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The cytogenetics of homologous chromosome pairing in meiosis in plants

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Abstract. Three activities hallmark meiotic cell division: homologous chromosome pairing, synapsis, and recombination. Recombination and synapsis are well-studied but homologous pairing still holds many black boxes. In the past several years, many studies in plants have yielded insights into the mechanisms of chromosome pairing interactions. Research in several plant species showed the importance of telomere clustering on the nuclear envelope (telomere bouquet formation) in facilitating alignment of homologous chromosomes. Homologous pairing was also shown to be tied to the early stages of recombination by mutant analyses in *Arabidopsis* and maize. In contrast, little is known about the mechanisms that guide homolog interaction after their rough alignment by the bouquet and before the close-range recombination-dependent homology search. The relatively large and complex genomes of plants may require additional mechanisms, not needed in small genome eukaryotes, to distinguish between local homology of duplicated genes or transposable elements and global chromosomal homology. Plants provide an excellent large genome model for the study of homologous pairing and dissection of this process.

Homologous chromosome pairing is one of the least understood meiotic processes. It encompasses interactions between chromosomes that lead to juxtaposition of homologs. Chromosome pairing takes place during the early stages of meiotic prophase I and coincides with two other major meiotic processes: recombination and synapsis. Recombination starts by formation of double-strand breaks (DSBs) in chromosomal DNA, which are later repaired, leading to crossovers between a single sister chromatid of each homologous chromosome. Synapsis is the installation of a protein that bridges the gap between homologs to hold them together. Pairing, synapsis, and recombination not only occur concurrently but there is also a great deal of coordination between the three processes (Pawlowski and Cande, 2005).

Plants have been at the forefront of homologous pairing research for several decades. With their large and conspicuous chromosomes, plants make excellent models to study chromosome pairing. Good genetic tools, particularly in *Arabidopsis* and maize, facilitate identifying genes regulating pairing and elucidation of their functions. Additionally, plants, unlike many other taxa, do not have meiotic checkpoints that arrest the progression of meiosis upon signs of recombination or synapsis abnormalities. This enhances functional dissection of meiotic genes by allowing examination of downstream defects in their mutations.

In plants, and most other taxa, meiotic chromosome pairing is DNA homology based. To understand the complexity of homologous pairing, one needs to consider the obstacles that homologs must overcome to pair. (i) Homologous loci may be spatially separated by relatively large distances in the nucleus at the start of meiosis. (ii) Compact heterochromatin may create difficulties in accessing an orthologous locus for homology recognition. (iii) Repetitive DNA sequences, such as transposable elements and large gene families, may obscure proper homology and lead to ectopic pairing interactions. Consequently, homologous chromosome pairing must include several distinct stages. First, homologous chromosomes must be co-aligned and brought into close proximity. Then, existence of homology between the partners must be established via DNA homology search. Finally, ectopic pairing interactions involving members of gene families, transposable elements, and other repetitive DNA sequences must be eliminated so that true homology can be established along the entire chromosome.

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Pre-meiotic chromosome pairing

In plants, homologous pairing of entire chromosomes is an activity uniquely tied to meiosis. However, evidence exists that specific chromosome regions can, in some cases, form homology-based pairing associations in pre-meiotic nuclei. Studies of *Arabidopsis* interphase nuclei show that high copy number repeats tend to cluster together, even though the arrangement of chromosome arms is largely random (Schubert et al., 2007). There is also evidence that heterochromatic regions of homologous chromosomes associate closely before meiosis in maize (Maguire, 1967). In polyploid wheat containing the *Ph1* gene, somatic association of centromeres has been reported (Martinez-Perez et al., 2001). Thus, there may be some activities prior to meiosis that favor homologous pairing. However, the intimate association of meiotic chromosomes along their entire length in plants, as well as in most other taxa, is always formed de novo during early meiotic prophase I.

