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Genetics of Meiotic Prophase I in Plants

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meiocyte, bouquet, pairing, synapsis, cohesion, recombination

Abstract

During meiotic prophase I, traits are reassorted as a result of a highly organized process involving sister chromatid cohesion, homologous chromosome alignment, pairing, synapsis, and recombination. In the past two years, a number of components involved in this pathway, including Structure Maintenance of Chromosomes, MRE11, the RAD51 homologs, BRCA2, MSH4, MER3, and ZIP1, have been characterized in plants; in addition, several genes that encode components unique to plants, such as POOR HOMOLOGOUS SYNAPSIS 1 and AMEIOTIC 1, have been cloned. Based on these recent data, essentially from maize and *Arabidopsis*, we discuss the conserved and plant-specific aspects of meiosis commitment and meiotic prophase I features.

Contents

INTRODUCTION

Plants, together with *Drosophila*, historically have served as model systems for the generation of many of the founding concepts of inheritance and meiosis. For instance, observation of chromosome segregation in plants led to the definition of the terms prophase, metaphase, and anaphase by the botanist E. Strasburger in 1875. The laws of heredity were originally discovered by Mendel in 1866; these laws were based on his analysis of pea phenotypes and re-established by three botanists, de Vries, Correns, and Von Tschermak, in 1900. Because of the accessibility of male meiocytes in the anther, plants have been very useful in the study of the conserved mechanism of meiosis at the cytological level. Maize (*Zea mays* L.) is one of the first model organisms in which the power of genetics was productively merged with cytology to create the new field of cytogenetics (reviewed in 150). Using maize, the link between recombination and the cytological observation of crossing-over was demonstrated in 1931 by Creighton & McClintock (45). Maize chromosome cytology has remained a fertile field for investigation (e.g., 14, 49, 129, 156) and has been accompanied by the development of sophisticated maize linkage maps (e.g., 47, 165; R.C.J. Wang & W.Z. Cande, unpublished data). Furthermore, many meiotic mutants were identified by forward genetics in plants (12, 67, 90, 95). In maize, in particular, a continuously growing collection of over 60 meiotic mutants has been isolated; this collection represents approximately 35 complementation groups (I.N. Golubovskaya, personal communication).

Until the 1990s, it had not been possible to isolate and characterize the genes affected in these plant mutants at a molecular level. However, this has begun to change with the identification of a number of meiotic mutants from *Arabidopsis thaliana*; these mutants have provided the means to clone a range of genes (34, 110). In addition, the use of reverse genetics in this organism has proven to be very powerful, and the functions of homologs of many yeast genes have been characterized in *Arabidopsis*. By comparison, reverse genetics in vertebrates has not been as successful, as homologs of some yeast genes involved in meiosis cannot be studied easily in vertebrates due to their shared mitotic functions and associated mutant lethality (usually not present in plants). Thus reverse genetics in plants can provide unique information on the functions of these genes during meiosis in multicellular organisms. Today, the improved and more accessible molecular tools and mutant banks extend the possibility to clone genes more easily in other plant model systems such as maize or rice.

Although this review is mainly focused on research conducted in *Arabidopsis* and maize,

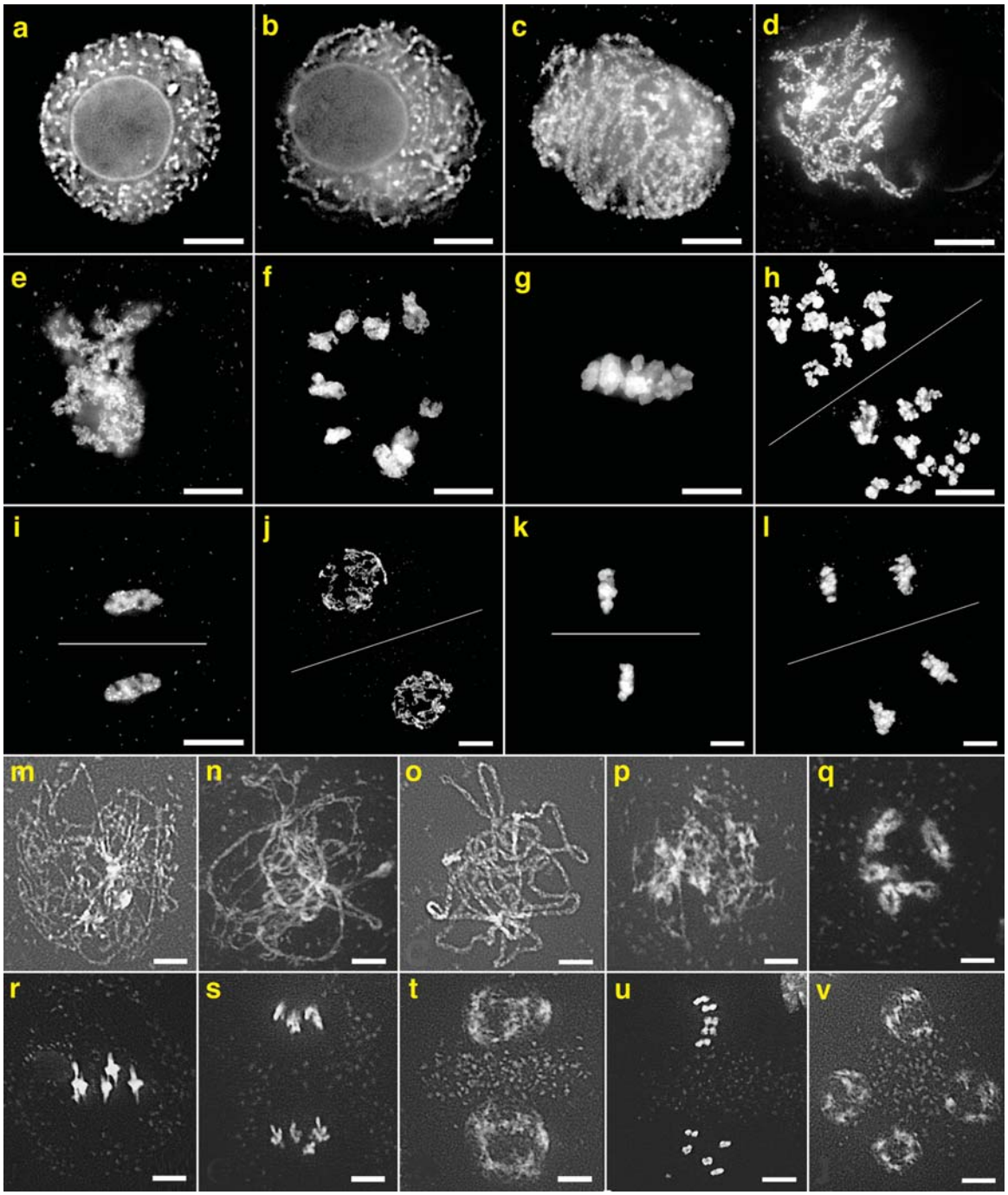
we predict that other plant species, in particular allopolyploid and dioecious species, will be used more often in the future as model organisms for meiosis because of their specific chromosome structure and behavior. Hybridization between two or more species leads to new species called allopolyploids. Important crops such as wheat are allopolyploids. Loci and chromosomes of the genomic sets of different parental origins are defined as homeologous, in contrast to the homologous alleles and chromosomes within the sets of one parent. Studying meiosis in these species will lead to the identification of mechanisms that function to distinguish between homologous and homeologous pairing (See the section Homeologous Pairing below). Furthermore, unlike established wild and cultivated allopolyploids that are genetically stable, allopolyploids of recent origin, such as *Arabidopsis suecica* or synthetic allotetraploids of *Arabidopsis thaliana*, display genomic and phenotypic instability that is in part caused by abnormal meiosis (40, 177). Therefore, allopolyploids are good models for analyzing both the control of meiosis and its evolution. In several dioecious species, heteromorphic chromosomes are associated with chromosomal sex determination (132). The novelty of these plant models is that the origin of sex chromosomes is much more recent in plants than in mammals. In Silene, this origin is estimated at approximately 25 million years, in contrast to 300 million years in mammals. The mechanisms of chromosomal sex determination, including the accumulation of genes determining sexual dimorphism, the controlled arrest of recombination along most regions of X and Y, Y chromosome genetic isolation, and X chromosome dosage compensation, are currently being unraveled and may shed new light on recombination and meiosis in general (35, 57, 108, 132, 133, 192).

MEIOSIS OVERVIEW

Meiosis is a highly conserved process in eukaryotes and occupies a central role in the life cycles of all sexually reproducing organisms, in particular by reassorting traits. Meiosis differs from mitosis in that a single round of DNA replication precedes two sequential cell divisions so that an initially diploid cell generates four haploid cells (**Figure 1**).

DSB: double-strand break

Commitment to meiosis occurs at premeiotic S-phase and the first meiotic markers, like the Cohesin protein REC8, are loaded onto the chromosomes. Initiation of meiosis can be cytologically recognized at the leptotene stage, as chromosome condensation begins and installation of the axial elements along the chromosomes is completed (204). Double-strand break (DSB) initiation is thought to occur between leptotene and zygotene, as it is accompanied by orchestrated chromatin structural changes. In maize, heterochromatin blocks such as knobs and centromeric heterochromatin elongate, and sister chromatids move slightly apart (31, 49). These transient structural changes may be required to initiate pairing interactions and recombination. Pairing of homologous chromosomes begins at zygotene, when the recruitment of recombination effectors, such as RAD51, on chromosomes reaches a peak. From zygotene to pachytene, homologous chromosomes pair and synapse: A central element is installed between the axial elements, now called lateral elements, to form a tripartite SC (78, 127, 191, 204). Pachytene is defined as the stage when synapsis and recombination is in its final stages. In diplotene, the SC falls apart and homologous juxtaposition ends. However, the homologues are held together as bivalents until metaphase I by chiasmata, the sites of recombination that lead to crossovers (137, 143). During diakinesis, the chromosomes condense further, thicken, and detach from the nuclear envelope. Homologs separate at anaphase I as cohesins are removed from chromosomes and chiasmata disassemble. The segregation of homologous pairs of chromosomes at the first division is therefore dependent on their prior pairing, synapsis, and recombination at earlier stages. The second meiotic division is equational and



separates the sister chromatids of each chromosome to give rise to the haploid gametes. Subsequent fertilization of female gametes by the male restores the diploid state.

While much has been learned, many questions remain unanswered, and new questions arise: (*a*) the mechanisms underlying homology recognition before and during pairing is mostly unknown; (*b*) despite decades of analysis, the role of the synaptonemal complex (SC) in relationship with pairing and recombination is still under debate; (*c*) how the meiocyte decides between a crossover or a noncrossover event is poorly understood; and (*d*) the centromere, with little sequence data available, remains an obstacle to understanding the biology of the meiotic chromosome. In this review we discuss the recent advances in the genetics of meiosis in angiosperms and focus on early events during prophase I. Other aspects of plant meiosis can be found in several recent reviews (34, 88, 110).

