Novak et al., 2001; Nakagawa and Kolodner, 2002a). MSH4 and MSH5 are two of several eukaryotic homologs of the bacterial MutS protein involved in DNA repair and are important for the formation of double-Holliday junctions; they form a heterodimer in vitro that recognizes Holliday juctions (Bocker et al., 1999; Snowden et al., 2004). *MER3* was first discovered as a mutant defective in meiosis and sequence comparison suggests that the MER3 protein is a DNA helicase containing a DEXDc-box. The DNA helicase activity was demonstrated by biochemical studies showing that MER3 can unwind double stranded DNA molecules, including Holliday junctions (Nakagawa and Kolodner, 2002b; Mazina et al., 2004).

The *msh4, msh5,* and *mer3* mutant meiocytes produce a small number of crossovers, about 10-15% of the normal levels. The remaining crossovers are randomly distributed; therefore, they are not sensitive to interference. In addition, mutations in the *MUS81* and *MMS4/EME1* genes that encode subunits of a heterodimeric endonuclease affect a small portion of the crossovers that are not sensitive to interference (Hollingsworth and Brill, 2004). Thus, there are two genetically separable pathways in yeast for crossover formation: one is responsible for the majority of crossovers, is sensitive to interference, and is dependent on the function of the *MSH4, MSH5,* and *MER3* genes; the other is less prominent, insensitive to interference, and requires the function of the *MUS81* and *MMS4/EME1* genes (Nakagawa and Ogawa, 1999; de los Santos et al., 2003; Bishop and Zickler, 2004; Hollingsworth and Brill, 2004; Stahl et al., 2004). In humans, statistical modeling also supports the existence of two pathways for crossing over, with only one of these being sensitive to interference (Housworth and Stahl, 2003). It has been observed that the numbers of crossovers dependent on the two different pathways vary in different organisms (Hollingsworth and Brill, 2004). In contrast to the budding yeast, in which the MSH4-MSH5 dependent pathway accounts for the majority of crossovers, fission yeast employs the MUS81-MMS4/EME1 pathway to generate most of the crossovers (de los Santos et al., 2003; Bishop and Zickler, 2004; Hollingsworth and Brill, 2004). *MSH4* and *MSH5* homologs are also important for meiosis in mammals (de Vries et al., 1999; Kneitz et al., 2000).

Several recent studies indicate that Arabidopsis, like yeast, also has two distinct pathways for crossover formation. Using genetic mapping studies with a *quartet* mutant that produces attached pollen grains that are the 4 products of each meiosis, it was shown that the distribution of crossovers in Arabidopsis is also consistent two different kinds of crossovers: interference-sensitive and insensitive ones (Copenhaver et al., 2002; Lam et al., 2005a). In addition, Higgins et al. (2004) reported that the *MSH4* homolog in Arabidopsis, *AtMSH4*, is essential for an interference-sensitive pathway for crossover formation. The AtMSH4

protein has 792 amino acids and is 35% identical and 57% similar to the human MSH4 in amino acid sequence. *AtMSH4* expression is highest in floral buds containing meiotic cells, reduced in mature flowers, and not detectable in vegetative organs. The AtMSH4 protein was found at numerous (~100) foci at mid-leptotene, gradually decreasing through zygotene, to only several sites at early pachytene, and completely disappearing by late pachytene, except at the ends of chromosomes (Higgins et al., 2004). This pattern of protein localization is consistent with the idea that AtMSH4 functions in recombination. This hypothesis is further supported by the observation that AtMSH4 and AtRAD51 have similar spatial and temporal patterns of distribution, although AtRAD51 might be loaded onto the chromosomes slightly earlier that AtMSH4. Furthermore, during synapsis in zygotene, AtMSH4 does not seem to remain on regions of chromosomes that have synapsed, as indicated by colocalization studies with ZYP1.

The importance of AtMSH4 in recombination, particularly crossover formation, was demonstrated by the analysis of *atmsh4* mutant and RNAi lines (Higgins et al., 2004). These plants have dramatically reduced fertility, producing only 7% of the normal number of seeds, and defective in bivalent formation. The mutant meiocytes have a small number (1.55) of chiasmata per cell, compared the normal ~10 per cell. In addition, the remaining crossovers in the *atmsh4* mutant are distributed randomly, indicating that Arabidopsis, like the budding yeast, also has a genetically separate pathway for interference-insensitive crossovers, which do not require the function of the *AtMSH4* gene (Higgins et al., 2004). Although juxtaposed chromosomes are observed at pachytene in light microscopic images, TEM analysis of chromosome spreads indicate that SC formation is not complete, with regions of desynapsed chromosomes, suggesting AtMSH4 is involved in promoting synapsis.

The analysis of the *AtMSH4* gene provides direct evidence for an interference-sensitive pathway of crossover formation in Arabidopsis. It is likely that other genes are also needed for this pathway. In yeast, the *MER3* gene is also required for this pathway. Is there an Arabidopsis *MER3* homolog that is necessary for crossover formation? Two recent studies have demonstrated that this is indeed the case (Chen et al., 2005; Mercier et al., 2005). Chen et al. (2005) identified a gene that is preferentially expressed in the Arabidopsis anther at the time of meiosis and found that it is expressed in meiocytes more than other tissues in the developing anther. This gene was named *ROCK-N-ROLLERS* (*RCK*) for the meiotic chromosome behavior

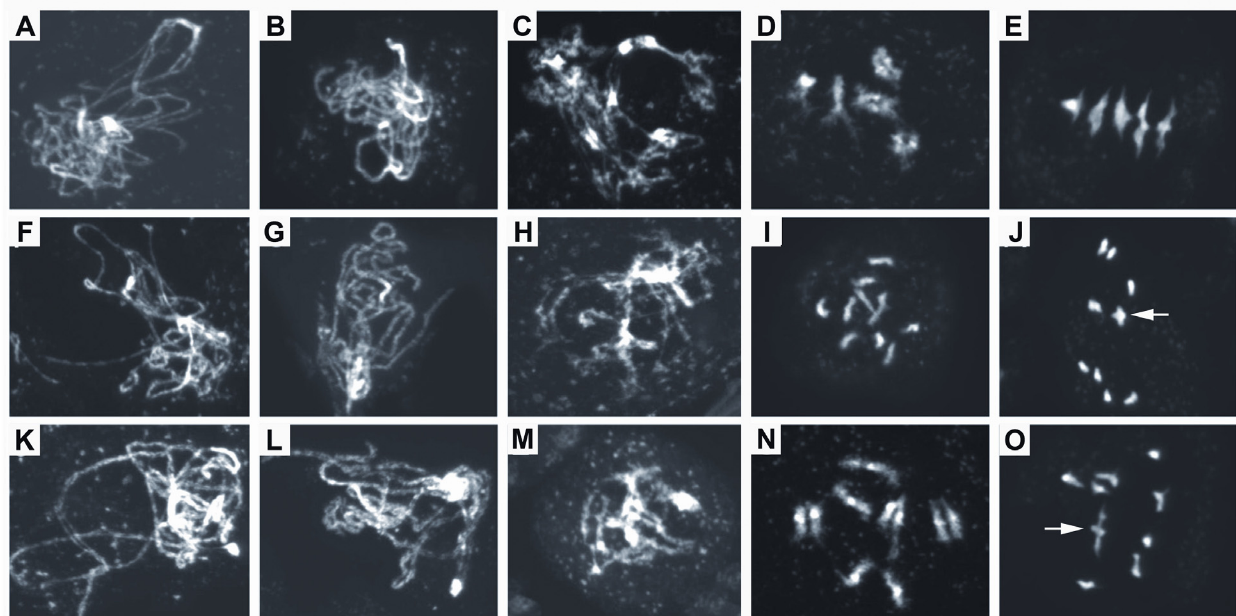

Figure 12. Male meiosis I in the wild type and *ptd* mutants. Shown are DAPI images from wild-type (A-E), *ptd-1* (F-J), *ptd-2* (K-O) cells. A, F, K, zygotene. B, G, L, pachytene, wild-type and *ptd* cells appear similar. C, H, M, diplotene. D, I, N, diakinesis, showing a clear contrast between 5 bivalents in the wild type and 10 univalents in *ptd* mutants. E, J, O, metaphase I. Unlike the 5 bivalents in the wild-type, the *ptd* cells have one bivalent (arrow) and 8 univalents. This figure is modified from Figure 4 of Wijeratne et al. (2006).

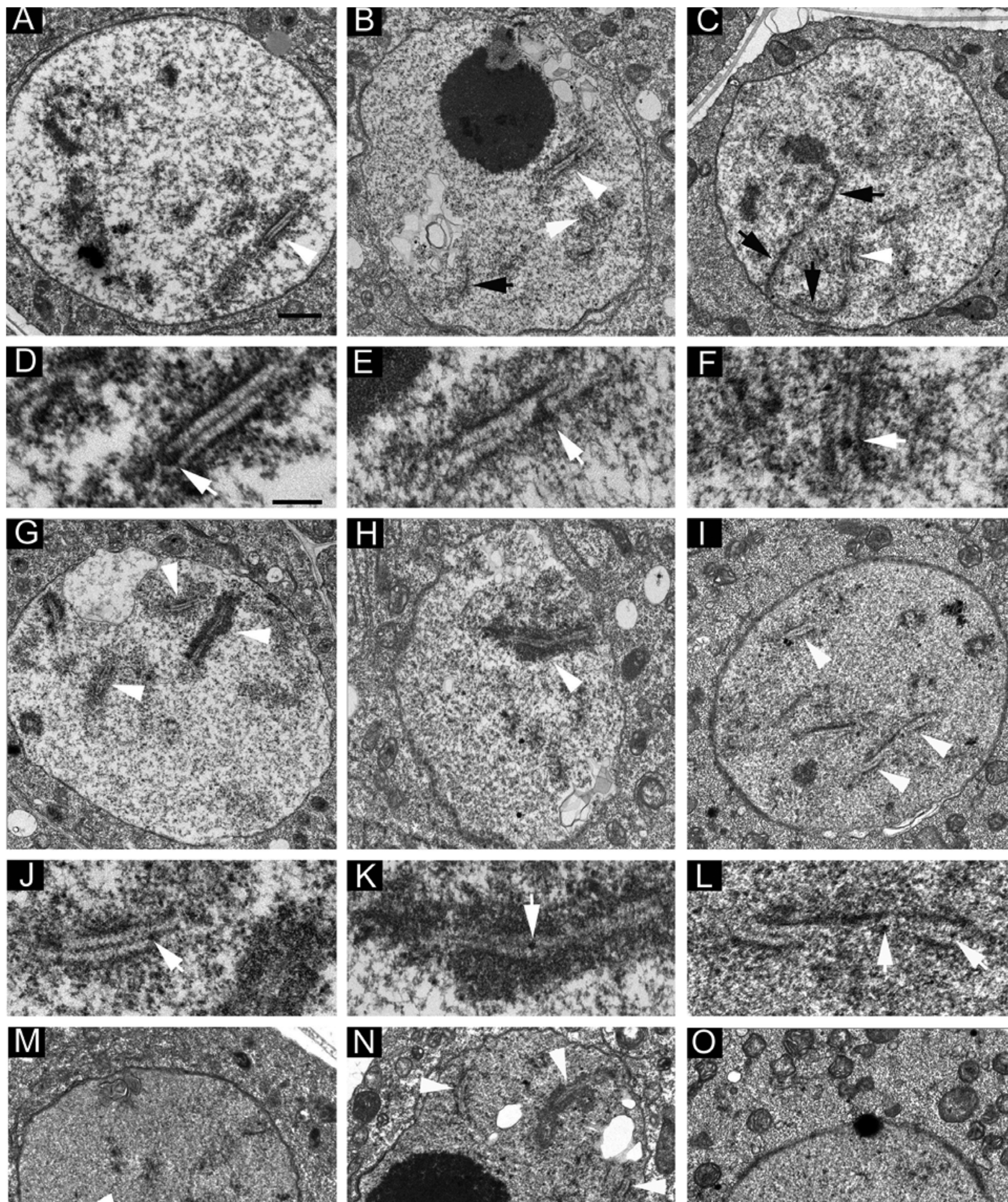

Figure 13. Analysis of meiotic prophase I in wild-type (A,D,G, J and M), the *ptd-1* (B,E,H,K and N) and the *ptd-2* (C,F,I,L and O) meiocytes (White arrowheads point to the synaptonemal complexes; black arrows point to unsynapsed elements and white arrows point to the recombination nodules). A, early pachytene, with synapsed chromosomes. B, SCs are found in *ptd-1at* a nearly normal level. C, SCs in *ptd-2* is present at a reduced level. Early recombination nodules seem to be present at normal levels in the *ptd-1* and *ptd-2* mutants (A and D, B and E, C and F). G and H, at mid-pachytene, SCs in the wild type and *ptd-1* have the same morphology. I, SC in *ptd-2* is delayed. J, K, L, late recombination nodules are found on mid-pachytene SCs. M, N, O, normal levels of SCs in both mutant alleles at late pachytene-early diplotene stages. D, E, F, J, K and L are magnified images from ones A, B, C, G, H and I, respectively. Bars, (A-C, G-I and M-O) =1000 nm; (D-F and J-L) =250 nm. This figure is modified from Figure 6 of Wijeratne et al. (2006).

caused by mutations in this gene. The analysis of the full-length *RCK* cDNA indicates that the predicted gene structure from the genomic sequence has a number of errors, resulting in a putative protein with a wrong sequence. The newly obtained sequence revealed that RCK is a highly similar to MER3, with three conserved domains.

