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Chromosome Dynamics in Meiosis

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Abstract Meiosis encompasses a large number of dynamic processes. Some of them are biochemical, such as formation and repair of meiotic double-strand breaks, while others are physical in nature, such as homologous chromosome segregation in anaphase I. Plants have been used as model species in meiosis studies for over 80 years. However, the past decade brought a dramatic improvement in the understanding of meiosis in plants at the mechanistic level, thanks to the adoption of genetic and molecular biology techniques in chromosome research and new microscopy methods.

1 Overview of Meiosis

Meiosis consists of two consecutive nuclear divisions (Fig. 1), a reductional division (meiosis I) and an equational division (meiosis II), without an intervening S phase between them. While meiosis II is essentially similar to a mitotic division, meiosis I is a specialized division, whose aim is to reduce the number of chromosomes in the nucleus and allow exchange of genetic material between maternal and paternal chromosomes. Based largely on chromosome dynamics, meiosis I is subdivided into four stages, prophase I, metaphase I, anaphase I, and telophase I. Meiotic prophase I, the most eventful of these stages, is further subdivided into five sub-stages: leptotene, zygotene, pachytene, diplotene, and diakinesis (Fig. 2). During **leptotene**, which follows the pre-meiotic S phase, decondensed chromatin becomes organized into chromosomes by the assemblage of a proteinaceous core. Meiotic recombination is initiated at this step by formation of double-strand breaks (DSBs) in chromosomal DNA (Pawlowski et al. 2004; Zickler and Kleckner 1999). In **zygotene**, homologous chromosomes pair. Pairing is followed by synapsis, when the central element of the synaptonemal complex (SC) is installed between the paired homologs and stabilizes pairing interactions (Page and Hawley 2004). By **pachytene**, SC formation is complete and meiotic recombination between homologs is resolved. In **diplotene**, the SC disassembles and chiasmata are visible. Chiasmata are the sites of crossovers (COs) and are responsible for holding homologous chromosomes together until their segregation in anaphase. Finally, in **diakinesis**, the chromosomes undergo the final stage of condensation.

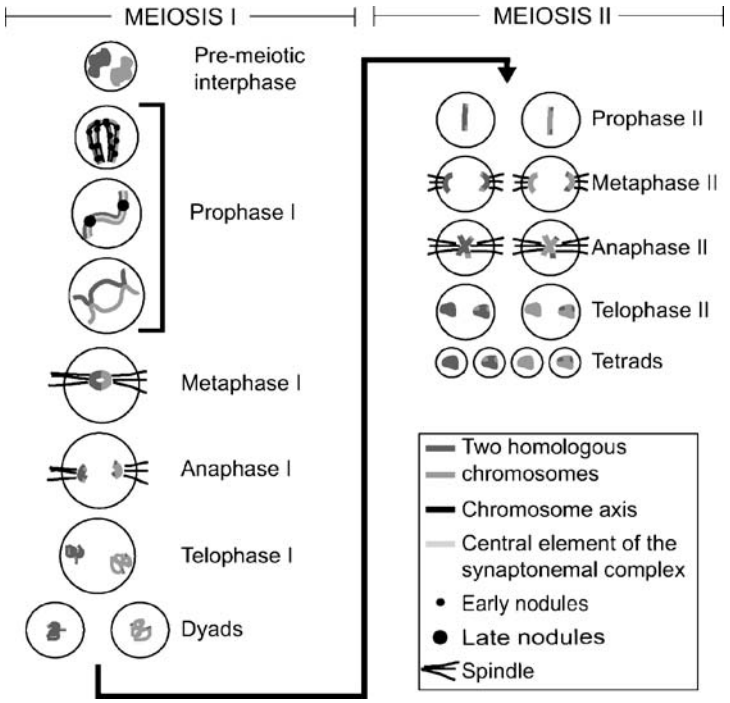

Fig. 1 A general overview of meiosis. Only one pair of chromosomes is shown and each homolog is a different shade of grey. Early and late recombination nodules are depicted as dots of different size. See Fig. 2 for a detailed list of prophase I substages and events

Recombination, pairing and synapsis are three main processes of meiotic prophase I. **Recombination** encompasses formation and repair of meiotic DSBs, including reciprocal chromosome arm exchanges. **Pairing** includes interactions between chromosomes, which involve homology recognition and lead to juxtaposition of homologs. Pairing is followed by **synapsis**, which is defined as the process of installation of the central element (CE) of SC, which binds the paired chromosomes along their entire length. These three processes are formally distinct but genetic and molecular analyses are now drawing a more complex scenario where these processes are intimately interconnected and show a great deal of coordination (Pawlowski and Cande 2005).

