

Chromosome Dynamics in Meiotic Prophase I in Plants

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Abstract

Early stages of meiotic prophase are characterized by complex and dramatic chromosome dynamics. Chromosome behavior during this period is associated with several critical meiotic processes that take place at the molecular level, such as recombination and homologous chromosome recognition and pairing. Studies to characterize specific patterns of chromosome dynamics and to identify their exact roles in the progression of meiotic prophase are only just beginning in plants. These studies are facilitated by advances in imaging technology in the recent years, including development of ultra-resolution three-dimensional and live microscopy methods. Studies conducted so far indicate that different chromosome regions exhibit different dynamics patterns in early prophase. In many species telomeres cluster at the nuclear envelope at the beginning of zygotene forming the telomere bouquet. The bouquet has been traditionally thought to facilitate chromosome pairing by bringing chromosome ends into close proximity, but recent studies suggest that its main role may rather be facilitating rapid movements of chromosomes during zygotene. In some species, including wheat and *Arabidopsis*, there is evidence that centromeres form pairs (couple) before the onset of pairing of

chromosome arms. While significant advances have been achieved in elucidating the patterns of chromosome behavior in meiotic prophase I, factors controlling chromosome dynamics are still largely unknown and require further studies.

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Meiotic prophase I is the longest and most complex stage of meiosis [Hsu et al., 1988; Ronceret et al., 2007]. Early steps of meiotic prophase are a period of dramatic reorganization of the nucleus which include changes in chromosome morphology as well as repositioning of chromosomes in the three-dimensional (3D) nuclear space. Changes in chromosome appearance during prophase I are used to subdivide it into several substages. In leptotene the decondensed chromatin becomes organized into chromosomes. In zygotene homologous chromosomes pair. In most species of plants, animals, and fungi chromosome pairing is preceded by clustering of telomeres at a single site on the nuclear envelope in early zygotene, a process known as the telomere bouquet formation [Harper et al., 2004; Scherthan, 2007]. At the beginning of pachytene the bouquet resolves, and completely paired chromosomes are visible as bivalents.

Upon entry into meiosis the nucleus contains 2 copies of each chromosome. In order to ensure that chromosomes segregate properly in anaphase I, the homologs

must pair, synapse, and recombine with each other [Zickler and Kleckner, 1999; Ronceret et al., 2007]. Homologous pairing is an interaction between chromosomes that leads to juxtaposition of homologs and the formation of bivalents [Zickler and Kleckner, 1999]. Synapsis closely follows pairing and is a process of installation of the proteinaceous synaptonemal complex between the homologous chromosomes in the bivalent [Zickler and Kleckner, 1999]. Recombination starts in leptotene by formation of meiotic double-strand breaks (DSBs) in chromosomal DNA. DSB repair during zygotene and pachytene leads to the formation of crossovers (reciprocal exchanges of chromosome arms) and non-crossovers (gene conversions) [Zickler and Kleckner, 1999]. Pairing, synapsis, and recombination occur concurrently during early prophase I, and there is a great deal of coordination between the 3 processes [Pawlowski and Cande, 2005].

Processes taking place in early meiotic prophase I impose major changes on the appearance and behavior of chromosomes. Thin, thread-like univalent chromosomes present at the beginning of zygotene thicken as a result of progressing chromatin condensation and form bivalents as a result of homologous pairing and synapsis. A number of meiotic processes, particularly bouquet formation and chromosome pairing, also require the ability of chromosomes to move across the nuclear space. However, despite the importance of the chromosome structure changes and chromosome motility, the dynamics of chromosomes in meiotic prophase I are poorly understood, particularly in plants.

Conspicuous chromosomes along with good genetics and cell biology tools that are available in a number of plant species, particularly in maize and *Arabidopsis*, make plants very suitable models for deciphering the patterns and functions of chromosome dynamics in meiotic prophase I. In this review we will present the newest imaging tools used for studying meiotic chromosome dynamics in plants, describe what is known about the patterns of early prophase chromosome dynamics in this group of organisms, and discuss factors that are likely to affect patterns of chromosome dynamics.

Imaging Methods for Studying Chromosome Dynamics in Meiotic Prophase: In Search of a High-Resolution 4D Picture

The complexities of chromosome behavior during the prophase of meiosis present serious challenges for imaging techniques. Fine details of chromosome organization

likely play critical roles in homology recognition and pairing of chromosomes. Consequently, the ability to see these structures is important for understanding chromosome interactions. Furthermore, to understand chromosome behavior, it has to be taken into account that chromosomes operate in a 3D space. Finally, chromosome behavior changes dynamically over time.

Our understanding of chromosome dynamics in meiotic prophase in plants so far comes almost entirely from analyses of fixed meiocytes. Despite its limitations this approach has been very successful in reconstructing the overall progression of meiotic events as well as elucidating many aspects of chromosome behavior. A number of new cytological techniques and improvements of traditional techniques have been introduced in the recent past. Particularly spectacular are the improvements in the quality of cytological images of the small chromosomes of *Arabidopsis thaliana* during the past 15 years [Armstrong et al., 2009].