Several T-DNA insertional lines were available for the *RCK* gene, and analysis of four insertional *rck* mutants

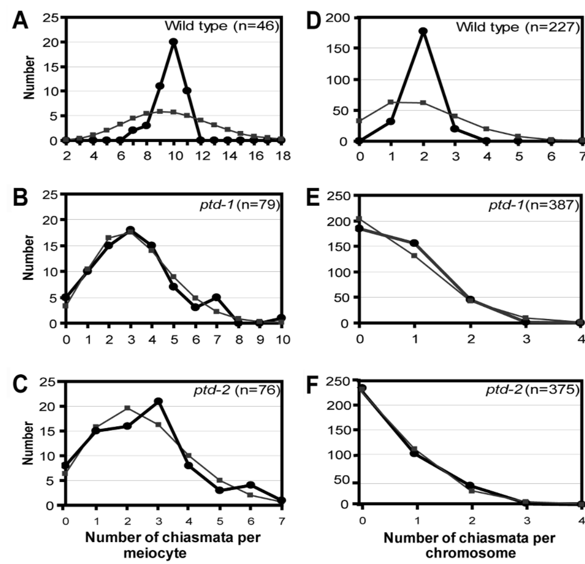

Figure 14. Distribution of chiasmata in wild type, *ptd-1* and *ptd-2*. A-C, chiasma distribution per meiocytes. D-F, chiasma distribution per chromosome. Black lines and closed circles indicate observed distributions whereas gray line and closed squares represent predicted Poisson distribution. A and D, the observed distributions of the chiasmata in the wild-type cells and on the wild-type chromosomes, respectively. B, C, E and F, the observed distributions of the chiasmata in mutant cells and on mutant chromosomes are close to the predicted Poisson distributions. This figure is Figure 5 of Wijeratne et al. (2006), with permission from Mol. Biol. Cell.

indicates that they are reduced in fertility (Chen et al., 2005; Mercier et al., 2005). Phenotypic characterization using light and electron microscopy showed that *rck* mutants were defective in meiotic homolog synapsis and crossover formation, resulting in reduced number of bivalents. Instead of ~10 chiasmata per cell as seen in wild-type meiocytes, *rck-1* meiocytes had an average of 3.10 chiasmata per cell. Similar to the *atmsh4* mutant, the remaining chiasmata found in the *rck-1* mutant had a distribution very close to the predicted Poisson distribution, suggesting that the residual chiasmata of the *rck* mutant are insensitive to interference. In contrast, in the wild type, chiasma distribution deviates significantly from the predicted Poisson distribution. These results provide further support for the presence in Arabidopsis of both interference-sensitive and -insensitive pathways and indicate that the Arabidopsis interference-sensitive pathway for crossover formation also requires a MER3-dependent function.

Crossing over via the interference sensitive pathway requires not only the formation of the double-Holliday junction, but also its resolution. Previous studies in yeast or animals have not uncovered a gene specific involved in a step after the Holliday junction. Nevertheless, a recent study in Arabidopsis has identified a potential key player in this process (Wijeratne et al., 2006). Using microarray analysis with RNAs from immature Arabidopsis anthers and other tissues, a number of putative meiotic genes were identified. One of these, At1g12790, was annotated as being similar to bacterial DNA ligases and is expressed in the anthers at approximately the time of meiosis. This gene was named as *PARTING DANCERS* (*PTD*) for its meiotic phenotypes (see below). RNA in situ hybridization experiments indicate that *PTD* is expressed in the floral meristem, the male and female meiocytes, and other tissues. Analysis of two independent T-DNA insertional lines in the *PTD* gene indicates that the mutants are normal in vegetative and flower development, but have reduced fertility. Reciprocal crosses indicate that both male and female fertility are affected in the *ptd* mutants.

An examination of pollen development quickly established that male meiosis is abnormal in the *ptd* mutants (Wijeratne et al., 2006). Further investigation using DAPI-stained chromosome spreads indicate that the *ptd* meiocytes exhibit normal meiotic chromosome morphologies from the onset of meiosis I through pachytene, forming juxtaposed chromosomes detected as "thick threads" (Figure 12). However, at diakinesis, the numbers of bivalents in the mutants are clearly reduced, though not absent, with an average of about 2.7 (*ptd-1*) or 1.8 (*ptd-2*) bivalents per cell, instead of the normal 5. Therefore, *ptd* mutants could not maintain the normal number of bivalents. TEM analysis further revealed that indeed the SC is formed in the *ptd* meiocytes, indicating that synapsis is not affected by the *ptd* mutations (Figure 13). Again using a social dance as a metaphor for the meiotic homolog interactions, the homologs in the *ptd* mutants initially dance in pairs, able to pair, juxtapose, and synapse, but then separating from each other prematurely. Thus the mutants were named "*parting dancers*."

The *ptd* mutant phenotypes are rather similar to those of the *atmsh4* and *rck/atmer3* mutants (Higgins et al., 2004; Chen et al., 2005; Mercier et al., 2005), suggesting that *PTD* might also be important for crossing over via the interference-sensitive pathway. This hypothesis was tested by investigating the distribution of the remaining chiasmata in *ptd-1* and *ptd-2* meiocytes (Figure 14) (Wijeratne et al., 2006). Unlike the wild-type chiasmata distribution, which deviated dramatically from the Poisson prediction (Figure 14A), the distribution of the residual chiasmata among cells in *ptd-1* and *ptd-2* mutant alleles (Figures 14B and C) were very similar to the predicted Poisson distribution. Furthermore, distributions of chiasmata per single chromosome are very different between the wild type (Figure 14D) and the *ptd* mutants (Figures 14E and 14F). Therefore, the chiasmata remaining in the *ptd* mutants appear not to be sensitive to interference, indicating that *PTD* is important for the interference-sensitive pathway.

Although *ptd* mutants are similar to the *atmsh4* and *rck/atmer3* mutants in terms of reduction of chiasmata and bivalents and the distribution of the remaining chiasmata, *ptd* mutants differ from *atmsh4* and *rck/atmer3* mutants in one critical aspect, the formation of complete SC in the *ptd* mutants (Wijeratne et al., 2006). In other words, the onset of meiotic defects in *ptd* is later than that in the *atmsh4* and *rck/atmer3* mutants, suggesting that *PTD* acts downstream of *AtMSH4* and *RCK/AtMER3*. It is possible that *PTD* may function after the formation of the double Holliday junctions, for example, by promoting their resolution. As mentioned earlier, late recombination nodules are correlated with crossing over and in Arabidopsis, *RCK/AtMER3* is involved in the formation of late recombination nodules. Therefore, the *ptd* mutants were examined in detail for the formation of recombinational nodules using serial TEM sections. At both mid-pachytene and late pachytene, the *ptd* mutant meiocytes have similar numbers of late recombination nodules to that in the wild-type, with associated normal appearing SCs. Therefore, late recombination nodules are formed at normal levels, indicating that *PTD* is not required for this step in the recombinational pathway and supporting the idea that *PTD* acts downstream of *AtMSH4* and *RCK/AtMER3*.

Clearly, *PTD* defines a critical step in the meiotic recombination pathway. What clues might its sequence provide in terms of the mechanisms of its actions? The *PTD* gene encode a 250 amino acid protein that has a putative rice homolog, OsPTD, with 63% amino acid sequence identity and 78% similarity over a 223-aa region, as well as putative homologs in other plants. Therefore, *PTD* represents a conserved function in plants, suggesting that its homologs in others may also have a similar meiotic function. Moreover, PTD shares low levels of sequence similarity in its C-terminal region to several DNA repair/recombination proteins, including ERCC1 from mammals, RAD10 from *S. cerevisiae*, the UvrC subunit of the ABC exonucleases in bacteria, and a bacterial NAD-dependent DNA ligase (Wijeratne et al., 2006). The level of sequence similarity is between 24 and 36% identity (45-58% similarity) over a region of up to 80 amino acids; therefore, it is unlikely that PTD has the same function as these proteins, although there may be some resemblance in biochemical activities. The *ptd* mutant phenotypes and the PTD protein sequence suggest that PTD may act to facilitate the resolution of the double Holliday junction, by cutting and/or ligating DNA. Furthermore, it is possible that PTD acts to resolve the double Holliday junction in favor of crossing over, rather than non-crossing over.

Relationship among Pairing, Synapsis, and Recombination

In meiotic prophase I, homologs pair and become juxtaposed; the SC is formed between homologs, and recombination also involve the same homologs. In addition, these processes occur during overlapping time periods and must be carefully coordinated and intimately coupled. However, the relationship between these processes is not always the same among different organisms. In yeast, synapsis and recombination are interdependent and closely coupled. In particular, genetic studies strongly support the idea that SC is dependent on the initiation of recombination by DSBs. On the other hand, in *C. elegans* and *Drosophila*, recombination, but not synapsis, is dependent on DSBs, indicating a different relationship in these invertebrate animals (Keeney et al., 1997; Dernburg et al., 1998; McKim et al., 1998; Peoples et al., 2002). The analysis of Arabidopsis genes provides direct evidence for the close relationship between pairing, synapsis and recombination in plants.

In plants, pairing is largely homology dependent; similarly, recombination necessarily relies on homology. From the above discussion of the phenotypes of several mutants defective in recombination, it is more than plausible that pairing and early recombination share the same molecular machinery. SPO11 induces DSBs, which allow homology verification between homologs. RAD51 is important for DSBs repair; therefore, it is reasonable that early RAD51 foci mark DSBs. Analysis in maize wild-type and mutant meiocyte have led to the proposal that early DSBs allow homology search for homolog pairing (Franklin et al., 2003; Pawlowski et al., 2003; Pawlowski et al., 2004). It has been shown that RAD51 is part of some early meiotic nodules in lily (Anderson et al., 1997) and that recombination nodules may be involved in pairing and synapsis (Anderson et al., 2001; Anderson et al., 2004). The analysis of the Arabidopsis *AtRAD51* gene provides genetic support for its role in homolog pairing and/or juxtaposition (Li et al., 2004). Furthermore, the phenotypes of *atrad51c* and *atxrcc3* mutants also support the function of these genes in both DSBs repair and pairing. It is known that early RAD51 foci, thus DSB sites, are more numerous than recombinational crossovers in maize and Arabidopsis (Franklin et al., 1999; Higgins et al., 2004). Therefore, most of SPO11-induced DSBs are probably generated to allow pairing, which involved base-pairing of DNA from two non-sister chromatids. After homology has been established, most of the DSBs are repaired without exchange of flanking sequences (crossing over), whereas a small fraction of DSBs remain to form crossovers and chiasmata, which maintain bivalent. This hypothesis predicts that all genes needed for the generation and repair of DSBs are also

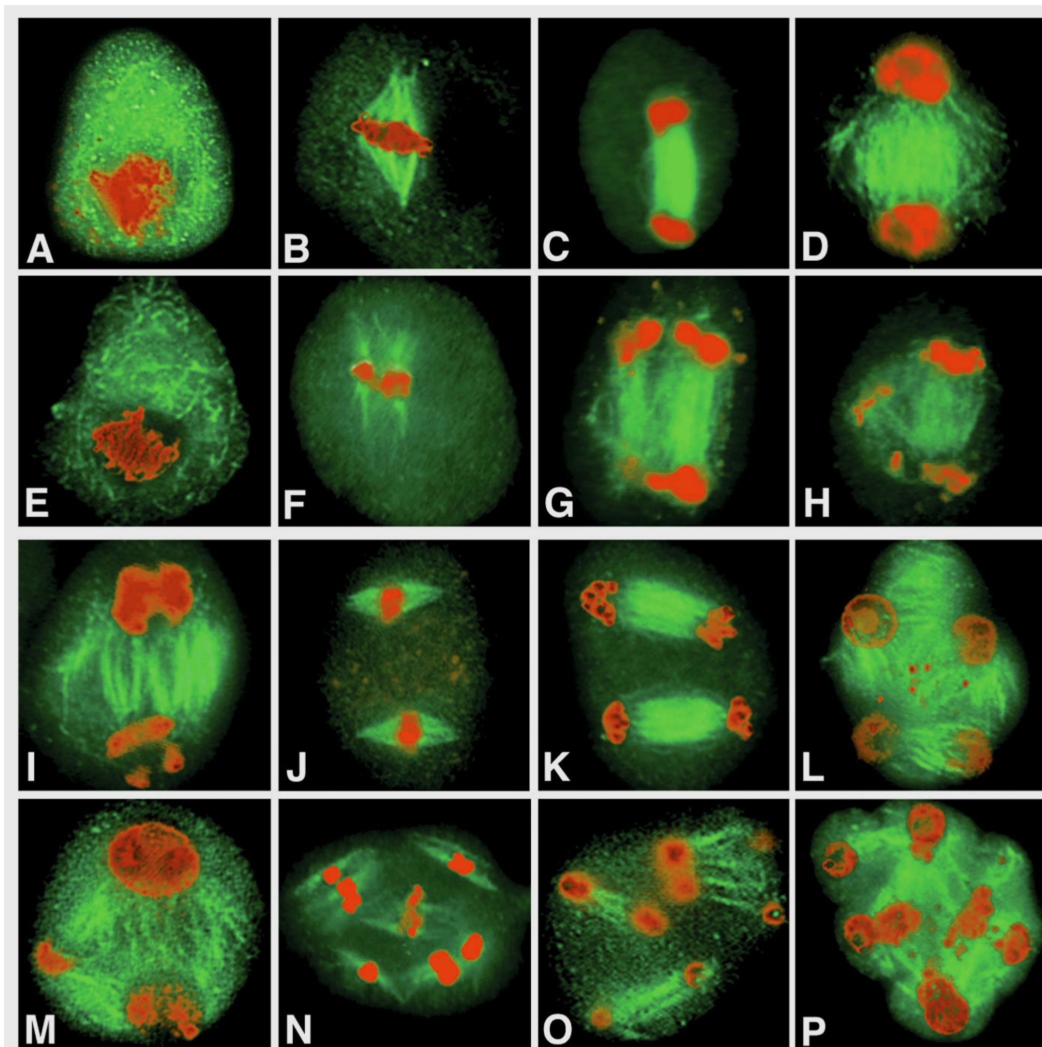

Figure 15. Spindle structures in wild-type and *atk1-1* male meiocytes. The microtubules (green) were visualized with anti-tubulin antibodies; DNA was stained with DAPI and the false red color was generated using Photoshop. A-D, I-L, wild-type. E-H, M-P, *atk1-1*. A and E, prophase I, with perinuclear microtubules. B and F, metaphase I; the *atk1* spindle is unfocused at the poles. C and G, anaphase I, showing abnormal chromosome segregation in the *atk1* cell. D and H, telophase I. I and M, prophase II. J and N, metaphase II, with multiple mini-spindles in the *atk1* mutant. K and O, anaphase II. L and P, telophase II, with more than 4 nuclei in the mutant. This figure is Figure 5 of Chen et al. (2002).

important for pairing, and that pairing is correlated with repair of DSBs, or gene conversion. In addition to *AtSPO11-1, AtRAD51, AtRAD51C, and AtXRCC3, AtMRE11,* and *AtRAD50* are also required for early recombination steps. Although details of homolog pairing in the *atmre11* mutant meiocytes are not available, the pachytene phenotypes are consistent with a pairing and/or synapsis defect. Further analysis of *atmre11* and other mutants to fully investigate their pairing properties is needed to verify the above proposed direct coupling of DSB repair and pairing.