The telomere bouquet and co-alignment of chromosomes

Homologous chromosome pairing in early meiotic prophase is accompanied by dynamic repositioning of chromosomes in the nucleus and formation of a cytological structure called the telomere bouquet. The bouquet consists of telomeres from all chromosomes clustered on the nuclear envelope. In most species, including maize, wheat, and rye, the bouquet forms in late leptotene and persists until early pachytene (Golubovskaya et al., 2002; Harper et al., 2004). Bouquet formation has been observed in most species of plants, as well as animals and fungi, with a notable exception being *Arabidopsis* (but see below). The timing of telomere bouquet formation just before the onset of chromosome pairing suggests that the bouquet may play a role in pairing. Indeed mutants defective in bouquet formation show slowed down and inefficient chromosome pairing (Niwa et al., 2000; Golubovskaya et al., 2002; Harper et al., 2004).

The formation of the telomere bouquet proceeds in two distinct stages. First, the telomeres attach to the nuclear envelope in late leptotene. Then, they slide to one location, close to the nucleolus, which in late leptotene migrates from the center of the nucleus to its periphery (Golubovskaya et al., 2002; Harper et al., 2004). Clustering of the telomeres is thought to be an active and sudden process (Bass et al., 1997).

As much as a link between bouquet formation and chromosome pairing may be intuitive, the molecular nature of this link is far from certain. It has been proposed that the bouquet affects pairing by (i) generating a telomere-centromere polarization, which leads to a rough co-alignment of homologs, and (ii) confining chromosomes to a limited nuclear space, which shortens the distances between chromosomes across which the homology search must operate (Scherthan, 2001). This model would imply that chromosome pairing starts at telomeres, which seems indeed to be the case, at least in plants (Bass et al., 2000). An alternative model for the bouquet function has recently been proposed in budding yeast and suggests that the bouquet affects early stages of meiotic recombination, which are known to be directly linked to the progression of homologous chromosome pairing (Wu and Burgess, 2006).

Little is known about factors that affect bouquet formation. The physical end of the chromosome is, interestingly, not required for attachment to the nuclear envelope because ring chromosomes in maize participate in bouquet formation (Carlton et al., 2003). However, several studies in yeast suggest that the protein complex normally present at the telomeres is required for telomere attachment to the nuclear envelope (Harper et al., 2004). Species-specific factors also affect the bouquet; Bass et al. (2000) showed that maize chromosomes in oat-maize addition lines (42 oat chromosomes plus two maize chromosomes) exhibit the bouquet dynamics typical for oat rather than maize. Work in yeast and mouse showed that progression through early stages of recombination also affects telomere clustering (Pandita et al., 1999; Liebe et al., 2006).

At the mechanistic level, the bouquet formation is best understood in yeast *Schizosaccharomyces pombe*. A number of genes encoding structural bouquet proteins have been identified in this species, and mutants in these genes allowed study of specific functions of these proteins (Harper et al., 2004; Chikashige et al., 2006). However, very few of these genes have sequence homologs in other groups of eukaryotes, including plants, suggesting that even though the overall bouquet structure is conserved, specific aspects of telomere clustering evolve more quickly. In plants, several mutants showing bouquet defects are known, including *pam1*, *dy1*, *dsy1*, *afd1*, and *phs1* in maize, and *sy1* in rye (Golubovskaya et al., 2002, 2006; Bass et al., 2003; Pawlowski et al., 2004; Sosnikhina et al., 2005). However, since the causes underlying nearly all these mutations are unknown, it is not clear which of these mutants represent specific bouquet defects and which are primarily defective in other meiotic processes, such as recombination, but also affect telomere clustering. A *bona fide* telomere clustering mutation and the best studied of these is the *pam1* mutation in maize (Golubovskaya et al., 2002). In the *pam1* mutant, telomeres attach to the nuclear envelope but fail to cluster. This leads to defects in many downstream meiotic processes, such as chromosome pairing and synapsis. On the other hand, the initiation and early progression of meiotic recombination are normal. Eventually, the defects in the *pam1* mutant make meiosis progression slow and inefficient. However, some cells do complete meiosis, suggesting that telomere clustering is not absolutely required for successful completion of meiosis.

Although the vast majority of eukaryotes show the presence of a telomere bouquet, *Arabidopsis* is one of the few exceptions. However, it has been observed that, instead, *Arabidopsis* telomeres cluster around the nucleolus in the pre-meiotic interphase, which may serve a similar function to that of the bouquet in other species (Armstrong et al., 2001).