MODEL PLANTS AND APPROACHES

Meiosis in angiosperms is organized rather differently in male and female organs, and the fates of the meiotic products are also divergent. In anthers, sporogenous cells proliferate by asynchronous mitotic divisions until the appropriate number of presumptive meiocytes (also called pollen mother cells) is achieved. In response to an appropriate signal, the pollen mother cells enter meiosis in synchrony and proceed through meiosis more or less synchronously to generate microspores (for review see Reference 110). In ovules, archesporial cells give rise to single embryosac mother cells, which undergo meiosis to produce four haploid cells (macrospores). In the anther, upon completion of meiosis, all the microspores undergo one round of mitosis and develop into pollen grains, whereas, in the ovule, usually three of the macrospores degenerate to leave one which develops into the embryo sac. The cellular organization of the embryo sac is less stereotyped than pollen and depends on the species as the number of degenerating macrospores and subsequent mitosis varies (e.g., in lily, orchids, and *Plumbagella*). Most of the data on meiosis in plants originate from studies of the pollen mother cell, due to its accessibility.

SC: synaptonemal complex

Forward Genetics

Primary screen: sterility. Both chemical (EMS, nitrosomethyl-urea) and insertional mutagenesis (transposons, T-DNA) have been used to produce populations of mutants in plants, mainly maize and *Arabidopsis* (10, 18, 34, 37, 69, 110, 122, 141, 153, 158, 171, 198). Screening for sterility however depends on the species : In maize, at anthesis, sterile plants exhibit nonprotruding

Figure 1

DAPI-stained chromosomes structure and behavior during male meiosis in maize and *Arabidopsis*. (*a–l*) Three-dimensional projections of male meiocyte nuclei in maize. (*a*) Leptotene. The nucleolus is at the center of the nucleus as chromosomes threads form. (*b*) Leptotene-zygotene. The nucleolus has moved to the periphery of the nucleus as telomere cluster. (*c*) Late zygotene. Synapsed (middle) and unsynapsed (edges of nucleus) chromosomes can be distinguished. (*d*) Pachytene. Chromosomes are fully synapsed. (*e*) Diplotene. (*f*) Diakinesis. Ten bivalents are individualized. (g) Metaphase I. (*h*) Anaphase I. X-shape chromosomes due to the maintenance of centromeric cohesion. (*i*) Telophase I. (*j*) Prophase II. (*k*) Metaphase II. (*l*) Anaphase II. Scale bar = 5 μm. (*m–v*) Male meiosis in *Arabidopsis thaliana*. (*m*) Leptotene. (*n*) Zygotene. Regions of pairing between homologs are indicated (*arrows*). (*o*) Pachytene. (*p*) Diplotene. (*q*) Diakinesis. The positions of the chiasmata (*arrows*) and the centromeric regions (*arrowheads*) are indicated. (*r*) Metaphase I. (*s*) Anaphase I. (*t*) Prophase II. The organelle band between the two groups of chromosomes is indicated (*arrow*). (*u*) Metaphase II. (*v*) Telophase II. The images were obtained from DAPI-stained chromosome spreads following the fixation of floral buds. These images were provided by W. Li. Scale bar = 5 μm.

anthers whereas the wild-type produce big anthers with a high pollen production rate. In *Arabidopsis*, the phenotypic screening for fruit length allows the discrimination between fertile (long silique) and sterile (short silique) plants. In rice, screening for sterility is based on the color of the fruit: Fertile fruits undergo a normal development during which glumes become brown; in contrast, in fruits that contain aborting ovules, glumes remain green (D. Grimanelli, personal communication).

Secondary screen: distinction between developmental and meiotic mutants. Secondary screens, based on cytological approaches, have been used to distinguish sterility due to meiotic defects from sterility due to defects in sporocyte initiation, anther dehiscence, or environmental factors (158, 198). Verification of the phenotype over several generations can also prove to be helpful to confirm that reduced fertility or sterility is caused by a genetic defect in meiosis (153).

At this step, maize presents several advantages that render this secondary screen more effective as cytogenetic approaches are easier in this organism. The meiocytes are embedded in a matrix of callose, and this material allows the dividing cells to be extruded mechanically from the cut end of an anther with minimal damage to the cells. Furthermore, because of their large size, chromosomes can be visualized by light microscopy in acetocarmine-stained, squashed meiocytes. This visualization allows for accurate screening of a great variety of phenotypes as we detail further in this review. In *Arabidopsis*, the smaller size of the chromosomes and the difficulty in obtaining intact meiocytes renders cytological examination of meiotic phenotypes more difficult, although improved cytogenetic techniques has permitted a more complete analysis of meiotic chromosome behavior (7, 9, 154). In addition, the *Arabidopsis* inflorescence produces new floral buds over a periods of weeks, particularly in sterile mutants; this characteristic allows cytological screens to be performed on the same plants identified as mutant in the primary sterility screen.

A comparison of the power of forward genetics in maize and *Arabidopsis*. Although more meiotic genes have been identified in *Arabidopsis*, there is a larger collection of meiotic mutants in maize than in *Arabidopsis*, more than 60 representing 35 complementation groups. There are several reasons why this has happened.

The initial screen for meiotic mutants in maize is based on the reduction of pollen production, whereas in *Arabidopsis*, fruit size is used as the primary screen. As (*a*) *Arabidopsis* plants produce normal-sized fruits even when the seed number is slightly reduced and (*b*) pollen is normally produced in excess, mutations that result in mild pollen reduction are not easily detected in the screens for reduced fruit size. In contrast, in maize, both the large population of pollen grains in the wild-type and the accessibility of anthers facilitate a relatively easy diagnosis of reduced pollen production. Furthermore, many of the mutants identified to date in *Arabidopsis* have been isolated through T-DNA insertional mutagenesis. Integration of the T-DNA in the genome occurs at a very specific time of plant development, particularly in the female gametophyte, and could bias against those mutations in genes that are important for female gametophyte development. In comparison, EMS and transposon mutagenesis approaches are not dependent on the tissue or the development stage, and have been successful in maize as well as *Arabidopsis* (10, 72, 141, 158, 198).

The future of forward genetics. The quality of the secondary screen is a determinant for the efficient isolation of meiotic mutants by forward genetics. Extended and detailed secondary cytological screens are currently done in maize because of its excellent chromosome morphology and meiocytes accessibility, and we can hope that the use of transposon-tagging strategies will facilitate the cloning

of the corresponding genes. Thanks to the still growing number of meiotic genes and phenotypes described in other organisms, yeast especially, candidate approaches will become more efficient towards cloning current maize and *Arabidopsis* mutants without going through map-based cloning protocol (72). With *Arabidopsis*, as cytological techniques improve, performing secondary screens on previously unsuccessful screening pools could provide new kinds of mutants. Furthermore, new forward genetics screens could be performed with *Arabidopsis* such as, for example, a screen based on *Arabidopsis* populations that express meiocyte markers that could be readily visualized or, alternatively, a screen to identify suppressors of known mutants.

Reverse Genetics

Isolation of candidate genes on the basis of sequence identity. Studies in budding and fission yeast over the past decades have identified meiotic genes (reviewed in: 48, 56, 152, 204) that subsequently have been found, based on their sequence homology, in plant genomes (120). A higher level of sequence identity is encountered for genes that encode proteins catalyzing homologous recombination, such as *DISRUPTED MEIOTIC cDNA* (*DMC*)*1* (55, 93, 159), *RAD51* (55), and *SPORULATION* (SPO) 11 (70, 75). When the level of sequence similarity is too low, the identification of a homolog is more difficult in silico. For instance, the yeast *ZIP1* and rat *Saccharomyces cerevisiae* calponin *(SCP) 1* genes encode proteins that comprise the transverse filaments of the SC. The similarity of these homologs is structural and functional, but cannot be predicted by the level of their sequence similarity (78). In this respect, all the *Arabidopsis* ZIP1 putative homolog sequences display little more than 20% sequence identity with ZIP1 (80). Finally, the absence of obvious homologs in plant genomes might suggest either that the nonplant meiotic protein function is dispensable, or that other proteins can perform the needed functions. Conversely, plant meiotic-specific genes have been identified (see below).

Isolation of candidate genes based on expression patterns. Another way to identify candidate meiotic genes is to focus on meiotically-expressed genes. Although expression of many *Arabidopsis* and maize meiotic genes can be detected in somatic tissues, some, such as *Arabidopsis* (*At*) *DMC1*, are upregulated during meiosis (93) or possess meiosis-specific splicing variants such as *SYNAPSIS (SYN)1*, *DETERMINATE*, *INFERTILE1(DIF) 1* (11). Approximately 200 genes specific to meiosis and gamete formation had been identified by classical methods by 2000 (146). Probably as many as 1500 genes show altered gene expression as analyzed by microarrays (4, 38, 146); this result leads to an estimate of the total of core genes specific for meiosis at 300 and those specific for sporulation/gametogenesis at 600 (165).

Acquisition of mutants by reverse genetics. Once the candidate gene has been identified, the functional analysis of the protein it encodes can be initiated either through screens of mutant banks or by transgenic approaches. Several mutant banks [e.g., Trait Utility System for Corn (TUSC), Cold Spring Harbor Laboratory (CSHL), Targeting Induced Local Lesions IN Genomes (TILLING)] are available in maize. The availability of the rice genomic sequence is an important resource because (*a*) rice is a diploid rather than an ancient tetraploid like maize, and (*b*) the rice genome is only one-fifth the size of maize (rice is ~480 Mb and maize is 2500 Mb). Furthermore, rice genes average over 85% identity to maize, therefore allowing the use of some of the rice molecular "toolkit" for characterizing maize genes. Finally, microarrays are now available in rice and maize. In *Arabidopsis*, the large number of mutant banks [e.g.,Feldmann, INRA-Versailles, SALK Institute, Syngenta Arabidopsis Insertion Library (SAIL), Institute for Molecular Agrobiology (IMA), Sainsbury Laboratory

Arabidopsis thaliana (SLAT), TILLING] ensures the possibility of getting an allelic series in almost every gene, and several approaches have been used to screen these tagged populations (26). Finally, it is also possible to generate knockdown plants by post-transcriptional gene silencing (73, 80, 170, 193) or by overexpression of a dominant-negative form of the protein.

Limitations of reverse genetics. In addition to imposing a requirement for a priori knowledge, a major limitation in assigning gene function by reverse genetics in plants arises from the finding that many plant genes belong to multigenic families, and there is widespread functional redundancy. For instance, the *Arabidopsis* genome has likely undergone one or more ancient duplication(s) that resulted in approximately 60% of the *Arabidopsis* genome being duplicated, although in some cases, the genes within these duplicated regions have undergone functional divergence (5). Thus, even in cases when putative meiotic genes can be identified on the basis of sequence similarity, there may be several potential candidates whose functions have to be investigated. Furthermore, forward genetics has led to the identification of meiotic genes, such as the maize *POOR HOMOLOGOUS SYNAPSIS* (*PHS*) *1* and *AMEIOTIC 1* (*AM1*), that are not present in nonplant genomes. Although reverse genetics is a powerful tool to study genetics of meiosis in plants, its limitations validate forward genetics as a worthwhile and complementary approach. See **Table 1** for a list of genes included in this review.