The interdependence of synapsis and recombination in plants is very well supported. Although synapsis was not directly observed in the *atspo11-1* mutant, the *atspo11-1* mutant phenotypes is consistent with synapsis being SPO11-dependent (Grelon et al., 2001). Furthermore, analysis using TEM on synapsis in the *atrad51-1* and *atrad51c-1* mutants (Li et al., 2004; Li et al., 2005) argue strongly for a close relationship between recombination and synapsis, similar to that in yeast. Moreover, the *atmsh4* and *rck/atmer3* mutants are also defective in synapsis. During normal meiosis, it is likely that DSBs are

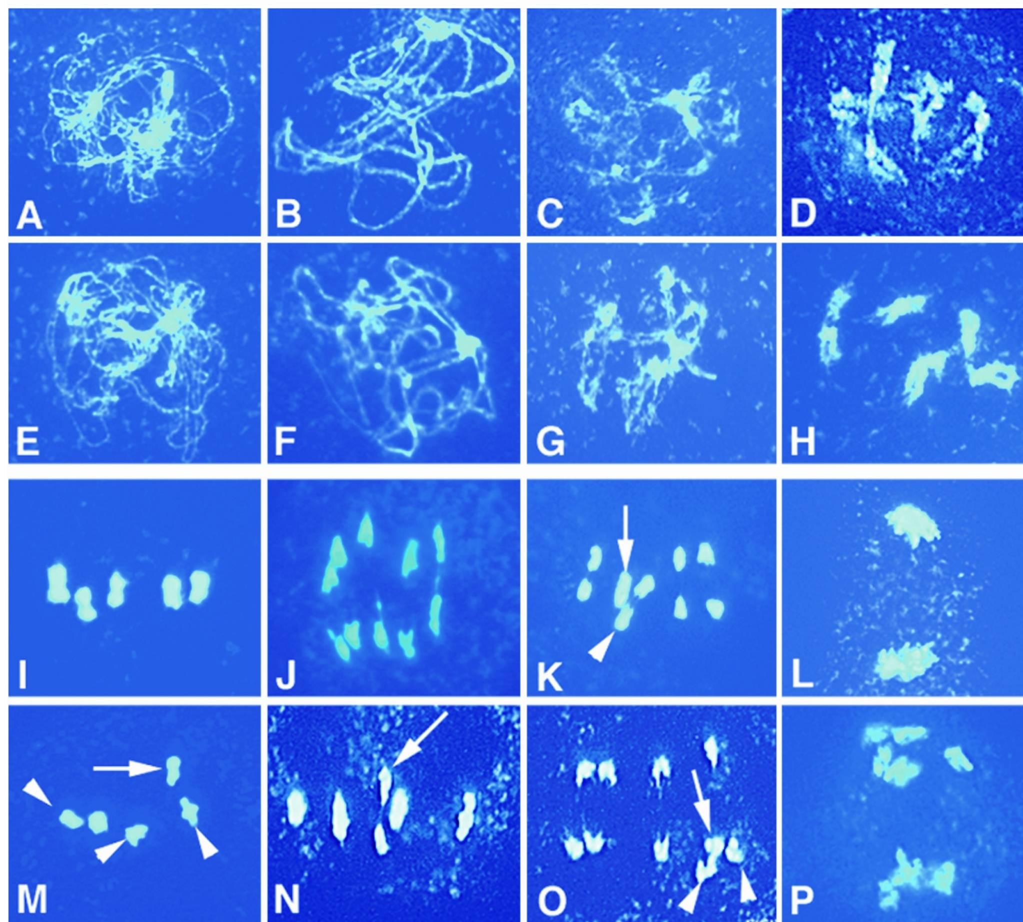

Figure 16. Wild-type and *atk1-1* male meiosis. A.-D., I., J., L., wild type; E.-H., K., M.-T. *atk1-1*. A. and E, zygotene. B and F, pachytene. C and G, diplotene. D and H, diakinesis. Mutant cells during prophase I seem normal. I and M, metaphase I; the position of bivalents are not normal in the *atk1-1* cell, with one bivalent (arrow) located slightly away from the equator; three others (arrowheads) are not aligned in parallel. K and N, metaphase I to anaphase I transition in *atk1*, showing asynchrony in homolog separation (arrows). J and O, anaphase I; in *atk1-1* meiocytes, some pairs of homologues are misaligned (arrowheads in K and O). L and P, telophase I; *atk1-1* has uneven chromosome segregation. This figure is modified from Figure 4 of Chen et al. (2002).

the sites of early recombination nodules. In addition, a subset of DSBs might be the sites for loading SC proteins, as supported by the analysis of ZYP1 foci, which initially number in 20-25 (Higgins et al., 2005). This can explain why in the absence of SPO11-1 induced DSBs, synapsis is defective. AtDMC1 is required for bivalent formation, indicating a critical role in crossover formation. However, unlike *AtRAD51, AtRAD51C,* and *AtXRCC3, AtDMC1* does not seem to be required for DSBs repair, suggesting that it may have a more specialized function for processing those DSBs targeted for crossover formation. It is possible that these DSBs correspond to late recombination nodules that require AtMSH4 and RCK/AtMER3, and that facilitate the loading of ZYP1. However, the localization of ZYP1 in *atmsh4* meiocytes indicates that AtMSH4 is not required for early loading of ZYP1 (Higgins et al., 2005). It is not known what effect *rck/atmer3* mutations might have on ZYP1 localization to the chromosomes.

Chromosome Separation and Segregation

The elaborate process of prophase I involving homolog pairing, synapsis and recombination results in formation of the bivalents at diakinesis. The purpose of this highly complex and tightly controlled "meiotic dance" is to make sure that homolog segregation at anaphase I is accurate. However, bivalent formation is not sufficient for proper segregation. Before the homologs can separate, the bivalents need to move to the metaphase plate, located at the center of the meiotic spindle. This process depends on a

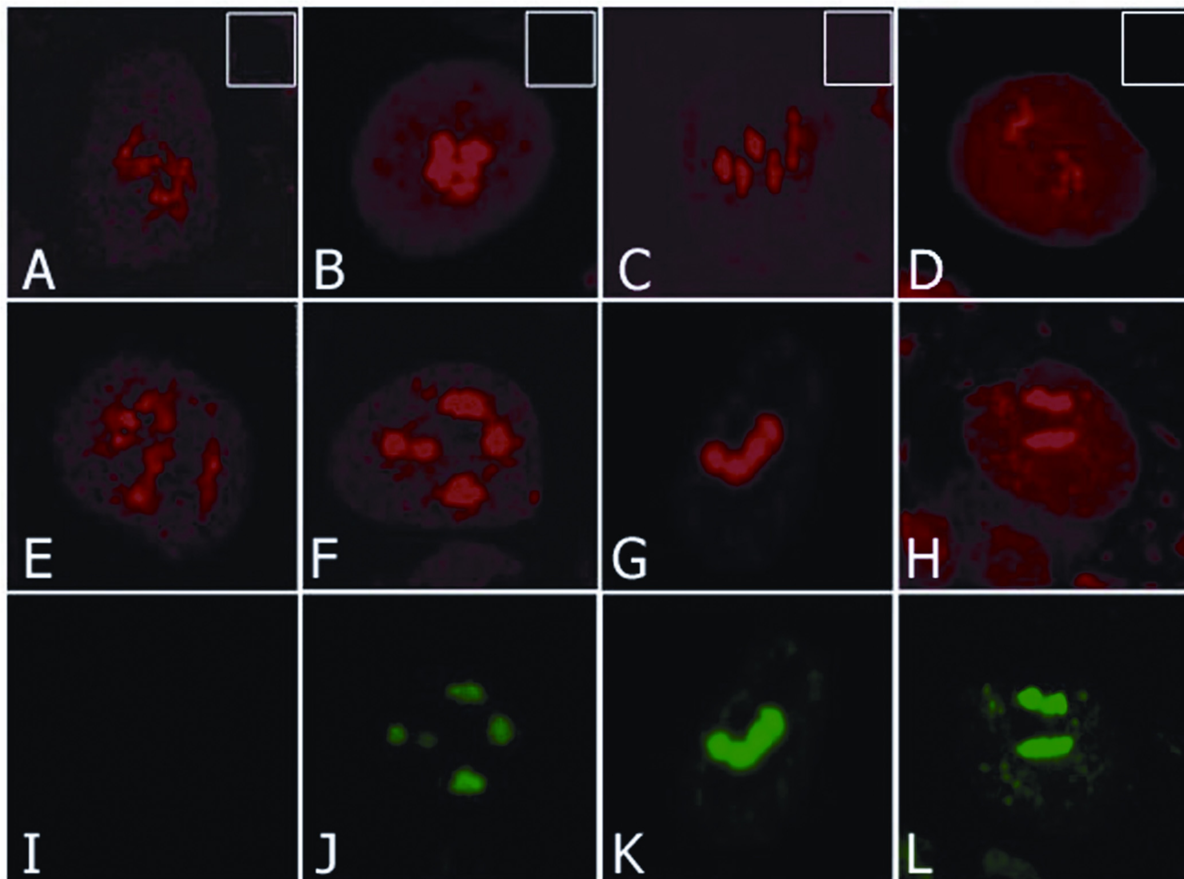

Figure 17. DNA breaks in *mmd1* microsporocytes detected by the TUNEL assay. A-D, wild type cells showing DNA (red), each with an inset in the upper right-hand corner showing no signal detected for DNA breaks (green). E-H, Chromosomes in *mmd1* cells; I-L, DNA breaks in the same *mmd1* cells as in E-H, respectively. A, diakinesis. B, prometaphase I. C, G and K, metaphase I. D, H and L, anaphase I. E and I, early diakinesis. F and J, late diakinesis. Chromosomes in *mmd1* cells are labeled with TUNEL starting at late diakinesis. Bar = 10mm. This figure is modified from Figure 4 in Yang et al. (2003b).

properly assembled spindle and other spindle associated functions. Following the alignment of bivalents along the equatorial plane of the spindle, sister chromatid cohesion along chromosomal arms must be removed via the proteolytic degradation of specific cohesin subunits, to allow homolog separation at the onset of anaphase I. Subsequently in anaphase I, the newly separated homologs are pulled by the forces of the spindle microtubules towards the two spindle poles.

The meiotic spindle provides the force for chromosome movement and is critical for normal chromosome segregation. During meiosis I, the spindle forms at prometaphase I into a bipolar structure (Franklin and Cande, 1999). In Arabidopsis, a mutant (*atk1*, for *Arabidopsis thaliana kinesin1*) was found to have greatly reduced male fertility and mutant male meiocytes form abnormal meiotic spindles (Chen et al., 2002). The *atk1* mutant meiotic spindles have unfocused poles, with disorganized microtubules (Figure 15). In addition, bivalents in mutant meiocytes fail to align at the metaphase I plate perfectly, with some bivalents seemingly lagging behind in their movement to the metaphase I plate (Figure 16). Therefore, a normal-shaped spindle may be needed to facilitate the alignment of the bivalents. The *atk1* mutant meiocytes also exhibit slightly asynchronous homolog separation (Figure 16). It is possible that uniform homolog separation requires an even distribution of microtubule forces from a properly formed spindle. The *ATK1* gene is the same gene as the previously cloned *KATA* gene, which encodes a kinesin protein (Mitsui et al., 1993; Liu et al., 1996; Chen et al., 2002). ATK1 has a predicted C-terminal motor domain and is highly similar to the *Drosophila* NCD and budding yeast KAR3 proteins; both NCD and KAR3 are important for meiotic and/or mitotic spindle morphogenesis (Hatsumi and Endow, 1992; Roof et al., 1992). Therefore, ATK1, NCD and KAR3 are structurally and functionally conserved proteins

important for meiotic spindle assembly. The abnormal unfocused spindle in the *atk1* mutant is also similar to that of the maize mutant *dv* (*divergent spindle*) (Staiger and Cande, 1990).

Both *ncd* and *kar3* mutants also have mitotic defects (Hatsumi and Endow, 1992; Roof et al., 1992; Endow et al., 1994). *ATK1* is also expressed in vegetative and floral organs, suggesting that it may also have a mitotic function. Careful examination of mitotically dividing cells of the *atk1* mutant revealed that the mitotic spindle is also affected and mitosis is prolonged in the mutant (Marcus et al., 2003); however, the spindle defect is not severe enough to cause gross developmental abnormalities (Chen et al., 2002). The mild defect in mitotic cells might be due to functional redundancy, because Arabidopsis has another gene, *ATK5*, which encodes a protein highly similar to ATK1, with an overall 83% amino acid sequence identity to ATK1. Both ATK1 and ATK5 proteins can bind to and move along microtubules in vitro (Marcus et al., 2002; Ambrose et al., 2005), suggesting that they have very similar functions. Whether they indeed have redundant in vivo functions will be determined by genetic studies.

At the metaphase I/anaphase I transition, the separation of homologs requires the resolution of sister chromatid cohesion. In Arabidopsis, it is not clear how cohesins are removed. Nevertheless, the *ask1* mutant is defective in chromosome separation and segregation at anaphase I (Yang et al., 1999). In *ask1* meiocytes, some chromosomes remain associated when they are pulled by the spindle and become stretched. In addition, the forces of the spindle also seem to cause chromosome fragmentation. The failure of chromosome separation then results in grossly uneven chromosome distribution. Cytokinesis then produces abnormal number of microspores contain abnormal amounts of DNA. *ASK1* encodes a homolog of the SKP1 protein (Yang et al., 1999), which is a critical component of SCF complexes (for SKP1, cullin/CDC53, F-box protein) (Zhang et al., 1995; Bai et al., 1996; Connelly and Hieter, 1996; Peters, 1998). As discussed before, the SCF complexes are E3 ubiquitin-protein ligases that control a number of biological processes by targeting specific proteins for degradation by the 26S proteosome (Zheng et al., 2002). The chromosome behavior of the *ask1* mutant meiocytes suggests that an *ASK1*-containing SCF ubiquitin ligase is involved in the removal of the cohesin complex along chromosomal arms. Recently, it was found that the ASK1 protein accumulates during early prophase I, primarily at leptotene to pachytene, at levels higher than those at other stages (Wang and Yang, 2005), suggesting that the effect of *ask1-1* mutation on chromosome separation might be indirect consequence of an early function of *ASK1*. In addition, the reduction of ASK1 function in the *ASK1/ask1-1* heterozygous plants is associated with an increase in the rate of recombination, suggesting that *ASK1* negatively regulates recombination (Wang and Yang, 2005). Further investigation is needed to understand the role of ASK1 and SCF complexes in meiosis.