Meiotic recombination and chromosome pairing

Meiotic recombination serves two purposes, first – to create genetic diversity and second – to provide mechanical stability for the paired chromosomes after the SC disintegrates and until chromosomes segregate in anaphase I. Extensive data suggest that a subset of meiotic recombination activities is also essential to promote pairing of homologous chromosomes in plants, as well as fungi and mammals, but, interestingly, not in *Caenorhabditis elegans* or *Drosophila* (Dernburg et al., 1998; McKim et al., 1998).

Meiotic recombination

The earliest recombination step is formation of double-strand breaks (DSBs) in the chromosomal DNA. The DSBs are resected from 5′ to 3′ to leave 3′ single-stranded overhangs. ssDNA is then bound by proteins that promote homologous recombination. The meiotic recombination pathway eventually leads to formation of crossover and non-crossover (gene conversion) events.

Meiotic DSBs in plants, as in all species examined thus far, are created by SPO11, a member of the type II topoisomerase protein family (Keeney et al., 1997). Unique from other taxa, plants possess multiple copies of SPO11: *Arabidopsis* and maize have three, while the rice genome contains four (Grelon et al., 2001; Pawlowski et al., unpublished). From the three *Arabidopsis* homologs, only SPO11-1 and SPO11-2 have a meiotic function (Grelon et al., 2001; Stacey et al., 2006). The phenotypes of *Arabidopsis spo11-1* and *spo11-2* mutants appear to overlap considerably. Neither are able to create crossovers to physically connect chromosomes, nor are they able to synapse chromosomes, which causes metaphase I to have entirely univalent chromosomes.

Once the DSBs are created, they are acted upon by the MRN protein complex, consisting of MRE11, RAD50, and NBS1 (Bundock and Hooykaas, 2002; Bleuyard et al., 2004; Puizina et al., 2004; Waterworth et al., 2007). *Arabidopsis* meiocytes deficient in MRE11 and RAD50 show chromosome breakage and meiotic sterility as a result of being unable to repair SPO11-induced DSBs (Bundock and Hooykaas, 2002; Bleuyard et al., 2004; Puizina et al., 2004).

Following the resection of the DSBs, a 3′ ssDNA overhang is generated. This overhang is bound by two recombination proteins, RAD51 and DMC1, which promote homologous recombination through single end invasion (SEI) of homologous double-stranded DNA. RAD51 exhibits both vegetative DNA repair and meiotic function while DMC1 is meiosis specific (Klimyuk and Jones, 1997; Doutriaux et al., 1998). *rad51* mutants in *Arabidopsis* show univalent chromosomes instead of bivalents at metaphase I caused by chromosome pairing defects and absence of chiasmata (Li et al., 2004). In addition, they exhibit chromosome breakage as a result of DSBs being unrepaired. In maize, plants deficient in RAD51 activity also exhibit chromosome breakage, as well as non-homologous synapsis, and, most strikingly, chiasmata between non-homologous chromosomes (Li et al., 2007). In contrast to the *rad51* mutant, DMC1-defective plants in *Arabidopsis* do not show chromosome fragmentation (Siaud et al., 2004). These observations indicate that DMC1 is chiefly responsible for DSB repair using the homologous chromosome as a template. RAD51 predominantly repairs meiotic DSBs using a sister chromatid as template, instead of the homologous chromosome.

Studies on yeast and mouse, show that following SEI, the meiotic recombination pathway splits into two parallel branches: one leading to crossovers (COs) and one to non-crossovers (NCOs) (Allers and Lichten, 2001; Hunter and Kleckner, 2001; Guillon et al., 2005). Crossovers are reciprocal recombination events that lead to the exchanges of chromosome arms. Non-crossovers (gene conversions) are generated through a non-reciprocal repair of DSBs, without a double Holliday junction intermediate. The presence of separate CO and NCO pathways may be universal in all meiotic species, including plants, although no direct data from plants exist so far.

Steps of meiotic recombination that affect chromosome pairing

A number of studies in a variety of species, including maize and *Arabidopsis*, indicate that homologous chromosome pairing is tightly linked to the progression of meiotic recombination. A strong connection between pairing and recombination exists also in mammals and fungi (Pawlowski and Cande, 2005). In contrast, chromosome pairing does not depend on recombination in *C. elegans* and *Drosophila* (Dernburg et al., 1998; McKim et al., 1998).