MEIOTIC COMMITMENT

Before meiosis commitment, a genetic control determines cell fate, e.g., that the cell will become a meiocyte, and the cell cycle is subsequently switched from mitotic to meiotic. In maize, the *multiple archaesporial cells (mac) 1* mutant produces an increased number of male sporocytes and lacks tapetal cells; this observation demonstrates a role for *MAC1* in cell fate determination (168, 169). Similar phenotypes have been observed in the *Arabidopsis excess microsporocytes (ems) 1/ extra sporogenous cells (exs) 1* and *tapetum determinant (tpd) 1* mutants as well as the rice *multiple sporocyte (msp) 1* mutant; molecular analyses of these mutants suggest that cell-cell communication between the tapetum and the meiocytes plays a role in cell fate determination (for review see Reference 110).

Little is known regarding the initiation of meiosis in plants. When anther explants are cultured before late S/G2, the pollen mother cell is induced to revert from meiosis to a mitotic division (87). This observation suggests that, as in yeast and animals, entrance into meiosis is probably determined during premeiotic S-phase. The isolation of two mutants, *ameiotic* 1 in maize and *switch1* in *Arabidopsis*, has showed that the meiotic switch is genetically determined.

Ameiotic1 and Switch1 Regulate the Initiation of Meiosis

In the *ameiotic1-1 (am1-1)* mutant, meiotic divisions in both male and female meiocytes are replaced by mitotic divisions (65, 149). In addition to a mitotic-like prophase and mitotic chromosome morphology, *(am1-1)* exhibits mitotic-like spindles and preprophase microtubule bands (179). Furthermore, meiocytes in the *(am1-1)* mutant do not contain RAD51 foci; this observation indicates that the meiotic recombination machinery is not installed in this mutant.

Six *am1* alleles have been found; for five of these, mutations in the alleles cause male meiocytes to undergo mitosis, and female meiocytes either to undergo mitosis or to arrest at interphase. In the sixth allele, *am1-prophase arrest* (*pra*) *1*, chromosomes display a leptotene morphology but do not form a telomere bouquet (see the section The Bouquet: Facilitating Pairing? below for a discussion of bouquet) and do not exhibit RAD51 foci; this result indicates that,

Table 1 List of genes in the review

Gene	Organism	Mutant	Allele	Homolog
ABSENCE OF FIRST DIVISION 1	Maize	*afd1*		*Rec8*
ARABIDOPSIS HOMOLOGUE PAIRING 2	*Arabidopsis*	*ahp2*		*Hop2*
AMEIOTIC 1	Maize	*am1*	*pra1*	—
ARABIDOPSIS SKP1-LIKE1	*Arabidopsis*	*ask1-1*		—
ASYNAPTIC 1	Maize	*asy1*		*Hop1*
BRCA2	*Arabidopsis*	*atbrca2*		*Brca2*
DIF1	*Arabidopsis*	*dif1*	*syn1*	*Rec8*
DISRUPTED MEIOTIC cDNA 1	*Arabidopsis*	*atdmc1*		*Dmc1*
DESYNAPTIC CS	Maize	*dsyCS*		—
DYAD	*Arabidopsis*	*dyad*	*ms4swi1*	—
MALE STERILE 4	*Arabidopsis*	*ms4*	*dyadswi1*	—
MALE STERILE 5	*Arabidopsis*	*ms5*	*tdm1pollenless3*	—
MER3	*Arabidopsis*	*atmer3*	*rck*	*Mer3*
MSH4	*Arabidopsis*	*atmsh4*		*Msh4*
MMS25	Maize	*mms25*		—
MRE11	*Arabidopsis*	*atmre11*		*Mre11*
PAIR2	Rice	*pair2*		*Hop1*
PH1	Wheat	*ph1*		—
PLURAL ABNORMALITIES OF MEIOSIS 1	Maize	*pam1*		—
POLLENLESS 3	*Arabidopsis*	*pollenless3*	*ms5tdm1*	—
POOR HOMOLOGOUS SYNAPSIS	Maize	*phs1*		—
PROPHASE ARREST	Maize	*pra*	*am1*	—
RAD50	*Arabidopsis*	*atrad50*		*Rad50*
RAD51	*Arabidopsis*	*atrad51*		*Rad51*
RAD51-B	*Arabidopsis*	*atrad51B*		*Rad51B*
RAD51-C	*Arabidopsis*	*atrad51C*		*Rad51C*
ROCK N' ROLLER	*Arabidopsis*	*rck*	*mer3*	*Mer3*
SMC3	*Arabidopsis*	—		*Smc3*
SOLO DANCERS	*Arabidopsis*	*sds*		*Cyclin*
SPORULATION 11-1	*Arabidopsis*	*atspo11-1*		*Spo11*
SWITCH	*Arabidopsis*	*swi1*	*dyadms4*	—
SYN1	*Arabidopsis*	*syn1*	*dif1*	*Rec8*
TARDY ASYNCHRONOUS MEIOSIS	*Arabidopsis*	*tam*		*Cyclin A*
THREE-DIVISION MUTANT	*Arabidopsis*	*tdm1*	*pollenless3ms5*	—
X-RAY CROSS COMPLEMENTING 2	*Arabidopsis*	*atxrcc2*		*Xrcc2*
X-RAY CROSS COMPLEMENTING 3	*Arabidopsis*	*atxrcc3*		*Xrcc3*

although meiosis is initiated, it subsequently arrests at the leptotene-zygotene transition in this mutant (66, 140). The *AM1* gene has recently been cloned (W. Pawlowski & W.Z. Cande, unpublished data) by using a Mutator-tagged *am1-489* allele and encodes a protein with unknown biochemical function but partial sequence similarity to the *Arabidopsis* SWITCH1 (SWI1) protein. Four *swi1* alleles have been isolated [*swi1-1*, *swi1-2*, *dyad* and *male sterile* (*ms4*)], which show similar defects in female meiosis: Ten univalents are

SCC: sister chromatid cohesion

SMC: Structure Maintenance of Chromosomes

observed at metaphase I, and sister chromatids segregate evenly into two daughter cells (a mitosis-like division) in *swi1-1*, *swi1-2*, *dyad* and *ms4* (122, 128, 171). This result strongly suggests that these two proteins are functional homologs. A role of SWI1 in initiating meiosis is compatible with its expression profile, as the protein is detected during premeiotic S phase but is absent by leptotene, as demonstrated by both GFP fusion and immunocytological analysis (119, 122).

However, male meiosis is initiated in *swi1* mutants. The *swi1-2* mutant exhibits the most extreme phenotype of the four alleles so far described (1, 122, 128). The *swi1-2* mutation results in a lack of bivalent formation, absence of RAD51 foci, and precocious loss of sister chromatid cohesion (SCC) during male meiosis (119, 122). These defects lead to the presence at metaphase I of 20 chromatids rather than five bivalents. At the molecular level, in the *dyad* allele of *swi1*, DMC1 is expressed, as indicated by a line that expresses ß-*glucuronidase (GUS)* under the control of the *DMC1* promoter (*pDMC1::GUS)* (1). The analyses of *am1* mutants suggest that cell division in *am1* meiocytes is more mitosis-like than that of *swi1* in male meiocytes (66). Assuming that *swi1-2* is a null allele, the differences between maize and *Arabidopsis* could be due to a difference in other genetic factors, such as the presence of *SWI1* homologs in the *Arabidopsis* genome, and also possibly in the maize genome. At this point one should stress that male meiosis in maize and *Arabidopsis* are not similar in the wild-type: In particular, maize male meiocytes undergo cytokinesis at the end of both Meiosis I and Meiosis II, whereas, in *Arabidopsis*, cytokinesis occurs only once at the end of Meiosis II. Another way to interpret these phenotypic differences is to propose that downstream components are differently regulated by AM1 and SWI1 in maize and *Arabidopsis*, respectively. In maize, the six *am1* alleles are epistatic to *absence of first division* (*afd*)*1-1*, which is defective in the maize *REC8* homolog (66; I.N. Golubovskaya, O. Hamant & W.Z. Cande, unpublished data). Similarly, the *swi1-2* mutant is epistatic to *syn1*, which is defective in the *Arabidopsis REC8* homolog (119). This result strongly suggests that the control of the initiation of meiosis occurs before regulation of meiotic SCC. Interestingly, the SWI1 protein contains a domain with weak similarity to Structure Maintenance of Chromosomes (SMC); this observation suggests an involvement of the protein in the SCC complex.

The Commitment Cascade

The control of meiosis progression is not wellunderstood. Sequence analysis suggests that the AM1 protein contains a coiled-coil domain that is likely to be involved in protein-protein interactions; this suggestion is consistent with the idea that commitment to meiosis might involve other regulators of the cell cycle. In addition, other meiotic proteins have been shown to contain either a domain related to or sequence similarity to cell cycle proteins (10, 195).

The *MS5* gene encodes a protein with no clear homology with other known proteins (64). However, stretches of the protein have limited similarity to the SC protein SCP1 from rat and the regulatory subunit of a cyclin-dependent kinase from *Xenopus* (64). There are two other genes in *Arabidopsis* with sequence similarity to *MS5* (*POLLENLESS3-LIKE1* and *POLLENLESS3-LIKE2*) (158). In the *ms5/three-division mutant* (*tdm*)*1/pollenless3* mutant, the pollen mother cells appear to undergo two rounds of normal meiotic division. However, at the end of meiosis II the cell attempts a third division without any further DNA replication (64, 153, 158). The chromosomes recondense, attach to a spindle, and random groups of chromatids eventually form interphase nuclei (153). This phenotype strongly ressembles that of the *polymitotic* mutant in maize (15).

Cyclins seem to play a major role during meiosis progression. The *Arabidopsis*

mutant *tardy asynchronous meiosis* (*tam*) is slowed in the progression of male meiosis (111) and has been shown to be defective in the A-type cyclin CYCA1;2 (195). By expressing a CYCA1;2-green fluorescent protein (GFP) fusion protein under the control of the *CYCA1;2* promoter, Wang et al. (195) showed that CYCA1;2-GFP is only detectable in prophase I. They also demonstrated that the duration of pachytene and meiosis II are longer in *tam* than in wild type. Therefore, CYCA1;2 seems to regulate both meiosis I and meiosis II even though there is little or no CYCA1;2 present after prophase I. Either the CYCA1;2 produced in prophase I indirectly regulates meiosis II progression, or a very low level of CYCA1;2 directly regulates meiosis II progression (195).