2 Initiation of Meiosis in Plants

The switch from the mitotic to the meiotic cell cycle in plants is preceded by a developmental pathway that assigns germ cell identity. First, archesporial

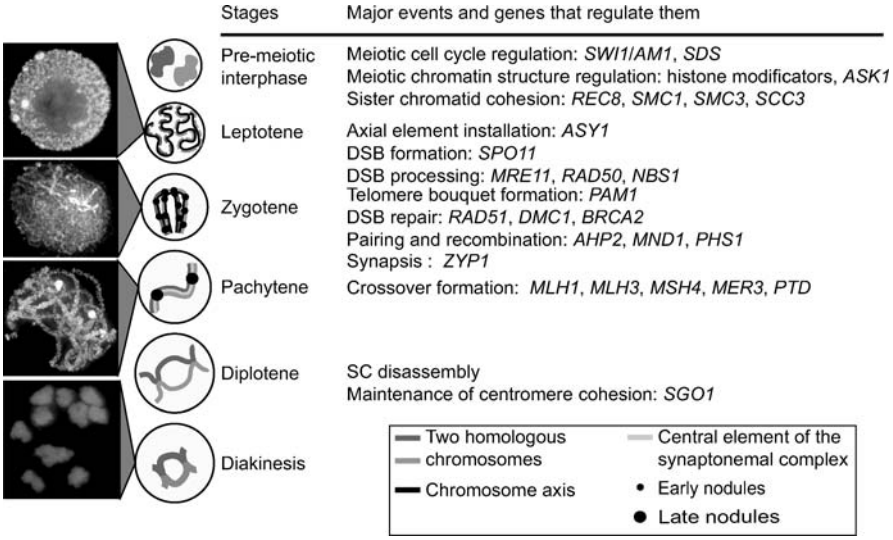

Fig. 2 Pre-meiotic interphase and meiotic prophase I in plants: substages, main events and key genes that regulate them. Only one pair of chromosomes is shown and each homolog is a different shade of grey. Early and late recombination nodules are depicted as dots of different size. *Images on the left* are chromosomes of maize male meiocytes stained with DAPI. The images are flat projections of three-dimensional image stacks collected with three-dimensional deconvolution microscopy

cells are differentiated from hypodermal cells in male and female reproductive organs. Then, the archesporial cells develop into sporocytes, which show features distinct from other cells and are destined to undergo meiosis (Ma 2005).

In contrast to the developmental events preceding the switch to meiosis, the initiation of meiosis in plants is less understood. The *ameiotic1* (*am1*) gene has been identified as a master controller of the switch from mitotic to meiotic cell cycle in maize (Golubovskaya et al. 1997, 1993) (Pawlowski et al., unpublished). Pre-meiotic cells in most *am1* mutants, instead of entering meiosis, undergo mitotic divisions. In severe cases, the progression of the cell cycle is arrested during interphase. All known specific aspects of meiosis, such as establishment of meiosis-specific chromatin structure, chromosome pairing, synapsis, recombination, and meiosis-specific chromosome dynamics require *am1*. In addition to its role in initiating meiosis, *am1* regulates the progression through the early stages of meiotic prophase (Golubovskaya et al. 1993) (Pawlowski et al., unpublished). This conclusion is based on the analysis of an unusual *am1* allele, *am1-praI*, in which pre-meiotic cells enter meiosis but arrest during meiotic prophase.

The *Arabidopsis* homolog of *am1*, *SWITCH1* (*SWI1*), also known as *DYAD*, has been shown to regulate several meiotic prophase processes, including es-

tablishment of meiotic chromosome structure, recombination and synapsis (Agashe et al. 2002; Mercier et al. 2001, 2003). However, the phenotypes of *swi1* mutants are less obvious than those of the maize *am1* mutants. Male meiocytes in *swi1* mutants either undergo a normal meiosis or show meiotic sister-chromatid cohesion (SCC) defects. Female *swi1* meiocytes undergo an equational division. However, this division is an abnormal meiosis rather than mitosis, since the meiosis-specific cohesin REC8 is loaded onto chromosomes, and *DMC1*, a gene encoding a meiosis-specific recombinase, is expressed. None of the *swi1* mutations result in meiocytes undergoing a normal mitotic division, as is the case in *am1* mutants. This suggests that initiation at the mechanistic level differs between maize and *Arabidopsis*.

Homologs of the AM1/SWI1/DYAD proteins are confined to plants. The molecular functions of AM1 and SWI1 are not known and the pathway downstream from these proteins that results in the transition from mitotic to meiotic cell cycle is poorly understood. The decision to enter meiosis is probably made before or at the beginning of the pre-meiotic S phase, although the evidence is mostly indirect. This timing is suggested by observations that female meiocytes in several *am1* mutants arrest at interphase and that the *Arabidopsis* SWI1 shows expression exclusively during pre-meiotic G1 and S. Overall, the mechanisms of meiosis initiation in plants is likely to corroborate conclusions from yeast and mammals indicating that the signaling cascade leading to meiosis initiation shows great diversity among species while the timing of meiosis initiation is a universal feature shared by all eukaryotes (Pawlowski et al. 2007).