Numerous plant mutants in early recombination genes show defects in chromosome pairing in addition to their recombination defects, indicating that pairing requires initiation of meiotic recombination and progression through early steps of the recombination pathway (Pawlowski and Cande, 2005; Hamant et al., 2006). In contrast, mutant studies show that late recombination steps, such as crossover formation, are not required for homologous pairing (Higgins et al., 2004; Jackson et al., 2006). Even though the pairing-recombination link has been well established, the nature of this interaction has not yet been resolved. It is possible that chromosome pairing utilizes the recombination pathway DNA intermediates. Alternatively, some recombination proteins may have dual functions, affecting both pairing and recombination. Homologous pairing in plants, as in mammals and fungi, requires recombination initiation. *Arabidopsis* mutants in the *SPO11-1* and *SPO11-2* genes, which fail to create DSBs, do not pair or synapse (Grelon et al., 2001; Stacey et al., 2006). This is also true for the DSB resection step: *atmre11* mutants are unable to pair homologs in about 90% of meioses (Puizina et al., 2004).

The strongest evidence linking recombination and pairing, however, is derived from the SEI step of meiotic recombination, which is facilitated by a protein complex that includes RAD51 and DMC1. In most meiotic species, these proteins form numerous foci on meiotic chromosomes in early meiotic prophase I (Fig. 1) (Terasawa et al., 1995; Franklin et al., 1999; Pawlowski et al., 2003; De Muyt et al., 2007). Franklin et al. (1999) suggested, based on the observations in maize, that the number of RAD51 foci vastly ex-

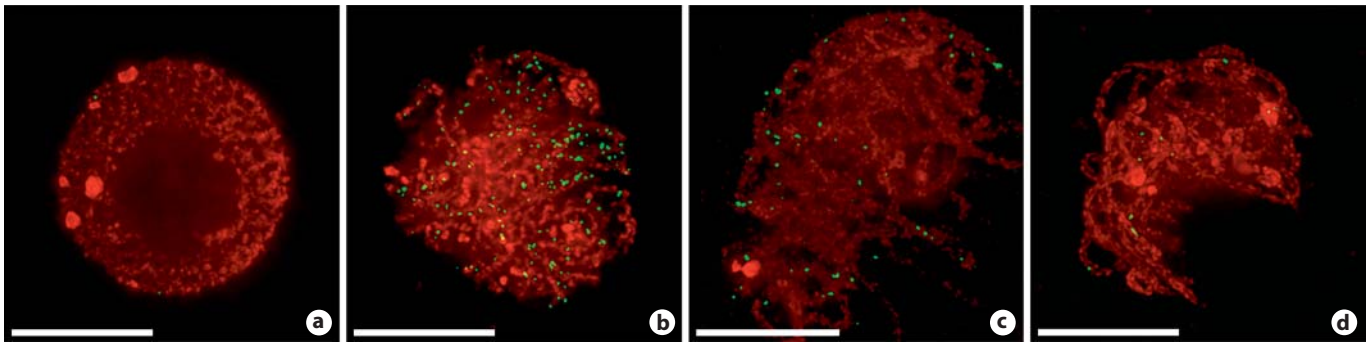

Fig. 1. Distribution of RAD51 foci in wild type maize meiocytes. Dynamic changes of RAD51 localization in meiotic prophase I, coincide with homologous chromosome pairing, which supports the proposed role of RAD51 in homology recognition. (**a**) Leptotene. (**b**) Mid-zygotene. (**c**) Late zygotene. (**d**) Pachytene. Red: chromatin; green: RAD51. Images are flat projections from several consecutive optical sections through 3-dimensional nuclei. Bar = 10 μm. Modified from Pawlowski et al. (2003).