In addition to *ASK1,* Arabidopsis has 20 other SKP1 homologs; among these, *ASK2* is most similar to *ASK1* in sequence and expression patterns (Zhao et al., 2003b; Kong et al., 2004). However, the fact that *ask1* is completely defective in male meiosis, resulting in total male sterility, indicates that *ASK2* is not able to fulfill the required function. *ASK2* is expressed at a level lower than that of *ASK1*; therefore, it is possible ASK2 is not expressed sufficiently for the male meiotic function. Alternatively, the ASK2 protein might be different from ASK1 in sequence, such that ASK2 is not able to interact with other proteins as well as ASK1. These ideas were tested using transgenic plants overexpressing *ASK2* (Zhao et al., 2003a); when *ASK2* is overexpressed in the *ask1* mutant background, the meiotic defects are partially corrected and the plants are partially fertile. However, the fact that these plants are still partially defective indicates that the ASK2 protein is functionally distinct from ASK1. This idea is further supported by the phylogenetic finding that ASK1 and ASK2 are members of separate clades which each have representatives of many plants (Kong et al., 2004). Therefore, ASK1 and ASK2 are not equivalent in male meiosis, although they do have redundant functions during embryo and seedling development (Liu et al., 2004).

Meiotic Progression: Potential Regulators

To ensure the success of a complex process such as meiosis, it is necessary to control the timing and sequence of various meiotic events. For example, chromosome condensation and sister chromatid cohesion are regulated by protein phosphorylation (Wei et al., 1998; Suja et al., 1999; Wei et al., 1999; De Souza et al., 2000). In yeast and nematode, the mitotic histone H3 phosphorylation is controlled by the balanced activities of both a protein kinase (Ipl1/Aurora) and a phosphatase (Glc7/PP1) (Francisco and Chan, 1994; Francisco et al., 1994; Hsu et al., 2000). Furthermore, it was shown that the maintenance of sister-chromatid cohesion in maize is correlated with phosphorylation of histone H3 (Kaszas and Cande, 2000), suggesting a role in the regulation of sister chromatid cohesion. Another study indicates that dissociation of sister chromatid cohesin is correlated with phosphorylation of the Xenopus XSA1/Scc3p cohesin subunit (Losada et al., 2000). Furthermore, purified cyclin B-CDK has been shown to phosphorylate XSA1 in vitro and reduce its ability to bind DNA or chromatin (Losada et al., 2000). These results together suggest that phosphorylation of several

proteins, at least in part mediated by cyclin-CDK, may be involved in the separation of sister chromatids.

Cyclins and CDKs were originally discovered as factors promoting the maturation of frog oocytes (MPF). In yeast, cyclins and CDKs (cdc2 from *S. pombe* and CDC28 from *S. cerevisiae*) play pivotal roles in controlling the mitotic cell cycle (Futcher, 1991; Nasmyth, 1993; Murray, 1994; Reed, 1996). In addition, they also function in regulating meiosis. For example, Clb1, Clb3, and Clb4 regulate events just before meiosis I (Grandin and Reed, 1993) and also meiosis II (Dahmann and Futcher, 1995). Moreover, the mitotic S-phase cyclins Clb5 and Clb6 are also required for the meiotic S phase (Dirick et al., 1998; Stuart and Wittenberg, 1998). Furthermore, Clb5 and CLb6 also seem to be important for the meiotic prophase I, including synapsis and recombination (Smith et al., 2001); however, the fact that these proteins are required for pre-meiotic S phase makes it different to determine whether the Clb5/Clb6 function in prophase I is direct. In mouse, the cyclin A1 is specifically expressed in the testis and mutant mice are male sterile and defective in male meiosis (Liu et al., 1998a). In the mutant, male meiosis arrests before metaphase I, with desynaptic chromosomes, and then undergo programmed cell death. Cyclin-CDK also plays critical roles in regulating sister chromatid cohesion during mitosis. Cyclin-CDK mediated phosphorylation regulates the activity of the APC E3 ubiquitin ligase, thereby controlling the timing of cohesin removal (Heo et al., 1999; Zachariae, 1999).

In Arabidopsis, a screen for fertility defects among a transposon insertional population identified a mutant with greatly reduced fertility (Azumi et al., 2002). This mutant was named *solo dancers* (*sds*) because meiotic chromosomes progress through prophase I largely as individuals, unlike the wild-type chromosomes, which undergo prophase I as pairs following homolog pairing. The *sds* mutant meiocytes are defective in homolog pairing/juxtaposition and recombination, resulting in greatly reduced bivalent formation. The *sds* mutant phenotypes are nearly identical to those of *atspo11-1* and *atdmc1* mutants, suggesting that *SDS* is required for the SPO11-dependent pathway for meiotic recombination. At the same time, in contrast to the *atrad51, atrad51c*, or *atxrcc3* mutants, the *sds* mutant meiocytes do not have fragmented chromosomes, suggesting that the *sds* mutant cells either do not have SPO11-induced DSBs or are able to repair the DSBs. In addition, *sds* mutant meiocytes exhibit premature partial separation of sister chromatids, suggesting a possible role in regulating cohesion. In situ hybridization experiments indicate that *SDS* is specifically expressed in male and female meiotic cells; in addition, *SDS* encodes a putative novel cyclin, which is phylogenetically distinct from other cyclins (Azumi et al., 2002; Wang et al., 2004a). It is likely that SDS activates one or more CDKs to regulate protein activities via phosphorylation, thereby coordinating multiple meiotic events, including pairing, synapsis, recombination, and possibly sister chromatid cohesion.

In addition, the Arabidopsis *tardy asynchronous meiosis* (*tam*) mutant is abnormal in the timing of meiotic divisions, resulting in the formation of intermediate structures such as dyads, which are not produced by wild-type meiosis (Magnard et al., 2001). In particular, in cells entering metaphase I or metaphase II, there is a clear indication of asynchronous meiosis (Magnard et al., 2001). Therefore, *TAM* may control the entry into metaphase, as further supported by the fact that the *tam* mutation is in the gene encoding the cyclin A CycA1,2 (Wang et al., 2004b). Using a GFP fusion, it was observed that TAM/CycA1,2 is present at its highest level at pachytene, becoming undetectable at diakinesis and subsequent meiotic stages, suggesting it regulates later stages of meiosis indirectly, presumably via the targets of TAM/CycA1,2-activated phosphorylation (Wang et al., 2004b). The function of TAM/CycA1,2 and the mouse cyclin A1 (Liu et al., 1998a) in meiosis suggests that plants and animals may share conserved aspects of the control of meiotic progression. Despite the asynchronous meiosis, the *tam* mutant is still fertile; therefore, it is possible that mutant screens for a reduction of fertility might have missed mutants that have altered meiotic timing but are fertile.

Another possibility for the small number of mutants in meiotic progression is that meiotic progression may share regulators with the mitotic cell cycle and mutations in such genes might have severe defects in mitotic growth, preventing their recovery as meiotic mutants. For example, the Arabidopsis homolog of the yeast *CDC45* gene is expressed in floral buds; a reduction of *CDC45* expression using an RNAi construct resulted in fertility defects (Stevens et al., 2004). Phenotypic characterization of these RNAi lines indicates that polyads instead of tetrad were formed from meiosis and nonviable pollen grains were produced. Furthermore, meiotic chromosomes in the RNAi transgenic plants are fragmented at late prophase I and the fragmentation becomes more severe at metaphase I and anaphase I. In yeast, CDC45 regulates mitotic DNA replication (Zou and Stillman, 1998, 2000), suggesting that CDC45 in Arabidopsis may regulate the pre-meiotic DNA replication; a defect in DNA synthesis provides an explanation for chromosome fragmentation during meiosis.

Another potential Arabidopsis regulator of meiosis was discovered by two independent studies, called *MALE MEIOCYTE DEATH1* (*MMD1*) or *DUET* (Reddy et al., 2003; Yang et al., 2003b). Mutations in the *MMD1/DUET* gene cause severe defects in the progression of meiosis and the

inability to produce any normal microspores. In prophase I, the *mmd1* mutant meiocytes appears normal up to diakinesis (Yang et al., 2003b). Subsequently through telophase II, mutant male meiocytes have chromosome fragmentation and cytoplasmic shrinkage; suggesting that the cells are undergoing programmed cell death. In addition, these mutant cells have positive TUNEL signal, indicating the presence of many DNA breakage in the chromosomes (Figure 17). The *mmd1* mutant meiotic cells die before cytokinesis, resulting in a failure to produce any microspores. The *mmd1* mutant was caused by a *D*s transposon insertion just downstream of the ATG codon, resulting in a likely null allele (Yang et al., 2003b). In situ hybridization experiments indicate that *MMD1* is preferentially expressed in the meiocytes. The predicted MMD1 protein contains a C-terminal PHD finger domain. PHD fingers are cystein-containing domains, some of which have been shown bind other proteins, DNA, or RNA (Aasland et al., 1995; Kennison, 1995; Coscoy and Ganem, 2003; Reddy et al., 2003; Yang et al., 2003b). The *duet* mutant has a milder phenotype than that of the *mmd1* mutant, with delayed meiotic cytokinesis that often results in dyads (two spores) instead of tetrads (Reddy et al., 2003). The *duet* microspores usually proceed with one or two mitotic divisions, and then undergo cell degeneration. The *duet* mutation is also caused by a *D*s insertion, which is approximately 500 amino acid residues downstream of the N-terminus and just upstream of the PHD finger. It is possible that the *duet* allele can produce a truncated protein that is partially functional. It is worth noting that in most plant meiotic mutants, the abnormal meiocytes do not undergo programmed cell death, unlike the meiotic mutants in animals. Therefore, the phenotypes of the *mmd1* mutant are rather unusually for a plant meiotic mutant, and may suggest a general regulatory function of the *MMD1* gene. The hypothesis that *MMD1* is a general regulator, rather a promoter of a specific meiotic event, is also supported by the lack of specific meiotic chromosomal defects in pairing, juxtaposition, synapsis, or segregation in the *mmd1* mutant.

Another potential regulatory gene was uncovered by the mutants *ms5* and *tdm1,* which are male sterile and defective in male meiosis (Chaudhury, 1993; Ross et al., 1997). Detailed phenotypic analysis of *tdm1* revealed that the mutant cells undergo meiosis I and II in a way similar to the wild-type cells; however, meiosis II is followed by an abnormal third division without a new round of DNA replication, resulting in the formation of polyads of 5-8 abnormal spores. Therefore, *MS5* normally regulates the completion of meiosis II by presenting another division. The T-DNA insertional *ms5-2/tdm1* mutation was used to clone the *MS5* gene (Glover et al., 1998), which is a member of a gene family conserved in plants, including multiple members in Arabidopsis and rice. The MS5 protein sequence is novel; although it has limited similarity to a synaptonemal complex protein from rat, it is not clear what the physiological significance of this similarity is, as the *ms5* mutant does not seem to have any defect in synapsis or other abnormal chromosomal behavior during prophase I.

Meiotic Cytokinesis

At the end of meiosis II, four clusters of chromosomes are formed and they become four new nuclei. Cell membranes and wall materials are then transported to the space between these nuclei and cytokinesis occurs to generate four haploid microspores. Using immuno-electron microscopy and electron tomography, Otegui and Staehelin (2004) showed that meiotic cytokinesis entails the formation of a distinctive post-meiotic-type cell plate. The formation of the post-meiotic cell plates depends on the function of mini-phragmoplasts and occurs simultaneously throughout the divisional plane. Presumably, vesicles carrying materials for cell plate are transported along microtubules of the mini-phragmoplast by microtubule associated motor kinesins. Fusion of these vesicles results in a tubular network, which fuses at its periphery with the plasma membrane, prior to the formation of the cell plate.

The Arabidopsis *stud* (*std*) and *tetraspore* (*tes*) mutants were found to have similar phenotypes and subsequently shown to be allelic (Hulskamp et al., 1997; Spielman et al., 1997). In the *std/tes* mutant meiocytes, meioses I and II proceed normally, but cytokinesis is defective and cell wall formation is incomplete. Consequently, a single microspore is formed with four nuclei that are partially separated by incomplete walls and a single cytoplasm. The abnormal microspore is able to continue through pollen development. In normal pollen development, the microspore first divides asymmetrically to form a large vegetative cell and a small generative cell, which is then internalized to be surrounded by the vegetative cells. The generative cell divides again inside the vegetative cell to form two sperm cells. During *std/tes* mutant pollen development within the large abnormal microspore, the four nuclei divide separately. The result is a very large pollen grain with four vegetative nuclei and up to eight sperm cells. Upon pollination, the large pollen grain produces a single pollen tube, allowing the fertilization of one ovule, resulting in greatly reduced male fertility (Hulskamp et al., 1997).

The *TES/STD* gene has been cloned using a mapped-based cloning strategy and it encodes a kinesin with an N-terminal motor domain (Yang et al., 2003a). The TES protein is most similar to two closely related tobacco kinesins, NACK1 and NACK2 (64% and 58% identity over the entire proteins, respectively), that are required for cell plate

expansion during cytokinesis (Nishihama et al., 2002). In addition, TES is highly similar (57% identity/73% similarity overall) to another Arabidopsis kinesin called HINKEL, which is involved in mitotic cytokinesis (Strompen et al., 2002). In the *tes* mutant meiocytes, the radial microtubule arrays that are found between the four meiotic nuclei at the end of meiosis II are not present, indicating that the TES kinesin is required for the formation and/or maintenance of such microtubule structures. In the absence of the microtubule arrays, material for cell plate formation cannot be properly distributed and cytokinesis is incomplete. This hypothesis is consistent with the known functions of NACK1/2 and HINKEL in mitotic cytokinesis (Nishihama et al., 2002; Strompen et al., 2002). The *TES* gene is expressed in both meiocytes and mitotically dividing cells, suggesting that it may also play a role in mitotic cytokinesis and possibly other processes. One explanation that the *tes/std* mutants are defective only in meiotic cytokinesis is that other (partially) redundant genes may provide the needed function in mitotic cell cycle, such as the *HINKEL* gene.