3 Regulation of Meiosis Progression

Several candidates for meiotic cell cycle regulators have been identified in *Arabidopsis* based on their functions and/or similarity to known cyclins. These proteins, CDC45, SOLO DANCERS (SDS), and TARDY ANSYNCHRONOUS MEIOSIS (TAM), are proposed to act at different times in meiosis. CDC45 functions during pre-meiotic S-phase (Stevens et al. 2004). SDS regulates chromosome pairing and synapsis in prophase I (Azumi et al. 2002), although the molecular mechanism of its function is not known. TAM has been proposed to regulate progression of both, meiotic prophase I and meiosis II, and its absence also leads to meiotic nuclear division becoming asynchronous with cytokinesis (Wang et al. 2004).

4
Meiotic Chromosome Structure

4.1
Dynamics of the Chromatin Structure and the Histone Code

The beginning of meiosis is associated with a dynamic re-organization of chromatin (Dawe et al. 1994; Zickler and Kleckner 1999). In fact, changes in chromosome structure and condensation have been recognized as landmarks to identify meiosis sub-stages. Specific histone H3 variant deposition (Okada et al. 2005), and histone modifications, such as methylation (Shi and Dawe 2006; Yang et al. 2006) and phosphorylation (Houben et al. 2005; Kaszas and Cande 2000) are observed in meiosis, suggesting a wide and dynamic meiotic reorganization of the histone code. In particular, phosphorylation of H3 at Ser10 correlates with the maintenance of sister chromatid cohesion (Kaszas and Cande 2000) and phosphorylation of H3 at Thr11 was found to correlate with meiotic chromosomes condensation (Houben et al. 2005). Insight into the regulation of these modifications is limited at present but an *Arabidopsis* SKP1 homolog, ASK1, was recently shown to play a role in this process (Yang et al. 2006). In hexaploid wheat, a change in chromatin conformation coincides with chromosome pairing and has been implicated in the process of homologous chromosome recognition (Prieto et al. 2004).

4.2
Chromosome Axis

The chromosome axis (axial element, AE), forming at the base of chromatin loops, contains components essential for SCC. The chromosome axis is credited with allowing preferential exchanges between homologous chromosomes rather than sister chromatids. The resulting chiasmata ensure correct segregation of chromosomes at the first meiotic division. AEs are also essential for homologous chromosome synapsis because they become, as lateral elements (LEs), components of the tripartite SC.

Loss of the ASYNAPTIC1 (ASY1) protein associated with the chromosome axis in *Arabidopsis* reduces fertility by 90% (Caryl et al. 2000; Ross et al. 1997). ASY1, proposed to be a homolog of the yeast Hop1 protein, forms foci on chromatin during pre-meiotic interphase and localizes to the entire length of chromosomes from leptotene to diplotene, except for the telomeric regions (Armstrong et al. 2002). The *Arabidopsis* genome also contains another homolog of *HOP1*, *ASY2* (At4g32200), whose function is non-redundant with *ASY1* (Caryl et al. 2000). Two *HOP1* homologs, *PAIR2* and *PAIR2c3*, were also found in rice. *PAIR2* is the ortholog of *ASY1*, since the *pair2* mutant exhibits the same phenotype as the *Arabidopsis asy1* mutant (Nonomura et al. 2004) and

the PAIR2 protein in rice shows the same localization pattern as ASY1 in *Arabidopsis* (Nonomura et al. 2006).

4.3 Sister-Chromatid Cohesion

During the S-phase DNA replication, the newly copied sister chromatids are kept associated, in meiosis as well as in mitosis, with a multi-subunit cohesin complex (Revenkova and Jessberger 2006). The cohesin complex is well conserved across kingdoms. The composition of the cohesin complex differs between mitotic and meiotic cells. In mitotic cells, it contains RAD21 and SCC3 proteins associated with two Structural Maintenance of Chromosomes (SMC) proteins, SMC1 and SMC3. In meiotic cells, RAD21 is replaced by a meiosis-specific cohesin REC8. Because SMC1, SMC3, and SCC3 function in both mitosis and meiosis, mutants defective in these proteins show embryonic defects and are embryo lethal in *Arabidopsis* (Chelysheva et al. 2005; Liu et al. 2002).

In contrast to fungi and animals, plants contain more than two genes encoding the RAD21/REC8 proteins. *Arabidopsis* has four RAD21/REC8 homologs (Dong et al. 2001) but only one of them, SYN1, also known as DIF1, has a meiotic function and is the true ortholog of REC8 (Bai et al. 1999; Bhatt et al. 1999; Cai et al. 2003; Peirson et al. 1997). Immunolocalization studies showed that SYN1 is present in meiocyte nuclei from interphase to metaphase I and localizes along chromosome arms while being devoid from the centromeric region (Cai et al. 2003).