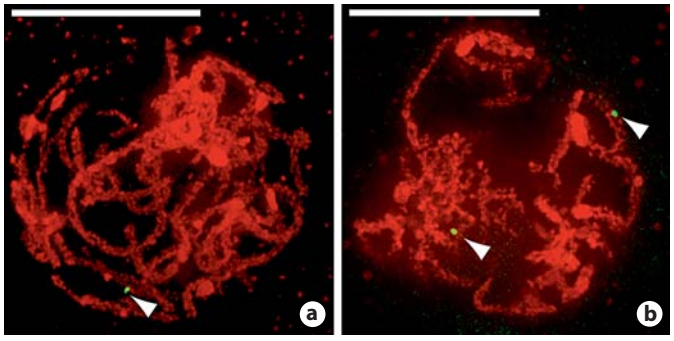

Fig. 2. Homologous pairing in a wild-type maize meiocyte in pachytene (**a**) and a pairing-like association of non-homologous chromosome association in a maize meiotic mutant *segII* (**b**) at the same stage of meiosis. Red: chromatin; green and marked with white arrows: 5S ribosomal RNA loci on maize chromosome 2. Images are flat projections from several consecutive optical sections through 3-dimensional nuclei. Bar = 10 μm.

ceeds what is required for formation of COs and proposed that the extra foci are utilized for the chromosome homology search. In vitro studies show that RAD51 and DMC1 coat the ssDNA overhangs, forming nucleoprotein filaments. In order for this short range mechanism of DMC1/RAD51 mediated homology search to be effective, the homologs must be in a close alignment. Evidence from yeast indicates that a resected ssDNA may extend to approximately 2 kb (Lee et al., 1998). Each nucleotide contributes about 0.6 nm to the length of a DNA strand (Murphy et al., 2004). Assuming that binding of RAD51 and DMC1 discourages DNA secondary structure, a linear nucleoprotein filament of 2 kb will extend to about 1200 nm. This is sufficient to bridge the roughly 400 nm distance between chromosomes brought together by an initial 'rough' alignment prior to pairing (Tesse et al., 2003). In the small genome budding yeast, the length of the RAD51/DNA filament would even be adequate to span the ~1-μm-wide telomere cluster (Trelles-Sticken et al., 1999). However, in the large genome maize, the bouquet cluster is much bigger, approximately 7 μm in diameter (Carlton et al., 2003), suggesting that an additional mechanism may be required to bring the homologs into a 'rough' alignment.

Coordination of pairing and recombination

Mutational analyses have not, thus far, uncovered any plant genes participating in the chromosome homology search that act completely independently of recombination. However, a small group of genes has been identified that encode proteins which are not primarily involved in DSB repair but, instead, coordinate pairing and recombination. Mutants in these genes show an interesting phenotype in which homologous chromosome pairing is replaced by associations between non-homologous partners (Fig. 2). In plants, this gene group contains *Phs1* described in maize (Pawlowski et al., 2004), and *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 several other species, including plants (Schommer et al., 2003; Domenichini et al., 2006; Kerzendorfer et al., 2006; Panoli et al., 2006). Studies in *Arabidopsis* as well as in yeast and mouse showed that HOP2 and MND1 form a heterodimer that is able to interact with DMC1 to stimulate its homology search activity (Petukhova et al., 2005; Pezza et al., 2006; Vignard et al., 2007). In *Arabidopsis*, HOP2/MND1 localize to chromatin from leptotene through pachytene (Vignard et al., 2007). This localization is not dependent on the SPO11-generated DSBs and does not significantly overlap with the DMC1 foci. Mutants lacking HOP2 or MND1 in *Arabidopsis*, yeast, and mouse are unable to proceed beyond DMC1/RAD51 loading and exhibit univalents at metaphase I (Leu et al., 1998; Tsubouchi and Roeder, 2002; Petukhova et al., 2003; Domenichini et al., 2006; Kerzendorfer et al., 2006). In the *hop2* mutant in yeast approximately 60% of synapsis takes place between non-homologous chromosomes (Leu et al., 1998). The molecular mechanism of action of the HOP2/MND1 complex is not yet

clear. While several authors proposed that its primary role is to directly facilitate the SEI invasion process (Tsubouchi and Roeder, 2002; Chen et al., 2004), Zierhut et al. (2004) suggested that it may have a more general function in affecting chromatin and/or higher order chromosome structures of the homologous target.