The SOLO DANCERS (SDS) protein, which represents a new type of cyclin in *Arabidopsis*, has been found to regulate synapsis and recombination in prophase I (10, 194). The mutant meiocytes fail to form normal pachytene bivalents, consistent with a defect in pairing and/or synapsis. In addition, the mutant meiocytes have greatly reduced levels of meiotic recombination. The sequence similarity of SDS to other proteins suggests that it may regulate several processes during prophase I. However, the SDS protein is distinct from other known or predicted cyclins; this fact suggests that its function may be different from those of other mitotic cyclins. In the rice genomic sequence, there is a putative ortholog of SDS; this observation suggests that SDS is conserved in flowering plants (194). In yeast, the Clb5/Clb6 cyclins are important for both premeiotic S phase and for events during prophase I, including synapsis and recombination (176). In mouse, cyclin A1 is required during spermatogenesis; although mutant meiocytes can undergo chromosome synapsis, they subsequently degenerate (106). It is possible that the *Arabidopsis* SDS, the yeast Clb5/Clb6, and the mouse cyclin A1 may have some conserved functions, although they clearly also have some differences.

CHROMOSOME STRUCTURE AND HOMOLOGY RECOGNITION

Chromatin Condensation

As outlined earlier, the commitment proteins are required for meiosis initiation and de facto are required for establishment of the structure of meiotic chromosomes during early prophase I. In *swi1-2*, premature loss of SCC and incomplete chromosome condensation is observed. On the basis of the phenotype of male meiocytes and a very weak sequence similarity of SWI to mammalian SMC proteins, a regulatory role of SWI1 in chromosome structure establishment and SCC was proposed (1, 122).

Sister chromatid cohesion (SCC). During premeiotic S-phase, newly replicated sister chromatids are associated via a multiprotein cohesin complex (reviewed in 101, 189). SCC is absolutely required for the control of chromosome structure and many subsequent meiotic events.

Cohesion proteins have been extensively studied in yeast and animals, and some homologs have been characterized in plants. The cohesin complex is ring shaped and comprises a core of three proteins (REC8/RAD21, SMC1 and SMC3) and several associated proteins such as SCC3 and PRECOCIOUS DISSOCIATION OF SISTERS (PDS) 5 (reviewed in 130). The REC8/RAD21 protein is the main regulator of the complex, as, at anaphase, its cleavage by separase releases cohesion and thus allows chromatids to segregate (71). In yeast, RAD21 is present in the mitotic cohesin complex and is mostly replaced at meiosis by REC8. In animal meiotic cells, SMC1ß replaces SMC1, and STROMAL ANTIGEN (STAG) 3 replaces SCC3 (145, 148). In plants, the distinction between the meiotic and mitotic cohesin complex is less clear; plant REC8 homologs like SYN1 or AFD1 display a stronger sequence similarity with yeast and animal RAD21 than yeast and

animal REC8, and the expression of the plant homologs is not meiosis specific (11, 138; I.N. Golubovskaya, O. Hamant & W.Z. Cande, unpublished data). Other meiotic effectors, such as *PHS1*, *ZmSGO1*, or *AM1*, are also expressed in both somatic and meiotic tissues in maize (72, 141; W. Pawlowski & W.Z. Cande, unpublished data). At this point, we can speculate that these differential observations may reflect the fact that in contrast to animals, a true germ line is absent in plants. Furthermore, there is only one SCC3 sequence and no STAG3 sequence in the *Arabidopsis* genome (36). However, at a functional level, analysis of mutants impaired in several cohesins have shown that the cohesion complex is absolutely required for regulation of chromosome structure during meiosis in plants.

Mutations in the *SYN1/DIF1/AtREC8* gene result in a complex meiotic phenotype that affects both male and female fertility (142). A defect can initially be detected as early as leptotene, when chromosome condensation appears irregular (11); however, chromosomes appear to be normally synapsed during pachytene (18). Recent analysis supports a function of SYN1/DIF1, in addition to SCC, in chromosome pairing and/or juxtaposition, (30). Extensive chromosome fragmentation is clearly observed by anaphase I. Further acentric fragments, together with chromosome bridges, are seen at anaphase II. Subsequent nondisjunction leads to the production of polyads with up to eight spores that contain variable amounts of DNA (18).

Two alternately-spliced *SYN1/DIF1* transcripts have been identified; these transcripts encode proteins that are 627 (expressed at low levels in all tissues) and 617 (expressed in buds only) amino acids long (11). Evidence that SYN1/DIF1 acts as a cohesin is supported by the fact that the protein can partially substitute for the Mcd1 mitotic cohesin protein in yeast complementation tests (54).

Recently, we have cloned *AFD*, the maize *SYN1* ortholog, and have initiated an analysis of its role in leptotene chromosome structure establishment (I.N. Golubovskaya, O. Hamant & W.Z. Cande, unpublished data). Using two *afd1* null alleles, we have demonstrated that AFD1/ZmREC8 is absolutely required for the maintenance of SCC, axial element elongation and homologous pairing (see the sections Homologous Chromosome Alignment and Installation of the Synaptonemal Complex). Surprisingly, in weak *afd1* alleles with reduced level of AFD1 expression, we observed leptotene chromosomes as well as bouquet formation (see the section The Bouquet: Facilitating Pairing?") at zygotene. We showed that this new *afd1* phenotype is due to the partial restoration of axial element elongation in the weak alleles, independent of the installation of the recombination machinery. This suggests that the establishment of early prophase I chromosome structure depends on the level of REC8 (I.N. Golubovskaya, O. Hamant & W.Z. Cande, unpublished data). It would be interesting to know if, conversely, the meiocyte has the ability to control the level of AFD1 to regulate the kinetics of early prophase I chromosome structure establishment. Consistent with our data, Chelysheva et al. (36) found that AtREC8 is involved in chromosome axis formation, and that this function is AtSPO11-1 independent in *Arabidopsis*.

In addition to SYN1 and AFD1, three other REC8/RAD21 proteins are present in each of the *Arabidopsis* and maize genomes (54). No mutant alleles of these genes are known. Furthermore, the function of these genes in the cohesin complex can be questioned, as two of the *Arabidopsis* RAD21 homologs cannot complement yeast cells that are deficient in the Mcd1 mitotic cohesin (54). The rice genome also contains four REC8/RAD21 sequences, and *OsRAD21-4* is the closest homolog of *AFD1* (O. Hamant & W.Z. Cande, unpublished data). The expression profile of OsRAD21-1 is not meiosis specific (200). It remains to be determined whether these putative RAD21 proteins share some of the REC8 functions during meiosis.

Homologs of SMC1, SMC3, and SCC3 have also been identified in *Arabidopsis* (36,

100) and are associated with meiotic chromosomes. Surprisingly, the *Arabidopsis* SMC3 protein is also associated with the meiotic and mitotic spindle from metaphase to telophase, but not with perinuclear microtubules during prophase. Analysis of the *syn1* mutant indicates that the chromosomal localization of SMC3, but not the spindle localization, is dependent on normal SYN1 function. Although no mutants have been described for *AtSMC1* and *AtSMC3*, these findings suggest that SMC proteins may have both conserved functions similar to those in other organisms, as well as novel functions specific to plants. AtSCC3 is present on chromosomes from leptotene until metaphase I and is required, together with SYN1, to maintain centromere cohesion and the monopolar orientation of the kinetochores at anaphase I (36; for review on chromosome segregation, see 50, 196). In addition, the *atscc3* mutant shows defective synapsis and abnormal condensation; this observation suggests a role of AtSCC3 in meiotic prophase chromosome structure establishment (36). According to present data, it is still difficult to address the contribution of the different cohesins in establishing prophase I chromosome structure in plants.

Histone modifications. The histone H3 phosphorylation pattern correlates well with the chromosome condensation during meiosis in mammals; however, in plants, the distribution of H3Ser10 phosphorylation correlates better with SCC (61, 89, 112). During the first meiotic division, entire chromosomes are highly phosphorylated, whereas, in the second division, H3 phosphorylation is restricted to the pericentromeric regions (89). During the second meiotic division, single chromatids, which result from equational division of univalents at anaphase I, show low levels of phosphorylation throughout the chromosome (61, 112). Furthermore, in the *afd1/zmrec8* mutant, which is defective in SCC, univalents at metaphase I showed high levels of H3Ser10 phosphorylation only in the pericentromeric regions, and unattached sisters at MII showed no staining at all (89). Therefore, plant H3 phosphorylation at Ser10 could regulate prophase chromosome condensation via a role in SCC.

One possibility to explain the differences between plants and animals is that both kingdoms may have evolved different histone codes. Recent observations have shown that H3 phosphorylation on Thr 11 in mammalian cells is restricted to the centromeric region (144). In contrast, in several plants (i.e., *Arabidopsis thaliana*, *Secale cereale*, *Triticum aestivum*, *Hordeum vulgare*, *Vicia faba*, *and Zea mays)*, H3Thr11 phosphorylation is distributed along the entire length of condensed chromosomes during meiosis (84; O. Hamant & W.Z. Cande, unpublished data). It is still not known if H3Thr11 phosphorylation in plants is tightly associated with condensation and independent from cohesion.

In addition to cohesins and chromatin modifications, the recruitment of axial elements of the SC is essential for the formation of leptotene chromosomes (see the section Installation of the Synaptonemal Complex below for a discussion of SC installation).

Homologous Chromosome Alignment

The biggest unresolved problem in meiosis is understanding the mechanism that allows homologous chromosomes to find each other and pair. Chromosome morphology, specific sequence distribution, and proteins bound to DNA (perhaps also involved in recombination) all may contribute to chromosome homology recognition, but the molecular mechanism remains to be established (for review, see 50).

Theoretically, the initial establishment of a few paired loci could allow the rest of the chromosome to zip-up mechanically, and therefore would increase the efficiency of homology recognition. The clustering of telomeres into a "bouquet" is one of the mechanisms that is thought to facilitate these initial contacts

leading to alignment and subsequent pairing (49, 74).

The bouquet: facilitating pairing? Before chromosomes synapse during zygotene, telomeres attach to the nuclear envelope and cluster to form a structure called the bouquet (74) (**Figure 2*b***). The bouquet stage has been observed in every plant species in which three-dimensional reconstructions have been performed (14). As synapsis typically is initiated near the telomeres (29), it has been proposed that the bouquet may help to facilitate pairing and synapsis. In addition to promoting initial contacts before pairing, the cluster may serve to confine homologous sequences to a small volume of the nucleus so as to promote synapsis.