Concluding Remarks

The development of cytological tools for Arabidopsis by pioneers of plant meiosis has opened the door for the molecular genetic investigations of the meiotic machinery. Efforts by a number of laboratories over the last decade have identified key genes important for processes from early prophase I to meiotic cytokinesis, not only demonstrating conservation of gene functions between yeast and plants, possibly also with vertebrate animals, but also uncovering novel aspects of a molecular portrait of Arabidopsis meiosis. These studies have firmly established Arabidopsis as an excellent model system for understanding not only plant meiosis, but also meiosis in general. As it is already indicated by a number of genes, meiosis depends on not only single-copy meiosis-specific genes, such as *AtSPO11-1, AtDMC1,* and *SDS*, that are readily discovered by forward genetics or examined using reverse genetics, but also requires genes that are functionally redundant or are also essential for mitotic growth, including the *BRCA2* genes. It is likely that future efforts will greatly expand the ranks of this second category of genes important for meiosis.

While currently available tools are invaluable in understanding meiotic gene functions, additional technology need to be developed. Meiosis in Arabidopsis is usually analyzed using chromosome spreading, which may distort mutant phenotypes, including exaggeration and loss of some details. In maize, meiotic chromosomes can be observed in three-dimensional images using deconvolution microscopy (Franklin et al., 1999; Franklin et al., 2003; Pawlowski et al., 2003; Pawlowski et al., 2004); such technology should also be applicable to Arabidopsis meiocytes. In addition, markers for observing meiosis in living cells will go a long way to providing physiological information about this still mysterious process. The tools from the post-genomic era of Arabidopsis biology will also greatly facilitate the process of identification and characterization of new genes and proteins important for meiosis. Therefore, whereas the past decade has been nothing short of amazing, the future of meiosis research promises to be even more exciting and satisfying.

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References

Aasland, R, Gibson, TJ, and Stewart, AF (1995). The PHD finger: implications for chromatin-mediated transcriptional regulation. Trends Biochem. Sci. **20**, 56-59.

Abe, K, Osakabe, K, Nakayama, S, Endo, M, Tagiri, A, Todoriki, S, Ichikawa, H, and Toki, S (2005). Arabidopsis *RAD51C* gene is important for homologous recombination in meiosis and mitosis. Plant Physiol. **139**, 896-908.

Agashe, B, Prasad, CK, and Siddiqi, I (2002). Identification and analysis of *DYAD*: a gene required for meiotic chro-

mosome organisation and female meiotic progression in *Arabidopsis*. Development **129**, 3935-3943.

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

Alonso, JM, Stepanova, AN, Leisse, TJ, Kim, CJ, Chen, H, Shinn, P, Stevenson, DK, Zimmerman, J, Barajas, P, Cheuk, R, Gadrinab, C, Heller, C, Jeske, A, Koesema, E, Meyers, CC, Parker, H, Prednis, L, Ansari, Y, Choy, N, Deen, H, Geralt, M, Hazari, N, Hom, E, Karnes, M, Mulholland, C, Ndubaku, R, Schmidt, I, Guzman, P, Aguilar-Henonin, L, Schmid, M, Weigel, D, Carter, DE, Marchand, T, Risseeuw, E, Brogden, D, Zeko, A, Crosby, WL, Berry, CC, and Ecker, JR (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science **301**, 653-657.

Ambrose, JC, Li, W, Marcus, A, Ma, H, and Cyr, R (2005). A minus-end-directed kinesin with plus-end tracking protein activity is involved in spindle morphogenesis. Mol. Biol. Cell **16:** 1584-1592.

Anderson, LK, Stack, SM, and Sherman, JD (1988). Spreading synaptonemal complexes from *Zea mays*. I. No synaptic adjustment of inversion loops during pachytene. Chromosoma **96**, 295-305.

Anderson, LK, Hooker, KD, and Stack, SM (2001). The distribution of early recombination nodules on zygotene bivalents from plants. Genetics **159**, 1259-1269.

Anderson, LK, Stack, SM, Fox, MH, and Zhang, CS (1985). The relationship between genome size and synaptonemal complex length in higher plants. Exp. Cell Res. **156**, 367-378.

Anderson, LK, Offenberg, HH, Verkuijlen, WMHC, and Heyting, C (1997). RecA-like proteins are components of early meiotic nodules in lily. Proc. Natl. Acad. Sci. U. S. A. **94**, 6868-6873.

Anderson, LK, Salameh, N, Bass, HW, Harper, LC, Cande, WZ, Weber, G, and Stack, SM (2004). Integrating genetic linkage maps with pachytene chromosome structure in maize. Genetics **166**, 1923-1933.

Anderson, LK, Doyle, GG, Brigham, B, Carter, J, Hooker, KD, Lai, A, Rice, M, and Stack, SM (2003). High-resolution crossover maps for each bivalent of *Zea mays* using recombination nodules. Genetics **165**, 849-865.

Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408**, 796-815.

Armstrong, SJ, and Jones, GH (2001). Female meiosis in wild-type *Arabidopsis thialiana* and two meiotic mutants. Sex. Plant Reprod. **13,** 177-183.

Armstrong, SJ, and Jones, GH (2003). Meiotic cytology and chromosome behaviour in wild-type *Arabidopsis thaliana*. J. Exp. Bot. **54**, 1-10.

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

Armstrong, SJ, Caryl, AP, Jones, GH, and Franklin, FC (2002). ASY1, a protein required for meiotic chromosome synapsis, localizes to axis-associated chromatin in *Arabidopsis* and *Brassica*. J. Cell Sci. **115**, 3645-3655.

Ashley, T, Westphal, C, Plug-de Maggio, A, and de Rooij, DG (2004). The mammalian mid-pachytene checkpoint: meiotic arrest in spermatocytes with a mutation in *Atm* alone or in combination with a *Trp53* (*p53*) or *Cdkn1a* (*p21/cip1*) mutation. Cytogenet. Genome Res. **107**, 256-262.

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

Bai, C, Sen, P, Hofmann, K, Ma, L, Goebl, M, Harper, JW, and Elledge, SJ (1996). SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell **86**, 263-274.

Bai, XF, Peirson, BN, Dong, FG, Xue, C, and Makaroff, CA (1999). Isolation and characterization of *SYN1*, a *RAD21-like* gene essential for meiosis in Arabidopsis. Plant Cell **11**, 417-430.

Barlow, C, Liyanage, M, Moens, PB, Tarsounas, M, Nagashima, K, Brown, K, Rottinghaus, S, Jackson, SP, Tagle, D, Ried, T, and Wynshaw-Boris, A (1998). *Atm* deficiency results in severe meiotic disruption as early as leptonema of prophase I. Development **125**, 4007-4017.

Bass, HW, Bordoli, SJ, and Foss, EM (2003). The *desynaptic* (*dy*) and *desynaptic1* (*dys1*) mutations in maize (*Zea mays L.*) cause distinct telomere-misplacement phenotypes during meiotic prophase. J. Exp. Bot. **54**, 39-46.

Bass, HW, Marshall, WF, Sedat, JW, Agard, DA, and Cande, WZ (1997). Telomeres cluster de novo before the initiation of synapsis: A three-dimensional spatial analysis of telomere positions before and during meiotic prophase. J. Cell Biol. **137**, 5-18.

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

Bhatt, AM, Lister, C, Page, T, Fransz, P, Findlay, K, Jones, GH, Dickinson, HG, and Dean, C (1999). The *DIF1* gene of *Arabidopsis* is required for meiotic chromosome segregation and belongs to the *REC8/RAD21* cohesin gene family. Plant J. **19**, 463-472.

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

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

Bishop, DK, Ear, U, Bhattacharyya, A, Calderone, C, Beckett, M, Weichselbaum, RR, and Shinohara, A (1998). Xrcc3 is required for assembly of Rad51 complexes in vivo. J. Biol. Chem. **273**, 21482-21488.

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

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

Bleuyard, JY, Gallego, ME, and White, CI (2004b). Meiotic defects in the *Arabidopsis rad50* mutant point to conservation of the MRX complex function in early stages of meiotic recombination. Chromosoma **113**, 197-203.

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

Bocker, T, Barusevicius, A, Snowden, T, Rasio, D, Guerrette, S, Robbins, D, Schmidt, C, Burczak, J, Croce, CM, Copeland, T, Kovatich, AJ, and Fishel, R (1999). hMSH5: a human MutS homologue that forms a novel heterodimer with hMSH4 and is expressed during spermatogenesis. Cancer Res. **59**, 816-822.

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

Braybrooke, JP, Spink, KG, Thacker, J, and Hickson, ID (2000). The RAD51 family member, RAD51L3, is a DNA-stimulated ATPase that forms a complex with XRCC2. J. Biol. Chem. **275**, 29100-29106.

Brenneman, MA, Weiss, AE, Nickoloff, JA, and Chen, DJ (2000). *XRCC3* is required for efficient repair of chromosome breaks by homologous recombination. Mutat. Res. **459**, 89-97.

Bundock, P, and Hooykaas, P (2002). Severe developmental defects, hypersensitivity to DNA-damaging agents, and lengthened telomeres in Arabidopsis *mre11* mutants. Plant Cell **14**, 2451-2462.

Cai, X, and Makaroff, CA (2001). The *dsy10* mutation of *Arabidopsis* results in desynapsis and a general breakdown in meiosis. Sex. Plant Reprod. **14**, 63-67.

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

Carpenter, AT (1975). Electron microscopy of meiosis in *Drosophila* melanogaster females: II. The recombination nodule: a recombination-associated structure at pachytene? Proc. Natl. Acad. Sci. U. S. A. **72**, 3186-3189.

Carpenter, AT (1987). Gene conversion, recombination nodules, and the initiation of meiotic synapsis. Bioessays 6, 232-236.

Cartwright, R, Tambini, CE, Simpson, PJ, and Thacker, J (1998). The *XRCC2* DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family. Nucleic Acids Res. **26**, 3084-3089.

Caryl, AP, Jones, GH, and Franklin, FC (2003). Dissecting plant meiosis using *Arabidopsis thaliana* mutants. J. Exp. Bot. **54**, 25-38.

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

Celerin, M, Merino, ST, Stone, JE, Menzie, AM, and Zolan, ME (2000). Multiple roles of Spo11 in meiotic chromosome behavior. EMBO J. **19**, 2739-2750.

Cervantes, MD, Farah, JA, and Smith, GR (2000). Meiotic DNA breaks associated with recombination in *S. pombe*. Mol. Cell **5**: 883-888.

Chaudhury, AM (1993). Nuclear genes controlling male fertility. Plant Cell **5,** 1277-1283.

Chelysheva, L, Diallo, S, Vezon, D, Gendrot, G, Vrielynck, N, Belcram, K, Rocques, N, Marquez-Lema, A, Bhatt, AM, Horlow, C, Mercier, R, Mezard, C, and Grelon, M (2005). AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. J. Cell Sci. **118**, 4621-4632.

Chen, C, Zhang, W, Timofejeva, L, Gerardin, Y, and Ma, H (2005). The *Arabidopsis ROCK-N-ROLLERS* gene encodes a homolog of the yeast ATP-dependent DNA helicase MER3 and is required for normal meiotic crossover formation. Plant J. **43**, 321-334.

Chen, C, Marcus, AI, Li, WX, Hu, Y, Calzada, JP, Grossniklaus, U, Cyr, RJ, and Ma, H (2002). The *Arabidopsis ATK1* gene is required for spinlde morphogenesis in male meiosis. Development **129**, 2401-2409.

Chen, J, Silver, DP, Walpita, D, Cantor, SB, Gazdar, AF, Tomlinson, G, Couch, FJ, Weber, BL, Ashley, T, Livingston, DM, and Scully, R (1998). Stable interaction between the products of the *BRCA1* and *BRCA2* tumor suppressor genes in mitotic and meiotic cells. Mol. Cell **2**, 317-328.

Chen, JJ, Silver, D, Cantor, S, Livingston, DM, and Scully, R (1999). BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway. Cancer Res **59**, 1752s-1756s.

Collis, SJ, Tighe, A, Scott, SD, Roberts, SA, Hendry, JH, and Margison, GP (2001). Ribozyme minigene-mediated *RAD51* down-regulation increases radiosensitivity of human prostate cancer cells. Nucleic Acids Res. **29**, 1534-1538.

Connelly, C, and Hieter, P (1996). Budding yeast *SKP1* encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell **86**, 275-285.

Connelly, JC, and Leach, DR (2002). Tethering on the brink: the evolutionarily conserved Mre11-Rad50 complex. Trends Biochem. Sci. **27**, 410-418.

Copenhaver, G, Browne, W, and Preuss, D (1997). Measuring genome-wide recombination and centromere functions in Arabidopsis meiosis. Mol. Biol. Cell **8**, 1557-1557.

Copenhaver, GP, Housworth, EA, and Stahl, FW (2002). Crossover interference in *Arabidopsis*. Genetics **160**, 1631-1639.

Coscoy, L, and Ganem, D (2003). PHD domains and E3 ubiquitin ligases: viruses make the connection. Trends Cell Biol. **13**, 7-12.

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

Cox, MM (1999). Recombinational DNA repair in bacteria and the RecA protein. Prog. Nucleic Acid Res. Mol. Biol. **63**, 311-366.

Cressman, VL, Backlund, DC, Avrutskaya, AV, Leadon, SA, Godfrey, V, and Koller, BH (1999). Growth retardation, DNA repair defects, and lack of spermatogenesis in *BRCA1-deficient* mice. Mol. Cell. Biol. **19**, 7061-7075.

Dahmann, C, and Futcher, B (1995). Specialization of B-type cyclins for mitosis or meiosis in *S. cerevisiae*. Genetics **140**, 957-963.

Dawe, RK (1998). Meiotic chromosome organization and segregation in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49,** 371-395.

Dawe, RK, and Cande, WZ (1996). Induction of centromeric activity in maize by *suppressor of meiotic drive 1.* Proc. Natl. Acad. Sci. USA **93**, 8512-8517.

Dawe, RK, Sedat, JW, Agard, DA, and Cande, WZ (1994). Meiotic chromosome pairing in maize is associated with a novel chromatin organization. Cell **76**, 901-912.

de los Santos, T, Hunter, N, Lee, C, Larkin, B, Loidl, J, and 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.

De Souza, CP, Osmani, AH, Wu, LP, Spotts, JL, and Osmani, SA (2000). Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*. Cell **102**, 293-302.

de Vries, SS, Baart, EB, Dekker, M, Siezen, A, de Rooij, DG, de Boer, P, and te Riele, H (1999). Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. Genes Dev. **13**, 523-531.