The maize *phs1* mutant also exhibits a severe pairing phenotype with 95% of chromosome pairing-like associations forming between non-homologous partners (Pawlowski et al., 2004). However, in this mutant, RAD51 fails to load on the sites of meiotic DSBs, suggesting that PHS1 acts earlier in meiosis than HOP2/MND1.

Synapsis and homologous pairing

Synapsis is the process of installing the central element (CE) between two paired chromosomes. Although synapsis normally takes place between homologs, aberrant meiosis can produce associations between non-homologous chromosomes, which become synapsed together. Such abnormal synapsis has been observed in haploids (De Jong et al., 1991) and in meiotic mutants defective on homology recognition (Leu et al., 1998; Pawlowski et al., 2004). This would suggest that synapsis is more of a 'default' process that does not take into account homology and will take place between non-homologous chromosomes if homologs are not available. However, unique from other model species, *Arabidopsis* mutants lacking the CE demonstrate the ability to associate non-homologous chromosomes as bivalents at metaphase I (Higgins et al., 2005), suggesting that synapsis may be important for homology recognition after all.

Chromosome pairing in polyploids

The complexity of chromosome pairing increases dramatically in polyploid species harboring genomes with similar (homeologous) chromosomes. To deal with this issue, polyploid species evolved genetic systems controlling recognition of homologous vs. homeologous chromosomes. The best known such system exists in hexaploid bread wheat, *Triticum aestivum*, which possesses three highly similar genomes, A, B, and D. Proper homology recognition, which ensures that, e.g., chromosomes from the A genome pair with their A genome homologs and not with homeologs from the B or D genome, is controlled by the *Pairing homeologous* (*Ph1*) locus (Riley and Chapman, 1958). The *Ph1* locus is likely to exert its influence by regulating the chromatin structure (Prieto et al., 2004) but the exact mechanism of its action remains unknown. It has been proposed that the action of *Ph1* results in disruption of pre-meiotic associations of centromeres of chromosomes from different genomes (Martinez-Perez et al., 2001). However, a counterargument stating that the *Ph1* locus acts during meiosis has also been made (Corredor et al., 2007).

Cloning of the *Ph1* locus proved difficult due to limited allelic diversity. The only *Ph1* mutations are deletions and generating new alleles through EMS mutagenesis has not been possible (Wall et al., 1971). Recent work has narrowed *Ph1* to a 2.5 Mb region containing a structure consisting of a segment of subtelomeric heterochromatin that inserted into a cluster of *cdc2*-related genes after polyploidization (Griffiths et al., 2006). This discovery suggests that *Ph1* (i) acts by epigenetically regulating the cell cycle machinery and (ii) is specific to polyploid wheat.

Outlook

Even though elucidating the chromosome behavior and interactions that lead to homologous pairing has been a research goal for many decades, there are still more questions than answers. The area of most progress in understanding pairing in plants is the link between the homology search step and the progression of meiotic recombination. This research is facilitated by the fact that recombination proteins are some of the most evolutionarily conserved meiotic players and it has been possible to utilize knowledge from small genome meiotic model systems, such as yeast, to understand the processes in plants. However, SEI mediated by the RAD51/DMC1 protein complex is unlikely to fully explain the process of pairing of homologous chromosomes, especially in complex genome species such as most plants. Such genomes most certainly require mechanisms that would prevent ectopic pairing interactions between repetitive DNA sequences. Consequently, it is likely that some mechanisms regulating pairing in plants are different from those in small genome eukaryotes. This underscores the need for original gene discovery to identify plant-specific pairing regulators. An example of the validity of this approach is the identification and cloning of *Phs1* in maize, which does not have obvious sequence homologs in species outside of the plant kingdom (Pawlowski et al., 2004). Another process, where finding plant-specific genes may be expected is telomere bouquet formation, since it appears that very few of the known yeast bouquet genes have sequence homologs in plants.

In addition to the plant-specific, or complex genome-specific aspects of pairing, there are remaining questions concerning the chromosome pairing mechanisms that are shared by all (or most) species. The first is the mechanism of alignment of homologous chromosomes within the effective range for SEI, part of which is undoubtedly the function of the telomere bouquet. The second is the coordination of SEI with other prophase I processes. In elucidating both these processes, plants can certainly play a major role.

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