The mechanism by which the bouquet is formed is unknown as very few bouquet mutants have been identified. The ability to culture rye anthers made it possible to obtain intermediates in bouquet formation, to correlate telomere distribution with elapsed time,

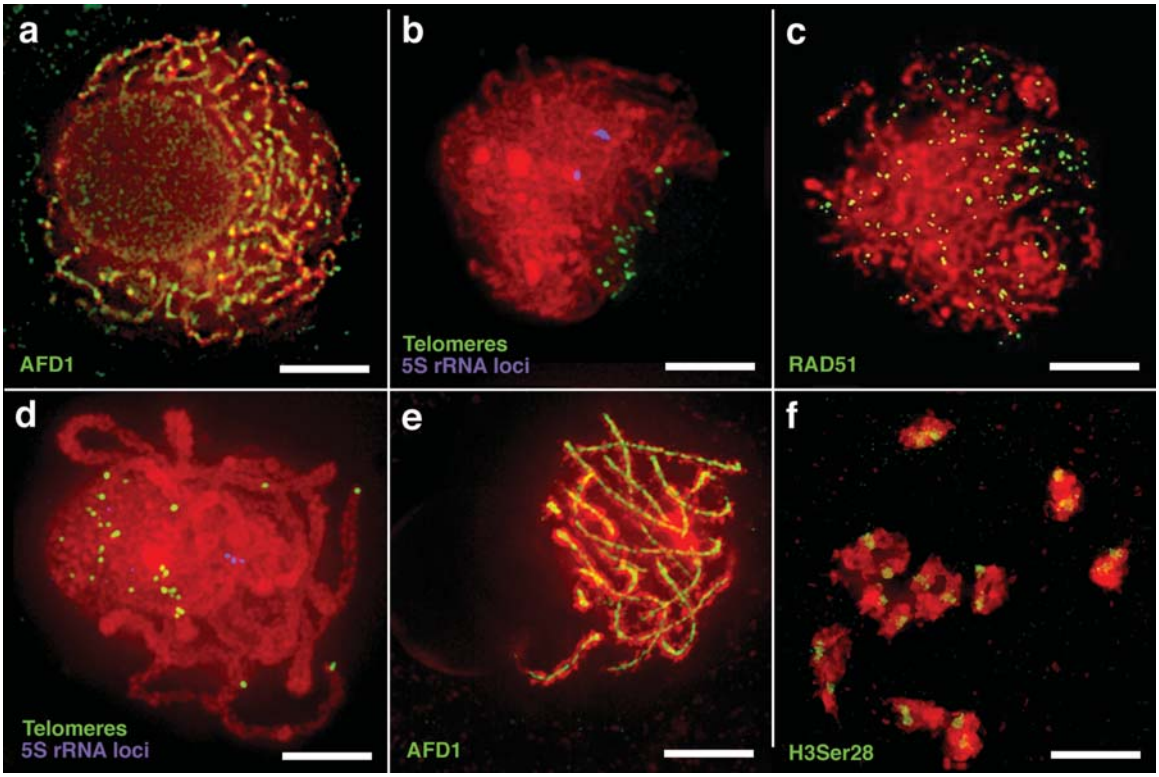


Figure 2

Some prophase I features in maize. DAPI-stained chromatin is shown in red. (*a*) Immunostaining of ABSENCE OF FIRST DIVISION (AFD) 1/ZmREC8, one of the earliest meiotic markers, at the leptotene-zygotene transition. (*b*) The teleomeric "bouquet," indicated by fluorescent in situ hybridization (FISH) at zygotene (see The Bouquet: Facilitating Pairing?); the telomere cluster (*green*) and unpaired 5S rRNA loci (*purple*) are visible. Image provided by I.N. Golubovskaya. (*c*) RAD51 immunostaining at zygotene. Image provided by W. Pawlowski. (*d*) Homologous pairing at pachytene shown by FISH: Paired 5S rRNA loci (*purple*) and spread telomeres (*green*) are visible. Image provided by I.N. Golubovskaya. (*e*) AFD1 immunostaining signal between the synapsed chromosomes at pachytene. (*f*) Histone H3Ser28 phosphorylation occurs at diakinesis; the immunostaining signal localizes to the centromeric and pericentromeric regions. Scale bar = 5 μm.

and thus to obtain an approximate time course of telomere clustering in rye (32). To determine whether the motion of chromosomes was random or directed, a computer simulation of bouquet formation was compared to empirical observations. Directed motion, as opposed to random diffusion, was required to reproduce the observations; this result implies that an active process moves chromosomes to cause telomere clustering. More generally, data from plants and mouse are consistent with a model in which the telomeres attach to the nuclear envelope at random, and then cluster thanks to an active process (14, 49, 160).

A simple hypothesis proposes that the polarized movement of telomeres is driven by the cytoskeleton. Using a monoclonal antibody to calf centrosomes (6C6), Schmit et al. observed staining at the ends of the zygotene chromosomes (162, 163). As the nuclear envelope functions as a microtubule-organizing center (MTOC) in plants, it is possible that nuclear-envelope-associated microtubules are involved in telomere movement. It was found that, in Lilium and Allium, colchicine reduces pairing when applied during bouquet formation (109, 180). Although bouquet formation in plants is sensitive to colchicine, other microtubule depolymerizing drugs, such as amiprophos methyl and vinblastine, do not inhibit telomere clustering in rye; this result suggests that it is not dependent on cytoplasmic microtubules (44). Whether a novel form of tubulin or some other colchicine-sensitive protein is involved in telomere clustering remains to be determined.

We have identified and characterized a maize meiotic mutant, *plural abnormalities of meiosis 1* (*pam1)*, that is deficient in the clustering but not the attachment of telomeres on the nuclear envelope (68). In *pam1*, leptotene chromosomes look completely normal using both deconvolution three-dimensional microscopy and transmission electron microscopy, telomeres attach normally to the nuclear envelope, are normally polarized in the nucleus, and undergo some initial stages of clustering by forming several small clumps of telomeres; however, telomeres cannot cluster into a normal tight bouquet (68). Interestingly, chromosomes in *pam1* behave similarly to rye chromosomes treated with colchicine.

Beyond bringing chromosomes into close proximity, what is the impact of bouquet formation on subsequent prophase events? The *pam1* meiotic nuclei have aberrant synapsis and a dramatic reduction in homologous pairing. However, RAD51 foci on zygotene chromosomes are normal in number; these observations suggest that (*a*) the early stages of recombination do not require bouquet initiation and (*b*) these two processes can be separated (68). Still, there is no evidence showing that the bouquet is absolutely required for pairing (63, 118). Even though the bouquet was restored in the weakest *afd1* allele, homologous pairing was still impaired, demonstrating a minor contribution of the bouquet in the AFD1-dependent homologous pairing pathway (I.N. Golubovskaya, O. Hamant & W.Z. Cande, unpublished data). Conversely, pairing does not impact bouquet formation as in maize *phs1*, *maize male sterile* (*mms)25*, and *desynaptic Chris Staiger* (*dsyCS*) mutants, morphologically normal bouquets are observed, but homologous chromosomes do not synapse and RAD51 complexes are not detected cytologically. To conclude, although pairing and bouquet formation are mutually independent, the clustering of telomeres is one of several possible mechanisms that may facilitate the initial homology recognition.

Homeologous pairing. The presence of a polyploid genome creates new problems for the pairing of homologous chromosomes. In polyploids, three or more chromosome sets, either from a given species (autopolyploids) or from related diploid species that sexually hybridized (allopolyploids), coexist. At meiosis, more than two homologous or genetically related (homoeologous) chromosomes can compete for synapsis and recombination. Pairing of homeologous partners would result in multivalent associations and improper

segregation of chromosomes at anaphase I to produce unbalanced and unviable gametes. To circumvent this problem, many allopolyploids show a diploid-like meiotic behavior with strict homologous pairing (166).

Little is known about the mechanism that allows the discrimination between homologous and homeologous pairing. In wheat, it has been proposed that the association of homologous chromosomes before meiosis prevent multivalent association and allows a true diploid-type homologous pairing (6, 114). In the wild-type, synapsis with a different partner at each end can occur, and a multivalent configuration results. In the course of prophase I, these multivalent associations are transformed into bivalents. In the *pairing homoeologous (PH)1* mutant, many of the multivalent associations persist until metaphase I (166). However, PH1 cannot prevent nonhomologous/homeologous chromosomes from associating via their centromeres when homologous chromosomes are absent in the hexaploid wheat-rye hybrid (115). This observation suggests that the discrimination between homologous and homeologous does not occur initially but is performed after chromosome alignment. In this scenario, PH1 would be required to resolve incorrect pairing (126, 151). Isolation of the *PH1* sequence should clarify the mode of action of this gene in wheat and its homologs, if any, in other species.

SYNAPSIS, RECOMBINATION, AND CROSSOVER FORMATION

Installation of the Synaptonemal Complex

Synapsis of homologous chromosomes is observed cytologically during zygotene as the installation of an evolutionarily conserved tripartite structure: the synaptonemal complex (SC) (for reviews see 62, 125, 137, 191, and 204). At leptotene, each chromosome forms chromatin loops that are attached to an axial element. During pairing, these axial elements come together to become the lateral elements of the SC with the central element between them, giving the SC its tripartite structure (**Figure 3**).

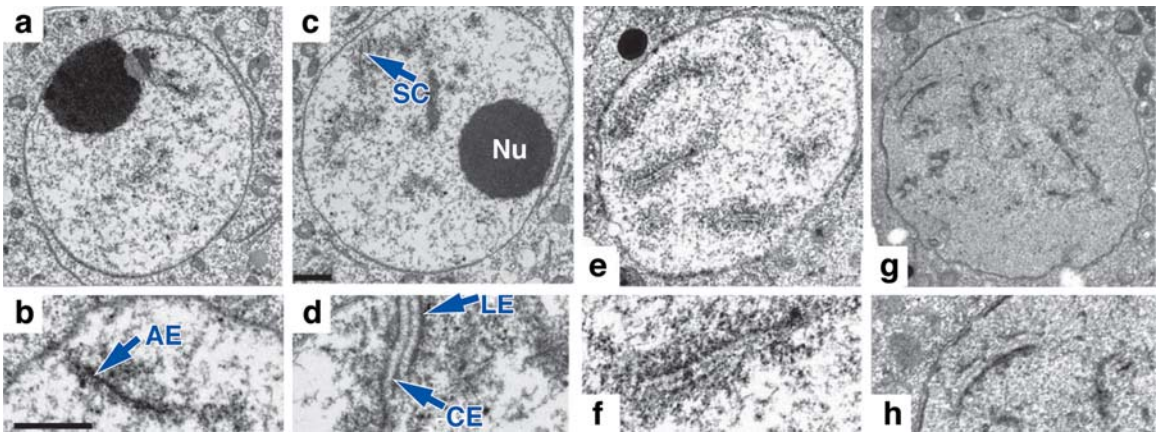


Figure 3

Transmission electron microscopy images of *Arabidopsis* male meiocytes. (*a*, *b*) Leptotene. Unsynapsed axial elements (AE); early nodules are associated with the axial element in *b*. (*c*, *d*) Zygotene. There are both unsynapsed and synapsed axial elements, called lateral elements (LE), in the nucleus. An early recombination nodule is present on the central element (CE) of SC in the synaptic fork I *d*. (*e*, *f*) Early pachytene. All axial elements have become LE of the SC; recombination nodules can be seen regularly on the central element of the SC. (*g*, *h*) Late pachytene. Only a few recombination nodules can be observed on the central elements of SCs. Images kindly provided by L. Timofejeva.