The analysis of a maize *rec8* mutant called *absence of first division1* (*afd1*) shows that the maize REC8 homolog is required for establishing the leptotene chromosome structure, the bouquet formation, as well as chromosome pairing, synapsis, and recombination (Golubovskaya et al. 2006; Pawlowski et al. 2003). AFD1 colocalizes with ASY1 from leptotene through pachytene, suggesting that both proteins are associated with the chromosome axis. ASY1 is present on chromosomes in *afd1* mutants, indicating that AFD1 is not required for ASY1 recruitment. A series of weaker *afd1* alleles revealed that the functions of REC8 in chromosome pairing, synapsis, and recombination may be independent from its function in establishing the meiotic chromosome structure (Golubovskaya et al. 2006).

5 Chromosome Dynamics of Early Meiotic Prophase—The Telomere Bouquet

The telomere bouquet is a temporary chromosome arrangement that occurs exclusively in meiosis and is found in most species examined to date (Harper et al. 2004; Zickler and Kleckner 1999). During prophase I, all telomeres

cluster at a single location on the nuclear envelope (NE), generally juxtaposed with the microtubule organizing region, causing the chromosomes to resemble a bouquet of flowers. The coincidental timing of the bouquet stage with homologous chromosome pairing, recombination, and synapsis suggests a role of the bouquet in these processes.

Within the plant kingdom, the bouquet has been studied in several species, mainly grasses such as rye, wheat, and maize (Bass et al. 2000; Cowan and Cande 2002; Martinez-Perez et al. 1999; Noguchi 2002).

5.1 Function of the Bouquet

In most organisms, the bouquet forms at the beginning of, or just before, zygotene and persists until the end of zygotene (Harper et al. 2004). In rye, wheat and maize, telomeres begin to attach to the NE in leptotene and the bouquet persists until early pachytene (Bass et al. 2000; Maestra et al. 2002; Martinez-Perez et al. 1999; Noguchi 2002). In addition to coincidental timing, there is evidence supporting a close relationship between the bouquet and pairing, although the exact nature of that relationship is still unknown. In mutants with defective bouquet formation, pairing is delayed and inefficient (Golubovskaya et al. 2002; Harper et al. 2004; Niwa et al. 2000; Trelles-Sticken et al. 2000). Attachment of telomeres and confinement of chromosomes to a small area may assist the sequence-dependent homology search by limiting the physical volume within which chromosomes may interact, and by creating constructive chromosome movements (Scherthan 2001). This could enhance the efficiency of pairing (Harper et al. 2004). Telomere clustering might also eliminate or reduce ectopic pairing interactions in highly conserved syntenic regions or regions of highly repetitive sequences (Niwa et al. 2000). Furthermore, synapsis in plants initiates at the telomeres and the bouquet may help catalyze this process (Maestra et al. 2002).

5.2 Genetics of Bouquet Formation

The most complete picture of bouquet formation in a single system exists in the fission yeast, *S. pombe*, where there are a number of mutants known to cause specific bouquet defects (Davis and Smith 2006; Harper et al. 2004; Jin et al. 2002). In contrast, little is known about the bouquet in higher eukaryotes. The best-characterized plant bouquet mutant is the *pam1* (*plural abnormalities of meiosis1*) mutant of maize (Golubovskaya et al. 2002). As the name indicates, the *pam1* mutation has several obvious meiotic defects, including asynchrony of meiocytes, inhibition of telomere bouquet clustering, and abnormal synapsis (Golubovskaya et al. 2002). The initiation of meiotic recombination in the *pam1* mutant is not affected. However, later recombination stages are likely de-

fective since it has been noticed that the RAD51 recombination protein persists on *pam1* chromosomes longer than in wild-type meiocytes.

5.3 Dynamics of Bouquet Formation

The "early bouquet" stage forms in late leptotene with the telomeres attached to the NE opposite the nucleolus, which has migrated from the center to the periphery of the nucleus. By zygotene, telomeres have migrated to coalesce with the nucleolus in the "mature bouquet" (Bass et al. 1997). Coincident with this migration, nuclear pores also migrate but in the opposite direction, away from the bouquet focus and nucleolus (Bass et al. 1997; Cowan and Cande 2002). On the basis of the low numbers of nuclei detected at early compared to late bouquet stages, Bass et al. determined that bouquet formation is a sudden and active process (Bass et al. 1997). By contrast, the termination of the bouquet seems to be a passive process with telomeres diffusing away from the focus.

The formation of the bouquet is most likely a two-stage process with telomeres first attaching to the NE, then migrating from a distal to a proximal position at or near the nucleolus (Bass et al. 1997). This model is supported by evidence from several maize meiotic mutants. In *pam1*, telomeres are able to attach to the NE in a wild-type fashion, but clustering is disrupted (Golubovskaya et al. 2002). Telomere attachment to the NE can also be disrupted as was shown in the allelic series of mutants in *afd1*, the maize homolog of *REC8* (Golubovskaya et al. 2006). In weak alleles of *afd1*, telomeres are able to attach to the NE, whereas in strong alleles, they cannot. Clustering is completely abolishing in all but the weakest *afd1* allele. Telomere attachment is not dependent on REC8 recruitment *per se* but on the formation of the AE over the entire length of the chromosome (Golubovskaya et al. 2006; Liebe et al. 2004; Trelles-Sticken et al. 2005). Evidence from mammals and fungi point to completion of DSB repair and CO formation (Liebe et al. 2006; Pandita et al. 1999; Trelles-Sticken et al. 2000) as the cue to exit from the bouquet, but there has not yet been evidence in plants for this hypothesis.