Deans, B, Griffin, CS, Maconochie, M, and Thacker, J (2000). *Xrcc2* is required for genetic stability, embryonic neurogenesis and viability in mice. EMBO J. **19**, 6675-6685.

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

Ding, ZJ, Wang, T, Chong, K, and Bai, SN (2001). Isolation and characterization of *OsDMC1*, the rice homologue of the yeast *DMC1* gene essential for meiosis. Sex. Plant Reprod. **13**, 285-288.

Dirick, L, Goetsch, L, Ammerer, G, and Byers, B (1998). Regulation of meiotic S phase by Ime2 and a Clb5,6-associated kinase in *Saccharomyces cerevisiae*. Science **281**, 1854-1857.

Dong, F, Cai, X, and Makaroff, CA (2001). Cloning and characterization of two *Arabidopsis* genes that belong to the RAD21/REC8 family of chromosome cohesin proteins. Gene **271**, 99-108.

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

Doutriaux, MP, Couteau, F, Bergounioux, C, and White, C (1998). Isolation and characterisation of the *RAD51* and *DMC1* homologs from *Arabidopsis thaliana*. Mol. Gen. Genet. **257**, 283-291.

Emmanuel, E, Yehuda, E, Melamed-Bessudo, C, Avivi-Ragolsky, N, and Levy, AA (2006). The role of *AtMSH2* in homologous recombination in *Arabidopsis thaliana.* EMBO Rep. **7**, 100-105

Endow, SA, Chandra, R, Komma, DJ, Yamamoto, AH, and Salmon, ED (1994). Mutants of the *Drosophila* ncd microtubule motor protein cause centrosomal and spindle pole defects in mitosis. J. Cell Sci. **107**, 859-867.

Esposito, MS, and Esposito, RE (1969). The genetic control of sporulation in *Saccharomyces*. I. The isolation of temperature-sensitive sporulation-deficient mutants. Genetics **61**, 79-89.

Fortin, GS, and Symington, LS (2002). Mutations in yeast *Rad51* that partially bypass the requirement for *Rad55* and *Rad57* in DNA repair by increasing the stability of Rad51-DNA complexes. EMBO J. **21**, 3160-3170.

Francisco, L, and Chan, CS (1994). Regulation of yeast chromosome segregation by Ipl1 protein kinase and type 1 protein phosphatase. Cell Mol. Biol. Res. **40**, 207-213.

Francisco, L, Wang, W, and Chan, CS (1994). Type 1 protein phosphatase acts in opposition to IpL1 protein kinase in regulating yeast chromosome segregation. Mol. Cell Biol. **14**, 4731-4740.

Franklin, AE, and Cande, WZ (1999). Nuclear organization and chromosome segregation. Plant Cell **11**, 523-534.

Franklin, AE, Golubovskaya, IN, Bass, HW, and Cande, WZ (2003). Improper chromosome synapsis is associated with elongated RAD51 structures in the maize *desynaptic2* mutant. Chromosoma **112**, 17-25.

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

Fransz, P, Armstrong, S, AlonsoBlanco, C, Fischer, TC, TorresRuiz, RA, and Jones, G (1998). Cytogenetics for the model system *Arabidopsis thaliana*. Plant J. **13**, 867-876.

Fransz, PF, Armstrong, S, de Jong, JH, Parnell, LD, van Drunen, C, Dean, C, Zabel, P, Bisseling, T, and Jones, GH (2000). Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: structural organization of heterochromatic knob and centromere region. Cell **100**, 367-376.

Futcher, AB (1991). *Saccharomyces cerevisiae* cell cycle: cdc28 and the G1 cyclins. Semin. Cell Biol. **2,** 205-212.

Gallego, ME, Jeanneau, M, Granier, F, Bouchez, D, Bechtold, N, and White, CI (2001). Disruption of the *Arabidopsis RAD50* gene leads to plant sterility and MMS sensitivity. Plant J. **25**, 31-41.

Game, JC (1993). DNA double-strand breaks and the *RAD50-RAD57* genes in *Saccharomyces*. Semin. Cancer Biol. **4**, 73-83.

Garcia, V, Salanoubat, M, Choisne, N, and Tissier, A (2000). An ATM homologue from *Arabidopsis thaliana*: complete genomic organisation and expression analysis. Nucleic Acids Res. **28**, 1692-1699.

Garcia, V, Bruchet, H, Camescasse, D, Granier, F, Bouchez, D, and Tissier, A (2003). *AtATM* is essential for meiosis and the somatic response to DNA damage in plants. Plant Cell **15**, 119-132.

Glover, J, Grelon, M, Craig, S, Chaudhury, A, and Dennis, E (1998). Cloning and characterization of *MS5* from

Arabidopsis: a gene critical in male meiosis. Plant J. **15**, 345-356.

Golubovskaya, I, Avalkina, N, and Sheridan, WF (1997). New insights into the role of the maize ameiotic1 locus. Genetics **147**, 1339-1350.

Golubovskaya, I, Grebennikova, ZK, Avalkina, NA, and Sheridan, WF (1993). The role of the *AMEIOTIC1* gene in the initiation of meiosis and in subsequent meiotic events in maize. Genetics **135**, 1151-1166.

Golubovskaya, IN (1979). Genetic control of meiosis. Int. Rev. Cytol. **58**, 247-290.

Golubovskaya, IN (1989). Meiosis in maize: *mei* genes and conception of genetic control of meiosis. In Adv. Genet., JG Scandalios, ed (San Diego: Academic Press), pp. 149-192.

Golubovskaya, IN, Harper, LC, Pawlowski, WP, Schichnes, D, and Cande, WZ (2002). The *PAM1* gene is required for meiotic bouquet formation and efficient homologous synapsis in maize (*Zea mays L.*). Genetics **162**, 1979-1993.

Grandin, N, and Reed, SI (1993). Differential function and expression of *Saccharomyces cerevisiae* B-type cyclins in mitosis and meiosis. Mol. Cell Biol. **13**, 2113-2125.

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

Grelon, M, Gendrot, G, Vezon, D, Pelletier, G, Mathilde, G, Ghislaine, G, Daniel, V, and Georges, P (2003). The *Arabidopsis MEI1* gene encodes a protein with five BRCT domains that is involved in meiosis-specific DNA repair events independent of SPO11-induced DSBs. Plant J. **35**, 465-475.

Haber, JE (1998). The many interfaces of Mre11. Cell **95**, 583-586.

Haber, JE (2000). Recombination: a frank view of exchanges and vice versa. Curr. Opin. Cell Biol. **12**, 286-292.

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

Habu, T, Taki, T, West, A, Nishimune, Y, and Morita, T (1996). The mouse and human homologs of *DMC1*, the yeast meiosis-specific homologous recombination gene, have a common unique form of exon-skipped transcript in meiosis. Nucleic Acids Res. **24**, 470-477.

Hamant, O, Ma, H, and Cande, WZ (2006). Genetics of meiotic prophase I in plants. Annu. Rev. Plant Biol. **57**, 267-302.

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

Hartung, F, and Puchta, H (1999). Isolation of the complete cDNA of the *Mre11* homologue of Arabidopsis (accession No. AJ243822) indicates conservation of DNA recombination mechanisms between plants and other eukaryotes. Plant Physiol. **121**, 312.

Hartung, F, and Puchta, H (2000). Molecular characterisation of two paralogous SPO11 homologues in *Arabidopsis thaliana*. Nuc. Acids Res. **28**, 1548-1554.

Hartung, F, and Puchta, H (2001). Molecular characterization of homologues of both subunits A (SPO11) and B of the archaebacterial topoisomerase 6 in plants. Gene **271**, 81-86.

Hatsumi, M, and Endow, SA (1992). The *Drosophila* ncd microtubule motor protein is spindle-associated in meiotic and mitotic cells. J. Cell Sci. **103**, 1013-1020.

Havre, PA, Rice, M, Ramos, R, and Kmiec, EB (2000). HsRec2/Rad51L1, a protein influencing cell cycle progression, has protein kinase activity. Exp. Cell Res. **254**, 33-44.

Hays, SL, Firmenich, AA, and Berg, P (1995). Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. Proc. Natl. Acad. Sci. U. S. A. **92**, 6925-6929.

He, CP, and Mascarenhas, JP (1998). *MEI1*, an *Arabidopsis* gene required for male meiosis: Isolation and characterization. Sex. Plant Reprod. **11**, 199-207.

Henning, W, and Sturzbecher, HW (2003). Homologous recombination and cell cycle checkpoints: Rad51 in tumour progression and therapy resistance. Toxicology **193**, 91-109.

Heo, SJ, Tatebayashi, K, and Ikeda, H (1999). The budding yeast cohesin gene *SCC1/MCD1/RHC21* genetically interacts with *PKA, CDK* and *APC*. Curr. Genet. **36**, 329-338.

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

Hirano, T (2000). Chromosome cohesion, condensation and separation. Annu. Rev. Biochem. **69**, 115-144.

Hirano, T (2002). The ABCs of SMC proteins: two-armed ATPases for chromosome condensation, cohesion, and repair. Genes Dev. **16,** 399-414.

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

Hollingsworth, NM, Goetsch, L, and Byers, B (1990). The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell **61**, 73-84.

Hollingsworth, NM, Ponte, L, and Halsey, C (1995). *MSH5*, a novel *MutS* homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev. **9**, 1728-1739.

Housworth, EA, and Stahl, FW (2003). Crossover interference in humans. Am. J. Hum. Genet. **73**, 188-197.

Hsu, JY, Sun, ZW, Li, X, Reuben, M, Tatchell, K, Bishop, DK, Grushcow, JM, Brame, CJ, Caldwell, JA, Hunt, DF, Lin, R, Smith, MM, and Allis, CD (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. Cell **102**, 279-291.

Hulskamp, M, Parekh, NS, Grini, P, Schneitz, K, Zimmermann, I, Lolle, SJ, and Pruitt, RE (1997). The *STUD* gene is required for male-specific cytokinesis after

telophase II of meiosis in *Arabidopsis thaliana*. Dev. Biol. **187**, 114-124.

Kans, JA, and Mortimer, RK (1991). Nucleotide sequence of the *RAD57* gene of *Saccharomyces cerevisiae*. Gene **105**, 139-140.

Kaszas, E, and Cande, WZ (2000). Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of the chromatin. J. Cell Sci. **113**, 3217-3226.

Kaul, MLH, and Murphy, TGK (1985). Mutant genes affecting higher plant meiosis. Thero. Appl. Genet. 70, 449-466.

Keeney, S (2001). Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. **52**, 1-53.

Keeney, S, Giroux, CN, and Kleckner, N (1997). Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88**, 375-384.

Keeney, S, Baudat, F, Angeles, M, Zhou, ZH, Copeland, NG, Jenkins, NA, Manova, K, and Jasin, M (1999). A mouse homolog of the *Saccharomyces cerevisiae* meiotic recombination DNA transesterase Spo11p. Genomics **61**, 170-182.

Kennison, JA (1995). The Polycomb and trithorax group proteins of *Drosophila*: trans-regulators of homeotic gene function. Annu. Rev. Genet. **29**, 289-303.

Khanna, KK, and Jackson, SP (2001). DNA double-strand breaks: signaling, repair and the cancer connection. Nat. Genet. **27**, 247-254.

Kim, PM, Allen, C, Wagener, BM, Shen, Z, and Nickoloff, JA (2001). Overexpression of human *RAD51* and *RAD52* reduces double-strand break-induced homologous recombination in mammalian cells. Nucleic Acids Res. **29**, 4352-4360.

Klein, F, Mahr, P, Galova, M, Buonomo, SB, Michaelis, C, Nairz, K, and Nasmyth, K (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell **98,** 91-103.

Klimyuk, VI, and Jones, JD (1997). *AtDMC1*, the *Arabidopsis* homologue of the yeast *DMC1* gene characterization, transposon-induced allelic variation and meiosis-associated expression. Plant J. **11,** 1-14.

Kneitz, B, Cohen, PE, Avdievich, E, Zhu, L, Kane, MF, Hou, H, Jr., Kolodner, RD, Kucherlapati, R, Pollard, JW, and Edelmann, W (2000). MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. Genes Dev. **14**, 1085-1097.

Kong, HZ, Leebens-Mack, J, Ni, W, dePamphilis, CW, and Ma, H (2004). Highly heterogeneous rates of evolution in the *SKP1* gene family in animals and plants: functional and evolutionary implications. Mol. Biol. Evol. **21**, 117-128.

Koonin, EV, Altschul, SF, and Bork, P (1996). BRCA1 protein products ... Functional motifs. Nat Genet **13**, 266-268.

Kurumizaka, H, Ikawa, S, Nakada, M, Eda, K, Kagawa, W, Takata, M, Takeda, S, Yokoyama, S, and Shibata, T (2001). Homologous-pairing activity of the human DNA-repair proteins Xrcc3 Rad51C. Proc. Natl. Acad. Sci. U. S. A. **98**, 5538-5543.

Lam, SY, Horn, SR, Radford, SJ, Housworth, EA, Stahl, FW, and Copenhaver, GP (2005a). Crossover interference on nucleolus organizing region-bearing chromosomes in *Arabidopsis*. Genetics **170**, 807-812.

Lam, WS, Yang, X, and Makaroff, CA (2005b). Characterization of *Arabidopsis thaliana* SMC1 and SMC3: evidence that AtSMC3 may function beyond chromosome cohesion. J. Cell Sci. **118**, 3037-3048.

Lambert, S, and Lopez, BS (2002). Inactivation of the *RAD51* recombination pathway stimulates UV-induced mutagenesis in mammalian cells. Oncogene **21**, 4065-4069.

Leu, JY, Chua, PR, and Roeder, GS (1998). The meiosis-specific Hop2 protein of *S. cerevisiae* ensures synapsis between homologous chromosomes. Cell **94**, 375-386.

Li, MJ, and Maizels, N (1997). Nuclear Rad51 foci induced by DNA damage are distinct from Rad51 foci associated with B cell activation and recombination. Exp. Cell Res. **237**, 93-100.

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

Li, W, Yang, X, Lin, Z, Timofejeva, L, Xiao, R, Makaroff, CA, and Ma, H (2005). The *AtRAD51C* gene is required for normal meiotic chromosome synapsis and double-stranded break repair in Arabidopsis. Plant Physiol. 138, 965-976.