By far, defects in synapsis are the most frequent phenotype among meiotic mutants in forward genetic screens. This result is mainly due to the fact that defective synapsis can arise as a consequence of several primary defects in SCC, homolog pairing, or recombination (88). In this respect, in both maize *afd1* and *Arabidopsis syn1* mutants, depletion of the key meiotic cohesin REC8 induces defective SC installation and absence of chiasmata; this result shows that SC formation and chiasmata maintenance depends on the presence of cohesion (11; I.N. Golubovskaya, O. Hamant & W.Z. Cande, unpublished data). Several desynaptic mutants have been characterized (for review, see Reference 110) but the corresponding genes have not yet been cloned.

Asynaptic1 (*asy1*) is an *Arabidopsis* mutant that exhibits a failure of homologous chromosome synapsis during prophase I (8, 153). The *ASY1* gene has been cloned and encodes a protein with significant homology to the yeast Hop1 protein over the N-terminal half. The C-terminal half of the ASY1 protein sequence has no obvious similarity to any other protein in the database (33). Yeast *hop1* mutants exhibit reduced levels of meiotic recombination and extremely low levels of spore viability (81). The Hop1 protein is first observed as multiple discrete foci during prophase I and is associated with the axial elements (82, 174). The Hop1 signal disappears at pachytene when full synapsis is completed (174). The phenotypes of the *hop1* mutants in yeast and related proteins in *C. elegans*, *Arabidopsis*, and rice are very similar; this observation suggests that the function of *HOP1* in SC formation is conserved across kingdoms (33, 82, 134, 199). Overall, the ASY1 protein exhibits the same immunostaining pattern in *Arabidopsis Brassica oleracea* and *Zea mays* (8; I.N. Golubovskaya, O. Hamant, R.C.J. Wang & W.Z. Cande, unpublished data) as Hop1 in yeast. However, ASY1 is first detected as early as meiotic interphase as punctate chromatin associated foci and is maintained longer in *Arabidopsis Brassica oleracea* than Hop1, which is lost when the chromosomes desynapse (8). Interestingly, immunogold labeling, which gave a higher resolution than standard immunostaining, revealed a discontinuous pattern along the axial/lateral elements. This observation could suggest a role of ASY1 in recruiting the bases of chromatin loops to the developing axial/lateral elements (8). In maize, the behavior of ASY1 and AFD1 is different during synapsis: ASY1 is released from the chromosomes when chromosomes synapse, whereas AFD1 is maintained. This suggests that AFD1, but not ASY1, has a role in the maturation of axial element into lateral elements during synapsis (I.N. Golubovskaya, O. Hamant, R.C.J. Wang & W.Z. Cande, unpublished data). The rice *pair2* mutant partially phenocopies the *asy1* mutant from *Arabidopsis*, although the *pair2* phenotype is more severe than that of *asy1*, as only univalents are observed at metaphase I. The *PAIR2* gene has been shown to encode a homolog of *ASY1* and *HOP1* (134).

Although central elements have been studied in detail in yeast and later in animals (137), little is known about plant central elements. Recently, the combination of a BLAST search and a prediction of the biochemical properties of the central element proteins led to the identification of two genes called *AtZIP1a* and *AtZIP1b* in the *Arabidopsis* genome (80). The presence of an AtZIP1 immunostaining signal in both *atzip1a* and *atzip1b* mutants as well as their identical mutant phenotypes suggest that *AtZYP1a* and *AtZYP1b* are functionally redundant. Both proteins are present in meiocytes during prophase I only. Double immunolocalizations demonstrated a central localization of AtZIP1, bordered by ASY1 on both lateral elements of the SC. Higgins et al. (80) also showed that the initiation of recombination is necessary for AtZIP1 recruitment but is not sufficient for its polymerization in a central element. RNAi lines were generated to deplete both *AtZYP1a* and *AtZYP1b*; knockdown of these genes resulted in delayed meiosis, absence of pairing and synapsis in most meiocytes. However, crossover distribution, as monitored by MUTL HOMOLOG

(MLH) 1 immunostaining, was not greatly affected; this result suggests that the loss of AtZYP1 does not prevent progression to the later stages of recombination. Finally, chiasmata observed in the *AtZYP1* RNAi lines were shown to occur between homologs and nonhomologs; this result demonstrates that the absence of AtZIP1 allows nonhomologous recombination to occur. Studies on budding yeast SC proteins have led to the proposal of a surveillance mechanism that monitors progression through prophase I (25). The analysis of AtZIP1 points towards strong similarities between *Arabidopsis* and budding yeast and suggests that the SC might act as a surveillance complex in plants to ensure a correct progression through recombination (80).

The Plant Recombination Pathway

Many genes have been identified that are important for meiotic recombination (for reviews, see 91, 105, 190). Given the high degree of conservation of recombination proteins among species, the reverse genetic approach has proven to be very powerful for the characterization of the functions of plant recombination proteins, in particular in *Arabidopsis* (**Figure 4**) (for reviews, see 3, 17, 110).

The recombination sequence. Meiotic recombination is initiated by DSBs generated by the Spo11 protein (16, 70, 91). The *Arabidopsis* AtSPO11-1 protein (one of three Spo11 homologs of *Arabidopsis*) is the functional homolog of yeast Spo11, as shown by its requirement for initiation of meiotic recombination (70, 75, 76). However, in *atspo11-1* meiocytes, a few bivalents at diakinesis are observed, suggesting that either recombination can still occur, or that bivalents form in a recombination independent way (70).

The initial DSBs are resected from 5′ to 3′ by the MRX complex, which is composed of Mre11, Rad50 and Xrs2/Nbs1. In yeast, the MRX complex is required for the formation of Spo11-induced DSBs and the processing of these DSBs ends (41, 175). *Arabidopsis* homologs of Rad50 (AtRAD50) and Mre11 (AtMRE11) have been shown to form a complex, and functional analysis of the *atrad50* and *atmre11* mutants strongly suggest that plants have a functional homolog of the MRX complex (22, 46, 60, 147).

Single-stranded DNA ends created by the coordinated action of SPO11 and the MRX complex invade the homologous double-stranded DNA. This step is catalyzed by RAD51 and DMC1, which are homologs of the RecA recombinase that possess single-stranded DNA-binding ability and DNA-dependent ATPase activity (2, 19). In vitro, these two proteins each have the ability to catalyze the strand exchange reaction, which is the motor of homologous recombination (83, 182). RAD51 functions in both the mitotic cell cycle and meiosis, whereas DMC1 is meiosis-specific (97, 117). Homologs of RAD51 and DMC1 have been identified in *Arabidopsis*, maize, lily, and rice (2, 53, 55, 58, 93, 103, 140). In *Arabidopsis*, the *atrad51* mutant is sterile and exhibits meiotic defects in pairing and synapsis as well as severe chromosome fragmentation; this observation suggests that RAD51 in plants is also involved in DSB repair (103). This hypothesis is further supported by the observation that chromosome fragmentation is absent in the *atrad51 atspo11-1* double mutant; this observation indicates that fragmentation is due to failure to repair *SPO11-1*-induced DSBs.

The *Arabidopsis atdmc1* mutant produces meiocytes with mainly univalents instead of bivalents at late prophase I; this observation indicates that *AtDMC1* is crucial for bivalent formation and chromosome segregation during meiosis in *Arabidopsis* (43). However, no chromosome fragmentation was detected in the *atdmc1* mutant; this result suggests that *AtDMC1* is functionally distinct from *AtRAD51*. Unlike yeast DMC1, AtDMC1 is expressed not only in reproductive tissues but also in leaves and cultured cell suspensions (43).

In maize, double mutants of the two RAD51 homologs have recently been obtained and are male sterile. Surviving female

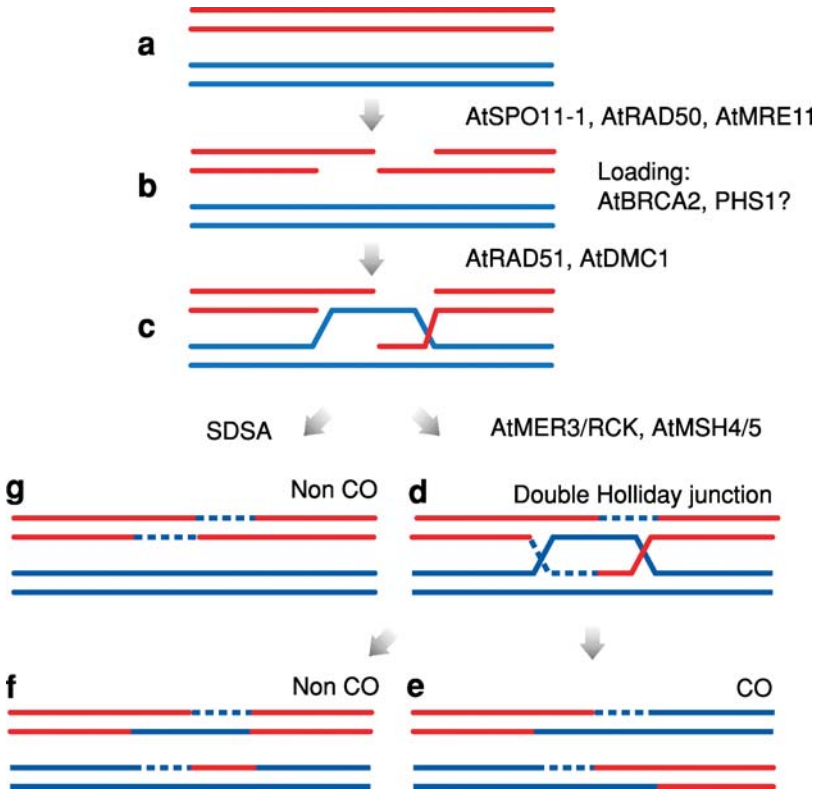


Figure 4

The plant recombination pathway. (*a*) Double-stranded DNA molecules of two nonsister chromatids. (*b*) A DSB is generated by SPO11, and 5′ strands are resected to produce 3′ single-stranded DNAs by the MRE11-RAD50-XRS2 (MRX) complex. (*c*) RAD51/DMC1 driven strand invasion occurs to produce a D-loop. (*d*) DNA synthesis followed by ligation results in a double-Holliday junction, which is stabilized by MUTS HOMOLOG (MSH) 4-MSH5 dimers. (*e*, *f*) Resolution of the double-Holliday junction in the opposite sense (*e*) leads to the formation of an interference sensitive crossover event (*CO*) whereas cleavage in the same sense (*f*) results in a noncrossover event (*Non CO*). (*g*) Alternatively, the Holliday junction progenitors may not be captured by MSH4–MSH5 dimers. At this point, if the invading end is rejected, synthesis-dependent strand annealing (SDSA) may be inititated and a noncrossover arise (*g*); in contrast, capture of the invading end may lead to an interference-insensitive crossover, a pathway in which no plant genes have been described and which is not shown on this figure.

gametes produced by ZmRAD51 double mutants are euploid and exhibit normal rates of crossing-over (P. Schnable, personal communication).