Studies indicate that telomere sequence and unknown host factors govern bouquet formation. Maize telocentric and ring chromosomes can enter the bouquet, indicating that the presence of the physical chromosome end is not necessary for bouquet formation (Carlton and Cande 2002). In another study, the meiotic behavior of an oat line carrying an addition of maize chromosome 9 was investigated (Bass et al. 2000). Normally in maize, all of the telomeres are localized in the bouquet at zygotene. In the oat-maize addition line, only about 70–90% of telomeres (both maize and oat) are localized to the bouquet during zygotene. Interestingly, the maize chromosome telomeres were observed to attach to the NE and enter the bouquet in a way similar to oat chromosomes, suggesting that telomere clustering in this line is controlled by the oat nuclei rather than by the telomeres themselves (Bass et al. 2000).

In addition to telomere sequences, there is mounting evidence that an intact cytoskeleton is required for bouquet formation. It has long been known that the microtubule (MT) depolymerizing drug colchicine applied during prophase I in plants and animals causes reduced chiasmata and impaired SC formation (Loidl 1989; Tepperberg et al. 1997). The application of colchicine to rye anthers inhibited telomere clustering but not telomere attachment to NE or nuclear pore migration, causing a resemblance to the maize *pam1* phenotype (Cowan and Cande 2002; Golubovskaya et al. 2002). Interestingly, this same effect was seen even when low levels of colchicine were applied that were insufficient to cause depolymerization of MTs. It is unknown if the target of colchicine is a cytoskeletal component on the inner NE or perhaps a membrane-associated β-tubulin (Cowan and Cande 2002; Harper et al. 2004).

5.4 Telomere Clustering in *Arabidopsis*

Arabidopsis does not form a bona fide telomere bouquet but it has been observed that *Arabidopsis* telomeres cluster around the nucleolus in pre-meiotic interphase (Armstrong et al. 2001), which could play a role equivalent to the role of the bouquet. However, this telomere clustering is also observed in mitotic interphase, and its early occurrence suggests the utilization of a chromosome arrangement existing after a previous cell division, rather than de novo clustering. In addition, a very loose bouquet-like arrangement of telomeres is observed in *Arabidopsis* in zygotene (Armstrong et al. 2001).

6 Meiotic Recombination

6.1 Formation of Meiotic DSBs and Early Recombination Steps

Meiotic recombination is universally initiated in all species by the introduction of DSBs into chromosomal DNA by SPO11, a protein belonging to the topoisomerase family (Keeney et al. 1997). In contrast to all other species, plants possess multiple SPO11 homologs. The *Arabidopsis* genome has three *SPO11* genes (Hartung and Puchta 2001), although only two of them, *AtSPO11-1* and *AtSPO11-2*, are essential for meiosis (Grelon et al. 2001; Stacey et al. 2006). Analysis of the *spo11-1* mutant demonstrated that the recombination defect is coupled with the inability of the mutant to synapse, leading to univalents, improper gamete formation, and ultimately, sterility (Grelon et al. 2001). Visualizing the presence of meiotic DSBs on maize meiotic chromosomes using the TUNEL assay showed that DSBs occur along the

entire length of chromosomes, are formed in very early leptotene, and persist until early pachytene (Pawlowski et al. 2004).

Downstream from SPO11, meiotic DSBs are resected by the MRN complex, which includes **M**RE11, **R**AD50, and **N**BS1 (Akutsu et al. 2007; Bleuyard et al. 2004; Daoudal-Cotterell et al. 2002; Gallego et al. 2001; Puizina et al. 2004). Like *spo11-1*, the *Arabidopsis rad50* and *mre11* mutants exhibit recombination defects and are unable to synapse. However, unlike *spo11-1*, they also show fragmented and dicentric chromosomes, resulting from unrepaired DSBs (Bleuyard et al. 2004; Gallego et al. 2001; Puizina et al. 2004). An analysis of *mre11*/*spo11-1* double mutants indicated that this fragmentation is SPO11-dependent (Puizina et al. 2004). In addition to the meiotic defects, the *rad50* and *mre11* mutants are hypersensitive to DNA damage agents and exhibit progressive telomere shortening in mitotically dividing cells (Gallego et al. 2001; Puizina et al. 2004). This indicates that the MRN complex in plants, similarly to its counterparts in yeast and mammals, is also involved in somatic DNA repair processes.