Lichten, M (2001). Meiotic recombination: breaking the genome to save it. Curr. Biol. **11**, R253-256.

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

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

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

Liu, B, Cyr, RJ, and Palevitz, BA (1996). A kinesin-like protein, KatAp, in the cells of Arabidopsis and other plants. Plant Cell 8, 119-132.

Liu, CM, and Meinke, DW (1998). The *titan* mutants of Arabidopsis are disrupted in mitosis and cell cycle control during seed development. Plant J. **16**, 21-31.

Liu, CM, McElver, J, Tzafrir, I, Joosen, R, Wittich, P, Patton, D, Van Lammeren, AA, and Meinke, D (2002a). Condensin and cohesin knockouts in Arabidopsis exhibit a titan seed phenotype. Plant J. **29**, 405-415.

Liu, D, Matzuk, MM, Sung, WK, Guo, Q, Wang, P, and Wolgemuth, DJ (1998a). Cyclin A1 is required for meiosis in the male mouse. Nat. Genet. **20**, 377-380.

Liu, F, Ni, W, Griffith, ME, Huang, Z, Chang, C, Peng, W, Ma, H, and Xie, D (2004). The *ASK1* and *ASK2* genes are essential for Arabidopsis early development. Plant Cell **16**, 5-20.

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

Liu, N, Lamerdin, JE, Tebbs, RS, Schild, D, Tucker, JD, Shen, MR, Brookman, KW, Siciliano, MJ, Walter, CA, Fan, W, Narayana, LS, Zhou, ZQ, Adamson, AW, Sorensen, KJ, Chen, DJ, Jones, NJ, and Thompson, LH (1998b). XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol. Cell **1**, 783-793.

Losada, A, Hirano, M, and Hirano, T (1998). Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. Genes Dev. **12**, 1986-1997.

Losada, A, Yokochi, T, Kobayashi, R, and Hirano, T (2000). Identification and characterization of SA/Scc3p subunits in the Xenopus and human cohesin complexes. J. Cell Biol. **150**, 405-416.

Lovett, ST (1994). Sequence of the RAD55 gene of *Saccharomyces cerevisiae*: similarity of RAD55 to prokaryotic RecA and other RecA-like proteins. Gene **142**, 103-106.

Lundin, C, Schultz, N, Arnaudeau, C, Mohindra, A, Hansen, LT, and Helleday, T (2003). RAD51 is involved in repair of damage associated with DNA replication in mammalian cells. J. Mol. Biol. **328**, 521-535.

Ma, H (2005). Molecular genetic analysis of microsporogenesis and microgametogenesis. Annu. Rev. Plant Biol. 56, 393-434.

Magnard, JL, Yang, M, Chen, YCS, Leary, M, and McCormick, S (2001). The Arabidopsis gene *Tardy Asynchronous Meiosis* is required for the normal pace and synchrony of cell division during male meiosis. Plant Physiol. **127**, 1157-1166.

Maloisel, L, Bhargava, J, and Roeder, GS (2004). A role for DNA polymerase delta in gene conversion and crossing over during meiosis in *Saccharomyces cerevisiae*. Genetics **167**, 1133-1142.

Marcus, AI, Li, W, Ma, H, and Cyr, RJ (2003). A kinesin mutant with an atypical bipolar spindle undergoes normal mitosis. Mol. Biol. Cell **14**, 1717-1726.

Marcus, AI, Ambrose, JC, Blickley, L, Hancock, WO, and Cyr, RJ (2002). *Arabidopsis thaliana* protein, ATK1, is a minus-end directed kinesin that exhibits non-processive movement. Cell Motil. Cytosk. **52**, 144-150.

Marmorstein, LY, Ouchi, T, and Aaronson, SA (1998). The *BRCA2* gene product functionally interacts with p53 and RAD51. Proc. Natl. Acad. Sci. U. S. A. **95**, 13869-13874.

Masson, JY, and West, SC (2001). The Rad51 and Dmc1 recombinases: a non-identical twin relationship. Trends Biochem. Sci. **26**, 131-136.

Masson, JY, Stasiak, AZ, Stasiak, A, Benson, FE, and West, SC (2001). Complex formation by the human RAD51C and XRCC3 recombination repair proteins. Proc. Natl. Acad. Sci. U. S. A. **98**, 8440-8446.

Masson, JY, Davies, AA, Hajibagheri, N, Van Dyck, E, Benson, FE, Stasiak, AZ, Stasiak, A, and West, SC (1999). The meiosis-specific recombinase hDmc1 forms ring structures and interacts with hRad51. EMBO J. **18**, 6552-6560.

Mazina, OM, Mazin, AV, Nakagawa, T, Kolodner, RD, and Kowalczykowski, SC (2004). *Saccharomyces cerevisiae* Mer3 helicase stimulates 3'-5' heteroduplex extension by Rad51; implications for crossover control in meiotic recombination. Cell **117**, 47-56.

McEntee, K, Weinstock, GM, and Lehman, IR (1980). recA protein-catalyzed strand assimilation: stimulation by *Escherichia coli* single-stranded DNA-binding protein. Proc. Natl. Acad. Sci. U. S. A. **77**, 857-861.

McKim, KS, Green-Marroquin, BL, Sekelsky, JJ, Chin, G, Steinberg, C, Khodosh, R, and Hawley, RS (1998). Meiotic synapsis in the absence of recombination. Science **279**, 876-878.

Mercier, R, Vezon, D, Bullier, E, Motamayor, JC, Sellier, A, Lefevre, F, Pelletier, G, and Horlow, C (2001). SWITCH1 (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. Genes Dev. **15**, 1859-1871.

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

Mercier, R, Jolivet, S, Vezon, D, Huppe, E, Chelysheva, L, Giovanni, M, Nogue, F, Doutriaux, MP, Horlow, C, Grelon, M, and Mezard, C (2005). Two meiotic crossover classes cohabit in *Arabidopsis*: one is dependent on MER3, whereas the other one is not. Curr. Biol. **15**, 692-701.

Meyerowitz, EM (1987). *Arabidopsis thaliana*. Ann. Rev. Genet. **21**, 93-111.

Meyerowitz, EM (1989). Arabidopsis, a useful weed. Cell **56**, 263-269.

Michaelis, C, Ciosk, R, and Nasmyth, K (1997). Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. Cell **91**, 35-45.

Mitsui, H, Yamaguchi-Shinozaki, K, Shinozaki, K, Nishikawa, K, and Takahashi, H (1993). Identification of a gene family (*kat*) encoding kinesin-like proteins in *Arabidopsis thaliana* and the characterization of secondary structure of KatA. Mol. Gen. Genet. **238**, 362-368.

Moens, PB (1969). Genetic and cytological effects f three desynaptic genes in the tomato. Can J. Genet. Cytol. 11, 857-869.

Murray, AW (1994). Cyclin-dependent kinases: regulators of the cell cycle and more. Chem. Biol. **1**, 191-195.

Nakagawa, T, and Ogawa, H (1999). The *Saccharomyces cerevisiae MER3* gene, encoding a novel helicase-like protein, is required for crossover control in meiosis. EMBO J. **18,** 5714-5723.

Nakagawa, T, and Kolodner, RD (2002a). *Saccharomyces cerevisiae* Mer3 is a DNA helicase involved in meiotic crossing over. Mol. Cell Biol. **22**, 3281-3291.

Nakagawa, T, and Kolodner, RD (2002b). The MER3 DNA helicase catalyzes the unwinding of holliday junctions. J. Biol. Chem. **277**, 28019-28024.

Nasmyth, K (1993). Control of the yeast cell cycle by the cdc28 protein kinase. Curr. Opin. Cell Biol. **5**, 166-179.

Nasmyth, K (1999). Separating sister chromatids. Trends Biochem. Sci. **24**, 98-104.

Neuffer, MG, Coe, EH, and Wessler, SR (1997). Mutants of maize. (Cold Spring Harbor: Cold Spring Harbor Laboratory Press).

Nishihama, R, Soyano, T, Ishikawa, M, Araki, S, Tanaka, H, Asada, T, Irie, K, Ito, M, Terada, M, Banno, H, Yamazaki, Y, and Machida, Y (2002). Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/MAPKKK complex. Cell **109,** 87-99.

Nonomura, KI, Nakano, M, Murata, K, Miyoshi, K, Eiguchi, M, Miyao, A, Hirochika, H, and Kurata, N (2004). An insertional mutation in the rice *PAIR2* gene, the ortholog of *Arabidopsis ASY1,* results in a defect in homologous chromosome pairing during meiosis. Mol. Genet. Genomics **271**, 121-129.

Novak, JE, Ross-Macdonald, PB, and Roeder, GS (2001). The budding yeast Msh4 protein functions in chromosome synapsis and the regulation of crossover distribution. Genetics **158**, 1013-1025.

Orr-Weaver, TL (1999). The ties that bind: Localization of the sister-chromatid cohesin complex on yeast chromosomes. Cell **99**, 1-4.

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

Osakabe, K, Abe, K, Yamanouchi, H, Takyuu, T, Yoshioka, T, Ito, Y, Kato, T, Tabata, S, Kurei, S, Yoshioka, Y, Machida, Y, Seki, M, Kobayashi, M, Shinozaki, K, Ichikawa, H, and Toki, S (2005). *Arabidopsis* Rad51B is important for double-strand DNA breaks repair in somatic cells. Plant Mol. Biol. **57**, 819-833.

Otegui, MS, and Staehelin, LA (2004). Electron tomographic analysis of post-meiotic cytokinesis during pollen development in *Arabidopsis thaliana*. Planta **218**, 501-515.

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

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

Parinov, S, Sevugan, M, Ye, D, Yang, WC, Kumaran, M, and Sundaresan, V (1999). Analysis of flanking sequences from dissociation insertion lines: a database for reverse genetics in Arabidopsis. Plant Cell **11**, 2263-2270.

Parisi, S, McKay, MJ, Molnar, M, Thompson, MA, vanderSpek, PJ, vanDrunenSchoenmaker, E, Kanaar, R, Lehmann, E, Hoeijmakers, JHJ, and Kohli, J (1999). Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. Mol. Cell. Biol. **19**, 3515-3528.

Pawlowski, WP, and Cande, WZ (2005). Coordinating the events of the meiotic prophase. Trends Cell Biol. **15**, 674-681.

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

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

Peirson, BN, Bowling, SE, and Makaroff, CA (1997). A defect in synapsis causes male sterility in a T-DNA-tagged *Arabidopsis thaliana* mutant. Plant J. **11**, 659-669.

Peirson, BN, Owen, HA, Feldmann, KA, and Makaroff, CA (1996). Characterization of three male-sterile mutants of *Arabidopsis thaliana* exhibiting alterations in meiosis. Sex. Plant Reprod. **9**, 1-16.

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

Peters, JM (1998). SCF and APC: the Yin and Yang of cell cycle regulated proteolysis. Curr. Opin. Cell Biol. **10**, 759-768.

Peterson, DG, Lapitan, NL, and Stack, SM (1999). Localization of single- and low-copy sequences on tomato synaptonemal complex spreads using fluorescence in situ hybridization (FISH). Genetics **152**, 427-439.

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

Pierce, AJ, Johnson, RD, Thompson, LH, and Jasin, M (1999). XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev. **13**, 2633-2638.

Pittman, DL, and Schimenti, JC (2000). Midgestation lethality in mice deficient for the *RecA*-related gene, *Rad51d/Rad51l3*. Genesis **26**, 167-173.

Pittman, DL, Cobb, J, Schimenti, KJ, Wilson, LA, Cooper, DM, Brignull, E, Handel, MA, and Schimenti, JC (1998). Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for *Dmc1*, a germline-specific RecA homolog. Mol. Cell **1,** 697-705.

Puizina, J, Siroky, J, Mokros, P, Schweizer, D, and Riha, K (2004). Mre11 deficiency in Arabidopsis is associated with chromosomal instability in somatic cells and Spo11-dependent genome fragmentation during meiosis. Plant Cell **16**, 1968-1978.

Reddy, TV, Kaur, J, Agashe, B, Sundaresan, V, and Siddiqi, I (2003). The *DUET* gene is necessary for chromosome organization and progression during male meiosis in *Arabidopsis* and encodes a PHD finger protein. Development **130**, 5975-5987.

Reed, SI (1996). G1/S regulatory mechanisms from yeast to man. Prog. Cell Cycle Res. **2**, 15-27.

Rinaldo, C, Ederle, S, Rocco, V, and La Volpe, A (1998). The *Caenorhabditis elegans RAD51* homolog is transcribed into two alternative mRNAs potentially encoding proteins of different sizes. Mol. Gen. Genet. **260**, 289-294.

Rinaldo, C, Bazzicalupo, P, Ederle, S, Hilliard, M, and La Volpe, A (2002). Roles for *Caenorhabditis elegans rad-51*

in meiosis and in resistance to ionizing radiation during development. Genetics **160**, 471-479.

Roeder, GS (1990). Chromosome synapsis and genetic recombination: their roles in meiotic chromosome segregation. Trends Genet. **6,** 385-389.

Romanienko, PJ, and Camerini-Otero, RD (1999). Cloning, characterization, and localization of mouse and human *SPO11*. Genomics **61**, 156-169.

Romanienko, PJ, and Camerini-Otero, RD (2000). The mouse *Spo11* gene is required for meiotic chromosome synapsis. Mol. Cell **6**, 975-987.

Roof, DM, Meluh, PB, and Rose, MD (1992). Kinesin-related proteins required for assembly of the mitotic spindle. J. Cell Biol. **118**, 95-108.

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

Ross, KJ, Fransz, P, Armstrong, SJ, Vizir, I, Mulligan, B, Franklin, FCH, and Jones, GH (1997). Cytological characterization of four meiotic mutants of *Arabidopsis* isolated from T-DNA-transformed lines. Chromosome Res. **5**, 551-559.

Rotman, G, and Shiloh, Y (1998). ATM: from gene to function. Hum. Mol. Genet. **7,** 1555-1563.

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

Sanders, PM, Bui, AQ, Weterings, K, McIntire, KN, Hsu, YC, Lee, PY, Truong, MT, Beals, TP, and Goldberg, RB (1999). Anther developmental defects in *Arabidopsis thaliana* male-sterile mutants. Sex. Plant Reprod. **11**, 297-322.

Sato, S, Hotta, Y, and Tabata, S (1995). Structural analysis of a *recA*-like gene in the genome of *Arabidopsis thaliana*. DNA Res. **2**, 89-93.