The strand invasion event can proceed down one of two pathways: the classical double-strand break repair (DSBR) pathway or the synthesis-dependent strand-annealing (SDSA) pathway (**Figure 4**) (20). In the DSBR pathway, DNA synthesis from the invading strands and ligation yield the double-Holliday junction. Resolution of the double-Holliday junction following cutting at alternative strands results in products with either crossovers or noncrossovers. X-ray cross complementing (Xrcc) 3, a paralog of Rad51, has been shown to play a role in mitotic recombination, DNA repair, and chromosome stability; it also participates in Holliday junction resolution in vertebrate cells (27, 107, 184, 185). Mutation in *AtXRCC3* induces meiotic chromosome

fragmentation, which leads to gametophytic lethality in *Arabidopsis* (24). Although the *atrad51* and *xrcc3* phenotypes are very similar, the phenotypes of the double mutants *atrad51 spo11-1* and *atxrcc3 spo11-1* are different. In *spo11-1* and *atrad51 spo11-1*, no fragmentation is observed during the second meiotic division (70, 103). In contrast, fragmentation is observed during the second meiotic division in *atxrcc3 atspo11-1* plants (23). Therefore, fragmentation during the second division in *atxrcc3 atspo11-1* is specifically caused by the lack of the AtXRCC3 protein. This late fragmentation results from unresolved sister chromatid events, as they are detectable upon the separation of sister chromatids at anaphase II. Based on the post-synapsis meiotic role of AtXRCC3 and the role of the vertebrate Xrcc3 and Rad51C protein in Holliday junction resolution (184), Bleuyard et al. (23) proposed that AtXRCC3 also contributes to Holliday junction resolution.

Four other RAD51 paralogs (XRCC2, RAD51B, RAD51C, and RAD51D) have been identified in mammals and retain distinct functions related to homologous recombination. (59, 98, 99, 123, 161, 172, 183). In vivo, two complexes have been identified, one containing RAD51B, RAD51C, RAD51D, and XRCC2, and the other containing RAD51C and XRCC3 (116, 197). A third one includes RAD51 and XRCC3 (161). *Arabidopsis* homologs of the RAD51B, RAD51C and XRCC2 have been identified in the genomic sequence (21). Two-hybrid analyses have confirmed that AtXRCC3 interacts with AtRAD51 and AtRAD51C (136). Furthermore, gamma-irradiation has shown that transcription of AtXRCC3 and AtRAD51C is induced in response to DNA damage (136). These data strongly suggest that AtXRCC3 and AtRAD51C are the functional homologs of XRCC3 and RAD51C. Recently, analysis of an insertional mutant indicated that *AtRAD51C* is required for the repair of SPO11-1 induced DSBs during meiotic prophase I (21, 104). Similar to the *atrad51* and *atxrcc3* mutants, the *atrad51c* mutant exhibit meiotic chromosome fragmentation in a SPO11-1 dependent fashion and is completely male- and female- sterile. In addition, *AtRAD51C* is important for both (*a*) normal homolog pairing and/or juxtaposition and (*b*) synapsis (104). In contrast, mutants in *AtRAD51B* and *AtXRCC2* are fertile and do not have detectable developmental defects, although they and the *atrad51c* mutant are all hypersensitive to the DNA-crosslinking agent Mitomycin C (21). Therefore, *AtRAD51B*, *AtRAD51C*, and *AtXRCC2* play a role in DNA repair during the mitotic cell cycle. *AtRAD51C* is required for meiotic prophase I and cannot be substituted for *RAD51* or other *RAD51* paralogs. Further analysis is needed to determine the precise roles of these *RAD51* paralogs.

Loading the recombination machinery. BRCA2 facilitates the loading of RAD51 on single-strand DNA (188). *Arabidopsis* possesses two closely related *BRCA2* homologs (170). To date, no *BRCA2* insertional mutant has been characterized in plants; however, an RNAi approach was used to reduce AtBRCA2 expression in *Arabidopsis* (170). In these meiocytes, chromosome fragmentation occurred, and univalents were formed, which led to uneven chromosome segregation; this phenotype is similar to that seen in cells mutant for *atrad51*, *atrad51c*, or *atxrcc3*. The absence of AtSPO11-1 function suppressed chromosome fragmentation in *BRCA2* RNAi plants; this result demonstrates that AtBRCA2 is required for meiotic recombination and acts downstream of AtSPO11-1 (170). Interestingly, yeast two-hybrid assays showed that AtBRCA2 interacts with AtRAD51 and AtDMC1; this result strongly suggests that the Brca2 function of loading Rad51 and/or Dmc1 on single-stranded DNA is conserved in plants (170).

In maize, Pawlowski et al. (141) identified a novel gene, *PHS1*, involved in loading the recombination machinery onto chromosomes. Observations by transmission electron

microscopy of silver nitrate stained chromosome spreads of male *phs1* meiocytes at different stages of prophase I indicated defects in homologous synapsis. The axial elements of SC are installed properly at pachytene but stretches of the apparently synapsed chromosome segments showed improper chromosome alignment and exchanges of synaptic partners (141). By monitoring homologous pairing using fluorescent in situ hybridization (FISH) to visualize the 5S rRNA locus, it was shown that, although the chromosomes appeared to be synapsed, none of the 5S rRNA loci were paired; this observation indicated that synapsis was completely nonhomologous. The *phs1* mutant meiocytes showed a dramatic decrease in the number of RAD51 foci during zygotene relative to the wild-type, with only three RAD51 foci on average per nucleus. However, the amount of the RAD51 protein in anthers remained the same as in the wild-type meiocytes. Based on the mutant phenotype, Pawlowski et al. (141) proposed that the PHS1 protein is a component of the meiotic recombination pathway and hypothesized that it may be involved in loading RAD51 complexes onto chromosomes. This analysis also showed a link between recombination and homologous pairing (see the section Coordination Between Pairing, Synapsis, and Recombination). The *PHS1* gene was cloned by transposon tagging and encodes a novel protein without significant similarity to any known protein and without any obvious functional features or domains; however, putative homologs are present in *Arabidopsis* and other plants. No homologs have been identified in yeast and animals (141).

Plant recombination proteins: conservation and specificities. Although the recombination pathway seems structurally and functionally conserved among kingdoms, a few phenotypic differences are highlighted among various organisms. Plant genomes contain multigene families of several of the recombination proteins. This is particularly the case for *SPO11* (three homologs in *Arabidopsis*) and *BRCA2* (two homologs in *Arabidopsis*) which, in contrast, are unique in yeast and animal genomes. In the case of *SPO11*, only *SPO11-1* has been shown to be important for meiosis, whereas both copies of *BRCA2* likely have similar meiotic functions. For *RAD51* homologs, functional analysis in fungal, plant, and animal kingdoms suggest that they have evolved distinct functions.

Another main difference is that single mutants in the *RAD51* gene family are embryonic-lethal in mammals but are viable in yeast, *Drosophila*, and plants. The yeast and *Drosophila* mutants are sensitive to radiation and chemicals that induce DNA breaks. In *Arabidopsis*, the mutants are either normal or sensitive to DNA-crosslinking agents. The yeast, *Drosophila*, and plant mutants are similar in that they are defective in meiosis. For instance, the mammalian *rad51* knockout is embryonic-lethal, whereas the *atrad51* mutant seems healthy under normal growth conditions and undergoes mitosis normally (103, 186). Therefore, the meiotic functions of these genes might be conserved between yeast, plants, and animals, but the mitotic DSB repair functions might not be universally critical.

Another way to explain these differences is to assume that members of a multigene family of recombination proteins display redundant functions during mitotic DNA repair, whereas, during meiosis, they have separate functions. The number of DSBs induced during meiosis is very high in comparison with somatic DNA damage, and, although other members of the recombination family could subsitute for an absent protein and repair mitotic DSB easily, that might not be the case during meiotic DSB repair. Alternatively, DSBs may be repaired by *RAD51*- and *XRCC3*-independent pathways during mitosis. To investigate this further, it would be helpful to determine whether plant recombination mutants are more sensitive to DSB-inducing agents.

Coordination from Pairing to Chiasmata Resolution

Coordination between pairing, synapsis, and recombination. Although SC formation appears normal in *phs1* mutants, pairing and recombination are uncoupled from synapsis. Knowing that RAD51 disruption in other organisms does not lead to extensive nonhomologous synapsis, another function of PHS1, in addition to loading RAD51, may be to coordinate pairing, synapsis, and recombination (for review, see 139). This coordination may be temporal: As synapsis is delayed in *phs1*, nonhomologous synapsis in *phs1* might result from delayed homologous pairing that requires a longer synapsis to stabilize chromosome associations. Knockouts of *hop2* in yeast show approximately 60% nonhomologous synapsis (102). This phenotype resembles that of *phs1*. Interestingly, HOP2 interacts with the RAD51/DMC1 complex (139). ARABIDOPSIS HOMOLOGUE PAIRING (AHP) 2, a Hop2 homolog, has been identified in plants. The *ahp2* mutant displays wild-type vegetative development, but is male- and female-sterile. However, contrary to yeast *hop2* and maize *phs1*, the *ahp2* mutant lacks a SC (164).

In addition to its role during recombination, RAD51, which acts in the DNA single-strand invasion process, could facilitate homologous recognition between DNA fragments. In maize, we have analyzed in meiotic nuclei the three-dimensional distribution of RAD51. Distinct RAD51 foci begin to appear at the end of leptotene, and reach a maximum number of approximately 500/nucleus in midzygotene (58). As pairing proceeds, the number of foci decreases until, in midpachytene, only a few (ten to twenty) foci persist. During zygotene, RAD51 foci are preferentially seen on unpaired chromosomes, whereas when chromosomes pair and synapse, foci on adjacent homologs appear to fuse together; by pachytene, only single foci are found (58). We also investigated RAD51 in maize meiotic mutants. Overall, we observed either the same or a reduced number of RAD51 foci. We found no or very few double RAD51 foci in several desynaptic mutants including *phs1*, *as1*, *dsy9901*, and *mtm99-25* (140). Thus, the behavior of RAD51 is consistent with the idea that it plays a role in homology identification as well as recombination (58, 140). In this respect, the number of RAD51 foci in the wild-type, and even in most of the meiotic mutants, is theoretically much higher than the number needed for crossover and formation of chiasmata. Conversely, in pachytene, the RAD51 foci disappeared more slowly in the meiotic mutants than in wild type; this observation suggests that successful completion of homologous pairing is required for removal of RAD51 from chromosomes (140).

The phenotypes of *atrad51c* and *atxrcc3* mutants (24, 104) suggest that these two *RAD51* paralogs may also be involved in the homology identification that is required for pairing. Furthermore, the *rock-n-roller* (*rck*, also named *atmer3*) mutants also exhibit a reduction in SC formation consistent with the reduced level of chiamata formation (37, 121; also, see below). Therefore, it is likely that, in plant meiosis, pairing, synapsis, and recombination are interdependent and co-regulated. As we mention above, the meiosis-specific cyclin SDS is required for homolog synapsis and recombination (10); it is possible that SDS plays a role in regulating these processes in a coordinated fashion.