The simple chromosome *squashing technique* [McClintock, 1929] is commonly used for routine observations of chromosome morphology and behavior, for example for characterization of new meiotic mutants. A more sophisticated *spreading technique* (fig. 1A), which involves removal of the cell wall material using a mixture of cellulase, pectinase, and other enzymes, allows for better separation of chromosomes and is widely used in chromosome research [Wang et al., 2006a; Stronghill and Hasenkampf, 2007; Armstrong et al., 2009]. The benefit of spreads is the ease with which 3D information is transformed into a 2D image. Most of the chromosome features, including centromeres, telomeres, and heterochromatic knobs, can be observed immediately without a need for an additional step of reconstituting the 3D organization from serial sections. The spreading and squashing techniques can be combined with fluorescent in situ hybridization (FISH) to identify specific DNA sequences on chromosomes and immunolocalization to localize specific proteins in the nucleus [Armstrong et al., 2009]. However, a serious limitation of the squashing/spreading techniques is that the preparation destroys the natural organization of the nucleus and can, therefore, introduce structural artifacts. For example, reliable information on the arrangement of chromosomes in the nucleus relative to each other cannot be obtained using spreads or squashes.

In *3D microscopy* a series of optical sections is collected across the entire nucleus and used to reconstruct the 3D object. This approach overcomes the problems associated with squashing and spreading as the 3D structure of