Savitsky, K, Sfez, S, Tagle, DA, Ziv, Y, Sartiel, A, Collins, FS, Shiloh, Y, and Rotman, G (1995). The complete sequence of the coding region of the *ATM* gene reveals similarity to cell cycle regulators in different species. Hum. Mol. Genet. **4,** 2025-2032.

Scherthan, H (2001). A bouquet makes ends meet. Nat. Rev. Mol. Cell. Biol. **2,** 621-627.

Scherthan, H, Weich, S, Schwegler, H, Heyting, C, Haerle, M, and Cremer, T (1996). Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing. J. Cell Biol. **134**, 1109-1125.

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

Schommer, C, Beven, A, Lawrenson, T, Shaw, P, and Sablowski, R (2003). *AHP2* is required for bivalent formation and for segregation of homologous chromosomes in *Arabidopsis* meiosis. Plant J. **36**, 1-11.

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

Scully, R, Chen, J, Plug, A, Xiao, Y, Weaver, D, Feunteun, J, Ashley, T, and Livingston, DM (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell **88**, 265-275.

Sharan, SK, Morimatsu, M, Albrecht, U, Lim, DS, Regel, E, Dinh, C, Sands, A, Eichele, G, Hasty, P, and Bradley, A (1997). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature **386**, 804-810.

Sharples, GJ, and Leach, DR (1995). Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. Mol. Microbiol. **17**, 1215-1217.

Sherman, JD, and Stack, SM (1995). Two-dimensional spreads of synaptonemal complexes from solanaceous plants. VI. High-resolution recombination nodule map for tomato (*Lycopersicon esculentum*). Genetics **141**, 683-708.

Sherman, JD, Herickhoff, LA, and Stack, SM (1992). Silver staining two types of meiotic nodules. Genome **35**: 907-915.

Shimazu, J, Matsukura, C, Senda, M, Ishikawa, R, Akada, S, Harada, T, Tabata, S, and Niizeki, M (2001). Characterization of a *DMC1* homologue, *RiLIM15*, in meiotic panicles, mitotic cultured cells and mature leaves of rice (*Oryza sativa L.*). Theor. Appl. Genet. **102**, 1159-1163.

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

Shu, Z, Smith, S, Wang, L, Rice, MC, and Kmiec, EB (1999). Disruption of *muREC2/RAD51L1* in mice results in early embryonic lethality which can be partially rescued in a *p53*(-/-) background. Mol. Cell Biol. **19,** 8686-8693.

Siaud, N, Dray, E, Gy, I, Gerard, E, Takvorian, N, and Doutriaux, MP (2004). Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. EMBO J. **23**: 1392-1401.

Siddiqi, I, Ganesh, G, Grossniklaus, U, and Subbiah, V (2000). The dyad gene is required for progression through female meiosis in *Arabidopsis*. Development **127**, 197-207.

Siddiqui, NU, Stronghill, PE, Dengler, RE, Hasenkampf, CA, and Riggs, CD (2003). Mutations in Arabidopsis condensin genes disrupt embryogenesis, meristem organization and segregation of homologous chromosomes during meiosis. Development **130**, 3283-3295.

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

Smith, KC, and Wang, TC (1989). recA-dependent DNA repair processes. Bioessays **10**, 12-16.

Smith, KC, Wang, TV, and Sharma, RC (1987). recA-dependent DNA repair in UV-irradiated *Escherichia coli.* J. Photochem. Photobiol. B **1,** 1-11.

Smith, KN, Penkner, A, Ohta, K, Klein, F, and Nicolas, A (2001). B-type cyclins CLB5 and CLB6 control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr. Biol. **11**, 88-97.

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

Snowden, T, Acharya, S, Butz, C, Berardini, M, and Fishel, R (2004). hMSH4-hMSH5 recognizes Holliday Junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. Mol. Cell **15**, 437-451.

Sonoda, E, Sasaki, MS, Buerstedde, JM, Bezzubova, O, Shinohara, A, Ogawa, H, Takata, M, Yamaguchi-Iwai, Y, and Takeda, S (1998). Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. EMBO J. **17**, 598-608.

Spielman, M, Preuss, D, Li, FL, Browne, WE, Scott, RJ, and Dickinson, HG (1997). *TETRASPORE* is required for male meiotic cytokinesis in *Arabidopsis thaliana*. Development **124**, 2645-2657.

Stack, S (1973). The synaptonemal complex and the achiasmatic condition. J. Cell Sci. **13**, 83-95.

Stack, SM, and Anderson, LK (2001). A model for chromosome structure during the mitotic and meiotic cell cycles. Chromosome Res. **9**, 175-198.

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

Stahl, FW, Foss, HM, Young, LS, Borts, RH, Abdullah, MF, and Copenhaver, GP (2004). Does crossover interference count in *Saccharomyces cerevisiae*? Genetics **168**, 35-48.

Staiger, CJ, and Cande, WZ (1990). Microtubule distribution in *dv*, a maize meiotic mutant defective in the prophase to metaphase transition. Dev. Biol. **138**, 231-242.

Staiger, CJ, and Cande, WZ (1991). Microfilament distribution in maize meiotic mutants correlates with microtubule organization. Plant Cell **3**, 637-644.

Staiger, CJ, and Cande, WZ (1992). *Ameiotic*, a gene that controls meiotic chromosome and cytoskeletal behavior in maize. Dev. Biol. **154**, 226-230.

Stevens, R, Grelon, M, Vezon, D, Oh, J, Meyer, P, Perennes, C, Domenichini, S, and Bergounioux, C (2004). A CDC45 homolog in Arabidopsis is essential for meiosis, as shown by RNA interference-induced gene silencing. Plant Cell **16**, 99-113.

StoopMyer, C, and Amon, N (1999). Meiosis: Rec8 is the reason for cohesion. Nature Cell Biol. **1,** E125-127.

Strompen, G, El Kasmi, F, Richter, S, Lukowitz, W, Assaad, FF, Jurgens, G, and Mayer, U (2002). The *Arabidopsis HINKEL* gene encodes a kinesin-related protein involved in cytokinesis and is expressed in a cell cycle-dependent manner. Curr. Biol. **12**, 153-158.

Stuart, D, and Wittenberg, C (1998). *CLB5* and *CLB6* are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev. **12**, 2698-2710.

Suja, JA, Antonio, C, Debec, A, and Rufas, JS (1999). Phosphorylated proteins are involved in sister-chromatid arm cohesion during meiosis I. J. Cell Sci. **112**, 2957-2969.

Sung, P (1997). Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. Genes Dev. **11**, 1111-1121.

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

Taki, T, Ohnishi, T, Yamamoto, A, Hiraga, S, Arita, N, Izumoto, S, Hayakawa, T, and Morita, T (1996). Antisense inhibition of the RAD51 enhances radiosensitivity. Biochem. Biophys. Res. Commun. **223**, 434-438.

Tarsounas, M, Morita, T, Pearlman, RE, and Moens, PB (1999). RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. J. Cell Biol. **147**, 207-220.

Thompson, LH, and Schild, D (2002). Recombinational DNA repair and human disease. Mutat. Res. **509**, 49-78.

Tsutsui, Y, Morishita, T, Iwasaki, H, Toh, H, and Shinagawa, H (2000). A recombination repair gene of *Schizosaccharomyces pombe, rhp57,* is a functional homolog of the *Saccharomyces cerevisiae RAD57* gene and is phylogenetically related to the human *XRCC3* gene. Genetics **154**, 1451-1461.

Tsuzuki, T, Fujii, Y, Sakumi, K, Tominaga, Y, Nakao, K, Sekiguchi, M, Matsushiro, A, Yoshimura, Y, and MoritaT (1996). Targeted disruption of the *Rad51* gene leads to lethality in embryonic mice. Proc. Natl. Acad. Sci. U. S. A. **93**, 6236-6240.

van Heemst, D, and Heyting, C (2000). Sister chromatid cohesion and recombination in meiosis. Chromosoma **109**, 10-26.

Wang, G, Kong, H, Sun, Y, Zhang, X, Zhang, W, Altman, N, DePamphilis, CW, and Ma, H (2004a). Genome-wide analysis of the cyclin family in Arabidopsis and comparative phylogenetic analysis of plant cyclin-like proteins. Plant Physiol. **135**, 1084-1099.

Wang, Y, and Yang, M (2005). The ARABIDOPSIS SKP1-LIKE1 (ASK1) protein acts predominately from leptotene to pachytene and represses homologous recombination in male meiosis. Planta. **223**, 1-5.

Wang, Y, Magnard, JL, McCormick, S, and Yang, M (2004b). Progression through meiosis I and meiosis II in Arabidopsis anthers is regulated by an A-type cyclin predominately expressed in prophase I. Plant Physiol. **136**, 4127-4135.

Watanabe, Y, and Nurse, P (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature **400,** 461-464.

Wei, Y, Mizzen, CA, Cook, RG, Gorovsky, MA, and Allis, CD (1998). Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in *Tetrahymena*. Proc. Natl. Acad. Sci. U. S. A. **95**, 7480-7484.

Wei, Y, Yu, L, Bowen, J, Gorovsky, MA, and Allis, CD (1999). Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. Cell **97**, 99-109.

Weinstock, GM, McEntee, K, and Lehman, IR (1981). Hydrolysis of nucleoside triphosphates catalyzed by the recA protein of *Escherichia coli*. Steady state kinetic analysis of ATP hydrolysis. J. Biol. Chem. **256**, 8845-8849.

Wiese, C, Collins, DW, Albala, JS, Thompson, LH, Kronenberg, A, and Schild, D (2002). Interactions involving

the Rad51 paralogs Rad51C and XRCC3 in human cells. Nucleic Acids Res. **30**, 1001-1008.

Wijeratne, A, Chen, C, Zhang, W, Timofejeva, L, and Ma, H (2006). The *Arabidopsis thaliana PARTING DANCERS* gene encoding a novel protein is required for normal meiotic homologous recombination. Mol. Biol. Cell **17,** 1331-1343.

Wong, AK, Pero, R, Ormonde, PA, Tavtigian, SV, and Bartel, PL (1997). RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*. J. Biol. Chem. **272**, 31941-31944.

Yang, CY, Spielman, M, Coles, JP, Li, Y, Ghelani, S, Bourdon, V, Brown, RC, Lemmon, BE, Scott, RJ, and Dickinson, HG (2003a). *TETRASPORE* encodes a kinesin required for male meiotic cytokinesis in *Arabidopsis*. Plant J. **34**, 229-240.

Yang, M, and McCormick, S (2002). The *Arabidopsis MEI1* gene likely encodes a protein with BRCT domains. Sex. Plant Reprod. **14**, 355-357.

Yang, M, Hu, Y, Lodhi, M, McCombie, WR, and Ma, H (1999). The *Arabidopsis SKP1-LIKE1* gene is essential for male meiosis and may control homologue separation. Proc. Natl. Acad. Sci. U. S. A. **96**, 11416-11421.

Yang, M, and McCormick, S (2002). The *Arabidopsis MEI1* gene likely encodes a protein with BRCT domains. Sex. Plant Reprod. **14,** 355-357.

Yang, X, Makaroff, CA, and Ma, H (2003b). The *Arabidopsis MALE MEIOCYTE DEATH1* gene encodes a PHD-finger protein that is required for male meiosis. Plant Cell **15**, 1281-1295.

Yoshida, K, Kondoh, G, Matsuda, Y, Habu, T, Nishimune, Y, and Morita, T (1998). The mouse *RecA-like* gene *Dmc1* is required for homologous chromosome synapsis during meiosis. Mol. Cell **1**, 707-718.

Yoshioka, K, Yumoto-Yoshioka, Y, Fleury, F, and Takahashi, M (2003). pH- and salt-dependent self-assembly of human Rad51 protein analyzed as fluorescence resonance energy transfer between labeled proteins. J. Biochem. (Tokyo) **133**, 593-597.

Yu, HG, Hiatt, A, Chan, A, Sweeney, M, and Dawe, RK (1997). Neocentromere-mediated chromosome movement in maize. J. Cell Biol. **139**, 831-840.

Yu, VP, Koehler, M, Steinlein, C, Schmid, M, Hanakahi, LA, van Gool, AJ, West, SC, and Venkitaraman, AR (2000). Gross chromosomal rearrangements and genetic exchange between nonhomologous chromosomes following BRCA2 inactivation. Genes Dev. **14,** 1400-1406.

Zachariae, W (1999). Progression into and out of mitosis. Curr. Opin. Cell Biol. **11**, 708-716.

Zenvirth, D, and Simchen, G (2000). Meiotic double-strand breaks in *Schizosaccharomyces pombe*. Curr. Genet. **38,** 33-38.

Zhang, H, Kobayashi, R, Galaktionov, K, and Beach, D (1995). p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase. Cell **82**, 915-925.

Zhao, D, Han, T, Risseeuw, EP, Crosby, WL, Ma, H. (2003a). Conservation and divergence of *ASK1* and *ASK2* gene functions during male meiosis in *Arabidopsis thaliana*. Plant Mol. Biol. **53**, 163-173.

Zhao, D, Ni, W, Feng, B, Han, T, Petrasek, MG, and Ma, H (2003b). Members of the *ASK* gene family exhibit a variety of expression patterns and may play diverse roles in Arabidopsis. Plant Physiol. **133**, 203-217.

Zheng, N, Schulman, BA, Song, L, Miller, JJ, Jeffrey, PD, Wang, P, Chu, C, Koepp, DM, Elledge, SJ, Pagano, M, Conaway, RC, Conaway, JW, Harper, JW, and Pavletich, NP (2002). Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature **416**, 703-709.

Zhong, XB, Hans de Jong, J, and Zabel, P (1996). Preparation of tomato meiotic pachytene and mitotic metaphase chromosomes suitable for fluorescence in situ hybridization (FISH). Chromosome Res. **4**, 24-28.

Zickler, D, and Kleckner, N (1998). The leptotene-zygotene transition of meiosis. Annu. Rev. Genet. **32**, 619-697.

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

Zou, L, and Stillman, B (1998). Formation of a preinitiation complex by S-phase cyclin CDK-dependent loading of Cdc45p onto chromatin. Science **280**, 593-596.

Zou, L, and Stillman, B (2000). Assembly of a complex containing Cdc45p, replication protein A, and Mcm2p at replcation origins controlled by S-phase cyclin-dependent kinases and Cdc7p-Dbf4p kinase. Mol. Cell. Biol. **20**, 3086-3096.