Recombination and crossover formation. Initial experiments in yeast to define the meiotic crossover and noncrossover pathways suggested that these pathways branch during recombination no later than at the single-strand invasion step (20). Findings by Borner et al. (25) suggest that the pathway generating crossovers is more complex than the noncrossover pathway and includes a checkpoint in late leptotene.

If a crossover occurs, the recombination event matures into a chiasma, which is visible at diplotene. During zygotene and pachytene, two types of recombination nodules have

been observed by transmission electron microscopy; these are termed early and late nodules, both of which are known to contain recombination enzymes. Early nodules are more numerous, contain Rad51 and Dmc1, and are thought to be the sites of initial single-strand invasion (2). Early nodules are lost by mid-pachytene; the nodules that remain, called late nodules, are thought to be sites of crossovers and are assumed to mature into chiasmata. MLH1, a mismatch repair protein, is a marker of the late nodules (3, 13, 86, 124).

There is almost always one crossover per chromosome arm per meiosis. This crossover guarantees proper alignment of bivalents on the equatorial plate of the first meiotic division and subsequent proper disjunction of homologous chromosomes. Furthermore, the presence of one crossover discourages additional crossovers in its vicinity, a phenomenon known as crossover interference. Several theorical models in yeast based on analyzing the distribution of crossovers in yeasts and animals suggest that crossover interference is tightly coordinated with synapsis (20, 139). Recent analysis in yeast has revealed two genetically separate pathways for crossover formation (51). One of these is dependent on *MER3*, *MSH4*, and *MSH5* genes and is sensitive to interference. The other is independent of these three genes and not sensitive to interference (**Figure 4**) (51). *MSH4* and *MSH5* are two of six homologs of the bacterial *mutS* gene (required for mismatch repair), and they encode subunits of a heterodimer that specializes in meiotic crossover formation (155). MER3 is a DNA helicase and has been shown to unwind double-stranded DNA. In yeast, mutations in *MER3*, *MSH4*, and *MSH5* cause a reduction in crossover formation to approximately 10–15% of the normal levels; the remaining crossovers do not exhibit interference. In mammals, *MSH4* and *MSH5* are required for normal meiosis as shown by mouse knockout mutants (52, 94), although the roles of these genes in the interference-sensitive pathway of crossover formation is not clear.

In maize, interference was detected genetically 50 years ago (150) and supported by cytological studies (178). Genetic analysis in *Arabidopsis* supports that idea that *Arabidopsis* has both interference-sensitive and insensitive pathways for crossover formation (42). In addition, there is evidence that two such pathways operate in humans (85). More recently, reverse genetic analysis has been conducted on the *Arabidopsis* homologs of *MSH4* and *MER3*. The *AtMSH4* gene is required for normal levels of crossover, as mutants or RNAi lines show a reduction of chiasmata (79). In addition, the remaining chiasmata are randomly distributed; this result suggests that the crossovers that lead to these chiasmata are not sensitive to interference. Similarly, the *Arabidopsis MER3* homolog *AtMER3/RCK* is also required for the interference-sensitive pathway of crossover formation (37, 121). This result is the first report of a role for a *MER3* homolog in crossover formation in a multicellular organism. Furthermore, transmission electron microscopy analysis of *rck* alleles indicate that the SC can form at a reduced level in these mutants; this observation suggests that crossover formation is important for SC establishment and/or maintenance (37).

Chiasmata resolution and sister chromatid cohesion. SCC is established during premeiotic S-phase and participates in the control of both chromosome structure and recombination. Release of SCC is also essential to resolve chiasmata. This function is crucial since it subsequently allows the proper segregation of chromosomes to opposite poles of the cell at anaphase I (reviewed in 135, 189). REC8 immunolocalizations simply illustrate this function. For instance, in *C. elegans*, REC8 is partially lost along chiasmata-distal portions of the arms at anaphase I (138). In *Arabidopsis* and maize, the immunolocalization of the REC8 homologs SYN1 and AFD1, respectively, indicates that these proteins are released at the end of prophase I to allow chiasmata resolution (30; I.N Golubovskaya, O. Hamant & W.Z. Cande, unpublished data).

The release of chromosome cohesion is triggered in most organisms by Separase, a cysteine protease that specifically cleaves RAD21/REC8 proteins; this cleavage subsequently leads to the opening of the ring-shape cohesin complex (39, 77, 187). Separase has not been studied in plants. However, some components of the proteolysis cascade have been shown to be involved in the release of SCC in *Arabidopsis*. The *Arabidopsis SKP1-like1* (*ASK1*) gene encodes a homolog of the human and yeast Skp1 proteins (198) and is involved in plant growth and development. In particular, ASK1 has been shown to interact with Unusual Floral Organ (UFO) to regulate flower organ identity (157, 202). The *ask1-1* mutant is also male-sterile, and chromosomes in this mutant fail to segregate at anaphase I; this failure leads to the production of polyads with spores of variable size and chromosome content (198). Interestingly, SYN1 immunostaining is maintained on chromosome arms during diplotene and diakinesis; this observation strongly suggests that ASK1 is required for the removal of SYN1 and SCC during late prophase I (D. Zhao, X. Yang, L. Quan, L. Timofejeva, C. Makaroff & H. Ma, unpublished data). Partners of ASK1 that form the SKP1-Cullin-F-box (SCF) complex, as well as downstream effectors of ASK1 need to be identified to further elucidate the cascade that leads to cohesion release. Data on ASK1 recall results obtained in other organisms: In yeast and *C. elegans*, the anaphase promoting complex (APC) activates Separase and is required for the release of the cohesin complex at the onset of anaphase I (28, 173). However, in addition to the APC-dependent proteolytic pathway that functions at anaphase, a second pathway, which takes place during prophase, is APC- and separase-independent in vertebrates (77, 181). The *ask1* phenotype might also rely on an alternative early pathway, in particular as SYN1 immunostaining in *ask1-1* is abnormal as early as leptotene (D. Zhao, X. Yang, L. Quan, L. Timofejeva, C. Makaroff & H. Ma, unpublished data).

Other SKP1 homologs may also play a role in meiosis in plants. For instance, the *ask1* chromosome separation defects can be partially rescued by a transgene of the closely related homolog, *ASK2* (201). There are at least 21 SKP1 homologs in *Arabidopsis* (96, 113). In *C. elegans*, a Skp1 homolog has been shown to be involved in pachytene progression; this observation demonstrates that there are SKP1 meiotic functions in animals (131).

OUTLOOK

Very few genes specifically involved in meiosis after prophase I have been identified, as most plant meiotic mutants exhibit defects during prophase I. One exception is maize shugoshin (ZmSGO1), which is specifically required for maintaining centromeric cohesion at metaphase I but has no apparent function during early prophase I (72). The genetic control of meiosis progression is still largely unknown and will need more research efforts in the coming years. Furthermore, after a very successful decade based on a gene-by-gene approach, the use of more global tools in the future, such as microarrays, proteomics, and modeling of genetic and biochemical pathways, should elucidate new genetic interactions and pathways that control the genetics of meiosis in plants.

SUMMARY POINTS

1. Meiosis is mitotic-like in the maize *ameiotic1* mutant and, to a lesser extent, in the *Arabidopsis switch1* mutant; this observation suggests that AM1 and SWI1 are two related proteins required for meiotic commitment.
2. Several meiotic cyclins such as SDS and TAM have been identified in *Arabidopsis*, but the control of meiosis progression remains largely unknown in plants.

3. Changes in chromosome morphology define the different meiotic prophase stages. Chromosome morphology is dependent on cohesion proteins (such as SYN1, AtSCC3 in *Arabidopsis*, and AFD1 in maize), histone modifications, and synaptonemal complex installation.
4. Telomere clustering (the bouquet) precedes homologous chromosome pairing in zygotene and has been proposed to facilitate homologous chromosome alignment by providing the initial paired foci.
5. Defects in synapsis are the most common phenotypes among meiotic mutants; this observation suggests that installation of the synaptonemal complex impacts or is impacted by several pathways, including homologous pairing, recombination, and sister chromatid cohesion establishment.
6. Mutants in genes homologous to *HOP1* have been identified in Arabidopsis (*asy1*) and rice (*pair2*); the relatively similar phenotypes of these mutants suggest that the function of HOP1 in synaptonemal complex formation is conserved.
7. The central element of the synaptonemal complex contains two redundant proteins: AtZIP1a and AtZIP1b. The analysis of the *atzip1* mutant points towards a role of the SC in verifying the correct progression of recombination.
8. The recombination pathway is highly conserved across kingdoms. Recombination is initiated by SPO11-induced double-strand breaks. Subsequently, the MRX complex generates a single stranded DNA that invades a neighbouring double-stranded DNA molecule, a RAD51/DMC1 controlled step. Strand invasion produces a D-loop. DNA synthesis followed by ligation results in a double-Holliday junction which is subsequently resolved by XRCC3. Homologs of all of those proteins have been characterized in plants.
9. Crossover versus noncrossover fate depends on the way the double-Holliday junction is resolved. Crossover formation is dependent on MSH4 and MER3/RCK and is cytologically visualized by the presence of MLH1 on pachytene chromosomes and chiasmata at diakinesis. Release of chiasmata requires the dissolution of cohesion, a process that is dependent on ASK1 in Arabidopsis.
10. Several proteins have been shown to be involved in the recruitment of the recombination machinery; these were identified by two-hybrid approaches (e.g., BRCA2) as well as by monitoring the distribution of RAD51 in mutants (e.g., PHS1, AM1, and AFD1).

FUTURE ISSUES TO BE RESOLVED

1. The genetics of meiosis in plants is an immature field that mainly relies on mutant screens for sterility or reverse genetics for identifying meiotic genes. In the future, one challenge will be to elucidate the genetic interactions that exist between these effectors. Identification of suppressors of meiotic mutant phenotypes is a powerful approach, as new genes as well as new interactions will be unraveled.

2. To date, few meiotic proteins have been characterized at the biochemical level. Given the abundance of male meiocytes in many plant model organisms, plants will make a major contribution, as most of the proteins we discuss in this review have unclear biochemical function and unknown partners.
3. Although the cell cycle in plants is relatively well characterized, control of meiosis progression is largely unknown (e.g., the presence of checkpoints is still under debate). A combination of microarrays and proteomics approaches on staged meiotic cells in wild-type and mutants will lead to the identification of proteins that drive meiosis forward, as well as proteins involved in meiosis checkpoints.
4. The involvement of small RNAs in meiosis has not been demonstrated in plants; given the role of nongenic micronuclear transcripts in genome rearrangements and chromosome dynamics, it is likely that small RNAs may have key meiotic functions.
5. The hierarchy of events needed for homologous chromosome pairing has not been elucidated. Future genetic analysis is needed to understand its molecular control.

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