6.2 Single-End Invasion and the Role of the RAD51 Family

Meiotic DSBs generated by SPO11 and the MRN complex are subsequently repaired by a complex of proteins containing two homologs of the bacterial RecA recombinase, RAD51 and DMC1. RAD51 and DMC1 exhibit extensive similarity at the amino acid level, but while RAD51 is expressed throughout the plant life cycle, DMC1 is only expressed during meiosis (Doutriaux et al. 1998; Jones et al. 2003; Klimyuk and Jones 1997; Li et al. 2004). RAD51 and DMC1 facilitate the single-end invasion (SEI) process, in which a single stranded DNA overhang, created by the MRN complex, invades a homologous double-stranded DNA region. Rad51 and Dmc1 in *S. cerevisiae* are known to form a complex that covers single stranded DNA ends, forming a nucleoprotein filament. This structure facilitates the recognition of the corresponding region in the homologous double helix (Neale and Keeney 2006).

RAD51 and DMC1 form distinct foci on meiotic chromosomes, presumably on the sites of DSBs (Franklin et al. 1999; Pawlowski et al. 2003; Terasawa et al. 1995). In maize, RAD51 foci first appear at the beginning of zygotene (Franklin et al. 1999; Pawlowski et al. 2003). The number of foci reaches its peak of roughly 500 per nucleus in mid zygotene, and later declines to about 10–20 in late pachytene. Observations in lily showed that most RAD51 and DMC1 foci colocalize (Terasawa et al. 1995).

The numbers and dynamics of RAD51/DMC1 foci resemble those of the early recombination nodules (EN), electron-dense structures observed in Transmission Electron Microscopy (TEM) in a number of species, including maize, lily and tomato (Anderson et al. 2003; Anderson et al. 2001). EN have been suggested to play a role in recombination and homologous chromosome

pairing. RAD51 and DMC1 are indeed components of early recombination nodules, as demonstrated by immuno-gold labeling (Anderson et al. 1997).

Arabidopsis rad51 and *dmc1* mutants show defects in meiotic recombination, chromosome pairing and synapsis (Couteau et al. 1999; Li et al. 2004). The *atrad51* mutant shows also extensive SPO11-dependent chromosome fragmentation, similar to the phenotypes observed in *Arabidopsis* mutants defective in MRE11 and RAD50 (Bleuyard et al. 2004; Li et al. 2004; Puizina et al. 2004). Interestingly, fragmentation is not observed in the *Arabidopsis dmc1* mutant (Couteau et al. 1999). It is likely that, in the absence of DMC1, meiotic DSBs are repaired by RAD51 using the sister chromatid as the template. Supporting this conclusion are observations that depletion of RAD51 in the *atdmc1* mutant background does lead to chromosome fragmentation (Siaud et al. 2004).

In addition to RAD51 and DMC1, the *Arabidopsis* genome encodes five other RecA homologs, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 (Lin et al. 2006). Of these, only RAD51C and XRCC3 have meiotic functions (Abe et al. 2005; Bleuyard and White 2004; Li et al. 2005), supporting the notion that different members of the RAD51 protein family have evolved to fulfill different requirements for meiosis and somatic DNA repair (Bleuyard et al. 2005).

The RAD51/DMC1 protein complex interacts with several other proteins in the process of DSB repair, including HOP2, MND1, and BRCA2. HOP2 and MND1 play roles in both recombination and homologous chromosome pairing and are discussed in the chromosome pairing section later in this chapter. BRCA2 is hypothesized to act in recruiting the RAD51/DMC1 complex to the sites of DSBs (Sharan et al. 2004). The *Arabidopsis* homolog of BRCA2 interacts with AtRAD51 and AtDMC1 through the conserved BRC motifs in the BRCA2 protein (Dray et al. 2006; Siaud et al. 2004). Silencing BRCA2 in *Arabidopsis* using RNAi leads to unrepaired DSB (Siaud et al. 2004).

6.3 Formation of Crossovers: Two Ways to Recombine Homologs

Only a subset of the numerous DSBs formed during meiosis produce COs, which lead to chiasmata and chromosome arm exchanges. The repair of most meiotic DSBs results in non-crossover (NCO) products (gene conversion). For example, in maize there are about 500 SEI events/nucleus that are marked by RAD51 foci, but only about 20 crossovers. Data from budding yeast indicate that COs and NCOs are made by two alternative pathways that branch very soon after DSB formation (Allers and Lichten 2001; Hunter and Kleckner 2001). In the CO pathway, SEI recombination intermediates form double Holliday junctions, which are subsequently resolved to give mostly CO products. The events that lead to NCOs are less clear, although it has been suggested that a synthesis-dependent strand-annealing mechanism is involved in their

formation (Allers and Lichten 2001). Although direct evidence is still lacking, similar two pathways may exist in *Arabidopsis* (Higgins et al. 2004).

Meiotic cells must ensure that each chromosome pair has at least one CO to form chiasmata that keep the homologous chromosomes together until anaphase I. The mechanism of this "crossover assurance" is unclear. A number of proteins that specifically promote CO formation have been identified, including members of the MutL and MutS DNA mismatch-repair protein families, MLH1, MLH3, MSH4, and MSH5. The *Arabidopsis* MLH1 homolog is expressed in vegetative and reproductive tissues (Jean et al. 1999) but MLH3 is specifically expressed during meiosis (Jackson et al. 2006). The MLH1 and MLH3 proteins form foci on chromosomes and colocalize in pachytene (Franklin et al. 2006). The *Arabidopsis* MSH4 protein also has a function in meiosis. The *msh4* mutant is defective in CO formation as well as synapsis (Higgins et al. 2004). Higgins et al. observed that a residual 16% of crossovers form independent of MSH4 and these events are randomly distributed on chromosomes (Higgins et al. 2004). Similar observations were made in several other *Arabidopsis* recombination mutants, *mlh3* (Jackson et al. 2006), *mer3*/*rock and rollers* (*rck*) (Chen et al. 2005; Mercier et al. 2005) and *parting dancers* (*ptd*) (Wijeratne et al. 2006). These observations are consistent with the proposal that there are two classes of meiotic COs: class I COs that have a regulated distribution and are subject to crossover interference, and class II COs that occur randomly, are not subject to interference (Copenhaver et al. 2002). CO interference is a poorly understood mechanism that prevents formation of two COs close to each other. The analyses of mutants in the *MSH4*, *MLH3*, *MER3*, and *PTD* genes indicate that they all are involved in class I CO formation. The *Arabidopsis* genome contains a homolog of *MUS81*, a gene thought to be involved in class II CO formation in yeast, but its meiotic function has not been studied.

At the cytological level, CO formation corresponds to the presence of late recombination nodules (LN) (Anderson et al. 2003; Stack and Anderson 2002). LN are less numerous then EN and likely form from a subset of EN. LN in the mouse contain recombination proteins involved in CO formation, MSH4 and MLH1 (Moens et al. 2002), and it is likely these enzymes are also present in the LN in plants.

7 Homologous Chromosome Pairing

Homologous chromosome pairing is one of the least-explored processes in meiosis. In most plants, with the exception of hexaploid wheat, chromosomes enter meiosis unpaired and pair de novo during zygotene. Intuitively, chromosome pairing must occur in two steps, a step in which the homologs are brought together into a close proximity and a homology search

step in which correct homologous chromosomes identify each other. The dynamic movement of chromosomes during early meiotic prophase I, including formation of the telomere bouquet, may be a part of the first step. Plants belong to a group of species, along with mammals and fungi, in which successful chromosome pairing depends on meiotic recombination (Pawlowski and Cande 2005). Consequently, it has been hypothesized that recombination plays a role in the homology search step of chromosome pairing in plants (Franklin et al. 1999; Li et al. 2004; Pawlowski et al. 2003). In addition to recombination, other mechanisms may be involved in the homology search, particularly in species with large genomes containing extensive repetitive DNA sequence families. Repetitive DNA would make identification of DNA sequence similarity insufficient alone to establish chromosome homology. However, even though existence of chromosome or chromatin-level homology recognition mechanisms may be intuitive (Stack and Anderson 2001), such mechanisms have not yet been experimentally identified.

7.1 Pairing and Recombination

Defects in chromosome pairing were observed in a number of *Arabidopsis* recombination mutants already mentioned in this chapter, including *spo11-1*, *rad50*, *mre11, brca2, rad51, dmc1, rad51c*, and *xrcc3*. In particular, members of the RecA family, DMC1, RAD51, and RAD51C, have been proposed to act in homologous chromosome pairing in addition to their roles in meiotic recombination (Franklin et al. 1999; Li et al. 2005; Pawlowski and Cande 2005; Pawlowski et al. 2003). Franklin et al. proposed a role for RAD51 in homologous pairing in maize based on the analysis of the dynamics of the distribution of chromosomal RAD51 foci in zygotene and pachytene (Franklin et al. 1999). This was supported by Pawlowski et al., who observed that, in a collection of meiotic mutants in maize, the degree of homologous pairing defects corresponded to the number of chromosomal RAD51 foci in zygotene (Pawlowski et al. 2003).

Recent years brought the identification of a small group of meiotic genes that affect both recombination and homologous chromosomes pairing and have been hypothesized to play major roles in coordinating recombination and pairing. This group contains the maize *Phs1* gene (Pawlowski et al. 2004), as well as *HOP2* and *MND1*, which were first identified in yeast (Leu et al. 1998; Tsubouchi and Roeder 2002) but recently also shown to have homologs in other species, including plants (Domenichini et al. 2006; Kerzendorfer et al. 2006; Panoli et al. 2006; Schommer et al. 2003). Mutants in these genes are defective in both pairing and recombination. The maize *phs1* mutant and the *hop2* mutant in yeast show a striking phenotype: in the absence of homologous pairing, nonhomologous chromosomes associate and synapse (Leu et al. 1998; Pawlowski et al. 2004). In *phs1*, this phenotype is particularly strong and only about 5%

of chromosome associations are between proper homologs. In the maize *phs1* mutant, meiotic DSBs are formed, but the repair process is delayed and most likely proceeds through an alternative pathway, since the RAD51 protein does not form foci on chromosomes in mutant meiocytes (Pawlowski et al. 2004). Mutants in the *Arabidopsis MND1* and *AHP2* (*Arabidopsis* homolog of *HOP2*) genes also show DSB formation but the DSBs are not repaired, leading to chromosome fragmentation, even though in the *mnd1* mutant a normal number of RAD51 foci is observed (Domenichini et al. 2006; Kerzendorfer et al. 2006; Panoli et al. 2006; Schommer et al. 2003). The molecular mechanism of the PHS1 protein function is not known. In contrast, the function of MND1 and HOP2 has been extensively studied in yeast, mammals, and *Arabidopsis*. The two proteins were shown to form a heterodimer (Kerzendorfer et al. 2006). Studies in mouse and yeast indicate further that this heterodimer interacts with the Rad51/Dmc1 complex. However, the details of this interaction remain unclear. It was proposed that the Hop2/Mnd1 complex acts directly to facilitate the SEI activity of Dmc1 (Chen et al. 2004; Tsubouchi and Roeder 2002). Another hypothesis suggests that Hop2/Mnd1 influence pairing and recombination indirectly by affecting chromatin and/or higher-order chromosome structures of the homologous target (Zierhut et al. 2004).

7.2 Other Pairing Mechanisms Independent of Recombination

Polyploid species may have developed additional mechanisms that allow distinguishing between homologous and homeologous (not homologous but similar) chromosomes (Moore 2000). For example, in wheat, homeologous associations between chromosomes of different genomes are prevented by the *Ph1* locus. *Ph1* was proposed to act by regulating centromere associations that are found in meiotic and somatic cells of wheat (Martinez-Perez et al. 2001). In the presence of *Ph1*, non-homologously associated centromeres separate at the beginning of meiosis. Chromatin structure is likely to be involved in this homolog recognition process (Mikhailova et al. 1998; Prieto et al. 2005; Prieto et al. 2004). The endeavor to fine-map and clone *Ph1* has recently focused on a structure that consists of a subtelomeric heterochromatin repeat that inserted into a cluster of Cdc2 gene repeats following the polyploidization event (Griffiths et al. 2006). Presence of this structure correlates with *Ph1* activity, making it a good candidate for the *Ph1* locus.

8 Synapsis

SC forms between the paired homologs along the entire chromosome length in zygotene (Page and Hawley 2004). The tripartite SC structure is com-

prised of two electron-dense LEs that flank a less-dense central region. LEs correspond to the AEs, which are formed in leptotene. Between the LEs are transverse filaments (TFs) that span the central region creating a zipper-like structure. Although the SC shows a high degree of structural conservation among species, the TF proteins of different species do not show significant similarity at the amino acid sequence level, which means that they have to be identified in each species de novo. The TF protein in *Arabidopsis*, ZYP1, has recently been identified by combining bioinformatics and protein immunolocalization approaches (Higgins et al. 2005). ZYP1 is encoded by two duplicated and highly redundant genes *ZYP1a* and *ZYP1b*. ZYP1 is first visible as foci on chromatin in late leptotene and then localizes to the region between synapsed homologous chromosomes in pachytene. However, in rye, the formation of the elongated ZYP1 structures along the entire chromosome length precedes synapsis and takes place as early as leptotene (Mikhailova et al. 2006). *Arabidopsis zyp1* RNAi mutants show that the SC is not formed in absence of ZYP1 but recombination is only slightly reduced. Interestingly, the distribution of chiasmata in these plants is normal showing that in plants SC is not required for interference (Higgins et al. 2005; Osman et al. 2006). In the absence of ZYP1, recombination can occur between homologous as well as non-homologous chromosomes, suggesting that ZYP1 may also affect homologous chromosome recognition.

9
Chromosome Segregation in Meiosis I and II

After completion of prophase I, the key events in meiosis are segregation of homologous chromosomes in meiosis I, followed by segregation of sister-chromatids in meiosis II. Both events require cleavage of the cohesin complex. In addition, homologous chromosome segregation requires SC disassembly, which takes place in diplotene. Cohesion in meiosis is removed in two steps. Cohesion along chromosome arms is released prior to anaphase I. However, in the centromere region, cohesion is preserved until anaphase II by the SUGOSHIN1 (SGO1) protein (Hamant et al. 2005). In maize, SGO1 is installed in centromeric and pericentromeric chromosome regions in early leptotene and requires the presence of REC8. A specialized protease, called separase, is responsible for cohesin cleavage (Liu and Makaroff 2006).

Segregation in meiosis I and II also requires spindle formation and chromosome attachment to spindle microtubules. A large group of proteins, kinesins, is thought to be involved in spindle morphogenesis. Recently, a meiosis specific kinesin ATK1 was shown in *Arabidopsis* to be specifically required for spindle formation in male meiocytes (Chen et al. 2002).

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