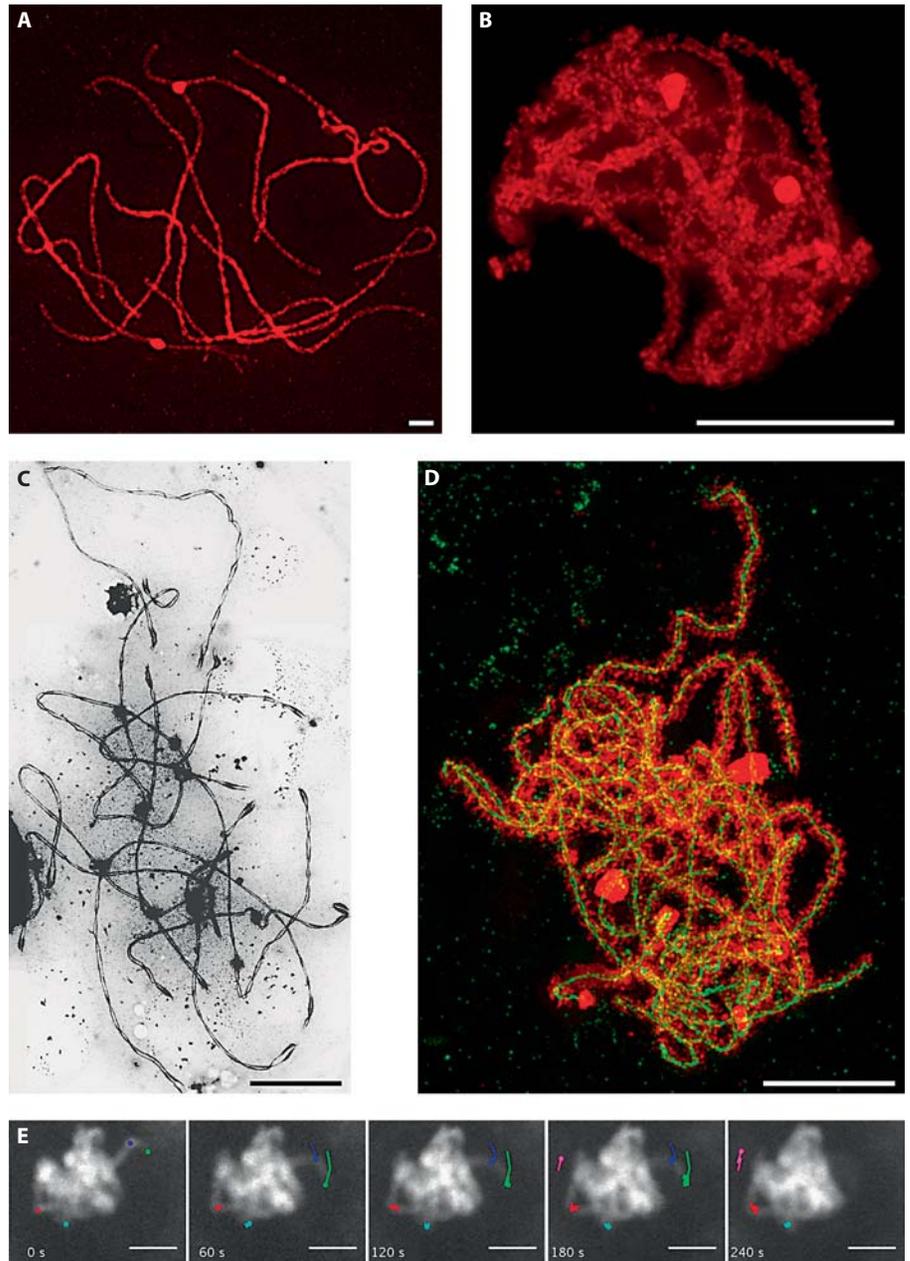


Fig. 1. Imaging of pachytene nuclei in maize using various microscopy methods. **A** A chromosome spread. Bar = 5 μ m. Image courtesy of C.-J.R. Wang. **B** A partial 3D projection of a nucleus imaged with 3D deconvolution microscopy. Bar = 5 μ m. **C** A synaptonemal complex spread of a nucleus stained with silver nitrate and imaged with transmission electron microscopy. Completely aligned lateral elements are visible as black lines. Bar = 5 μ m. Modified from Pawlowski et al. [2004]. **D** A projection of a nucleus imaged by 3D structured illumination microscopy, stained with DAPI to visualize chromatin (red) and immunostained with an antibody against a meiotic cohesin AFD1 [Golubovskaya et al., 2006] to visualize the chromosome axis (green). Bar = 5 μ m. Image courtesy of C.-J. R. Wang. **E** A live nucleus imaged every 60 s for 240 s using multiphoton excitation microscopy. Images are overlaid with trajectories of 5 chromosome marks (blue, cyan, green, magenta, and red). Bar = 5 μ m. Modified from Sheehan and Pawlowski [2009].

the cell is preserved. Consequently, this method is particularly well suited for examining nuclear organization. In addition to 3D reconstruction, out-of-focus light is removed from the microscope image by using either confocal microscopy or deconvolution methods [Dawe et al., 1994; Martinez-Perez et al., 2001; Prieto et al., 2007] which results in an image that is sharper and crisper (fig. 1B). Successful examples of using 3D microscopy include studying changes in chromosome morphology during the leptotene-zygotene transition [Dawe et al.,

1994], telomere clustering in zygotene [Bass et al., 1997; Golubovskaya et al., 2002], and homologous chromosome pairing [Pawlowski et al., 2004; Ronceret et al., 2009]. 3D microscopy is frequently combined with FISH [Bass et al., 1997] and immunolocalization [Franklin et al., 1999; Golubovskaya et al., 2006; Ronceret et al., 2009] to monitor location and behavior of specific chromosome regions and to localize meiotic proteins. A particularly good example of using the capabilities of 3D microscopy to investigate chromosome dynamics is the study of Bass

et al. [1997] who conducted a 3D reconstruction of the progression of telomere clustering in male meiocytes of maize. To understand the dynamics of the process, the authors analyzed the positions of the telomeres relative to each other in the nuclear space before and during the bouquet formation as well as following its resolution. Such an analysis would not be possible using 2D spreads or squashes.

A great deal of information on chromosome organization has been generated using *electron microscopy (EM)* methods which can show elements of chromosome structure specifically involved in meiosis, such as the synaptonemal complex, with superior resolution (fig. 1C) [Anderson et al., 2003; Lopez et al., 2008]. Serial sections can be used to analyze the nucleus in 3 dimensions [Gillies, 1973]. However, 3D reconstruction of fine sections is very difficult and laborious and, therefore, this method is not well suited for analyzing rapid chromosome dynamics. For this reason most studies on meiotic chromosomes in plants using EM have been done with the spreading technique [Gillies, 1981; Anderson et al., 2003; Lopez et al., 2008]. Antibodies coupled with gold particles can be used for protein immunolocalization in EM [Anderson et al., 1997; Lohmiller et al., 2008] although, inevitably, the number of different antibodies that can be used at the same time is very limited.

The recently developed *structured illumination microscopy (SIM)* is a light microscopy method that can overcome the 200 nm diffraction limit of conventional light microscopy and provide a near-EM resolution of at least 100 nm while allowing 3D optical sectioning (fig. 1D) [Gustafsson, 2005; Schermelleh et al., 2008]. All FISH and immunolocalization tools developed for light microscopy can be used with SIM. Using this technique coupled with antibodies marking the chromosome axes, Wang and colleagues [2009] recently examined the progression of synapsis during zygotene and pachytene in maize. Owing to the capabilities of 3D-SIM they were able to resolve several aspects of chromosome behavior that were previously elusive for light microscopy studies because of insufficient resolution and for EM because of the difficulties in using antibodies. For example, they observed that the axes of the 2 chromosomes were forming a bivalent coil around each other, and they could follow the progression of coiling during prophase I [Wang et al., 2009]. Although coiling was previously observed in EM studies [Moens, 1972; Zickler and Kleckner, 1999], the dynamics of this process was not well documented as reconstructions of chromosome dynamics using EM are very laborious.

While the static approaches using fixed cells continue to provide a wealth of information on meiotic prophase chromosome behavior, they are unavoidably limited in depicting the true chromosome dynamics. *Live microscopy* studies in unicellular lower eukaryotes, fission yeast *Schizosaccharomyces pombe* and budding yeast *Saccharomyces cerevisiae*, have shown that chromosomes can display vigorous motility patterns during meiotic prophase [Chikashige et al., 1994, 2007; Conrad et al., 2008; Koszul et al., 2008]. In higher eukaryotes, including plants, such studies have not been conducted until very recently, because isolated meiocytes in these species cannot be easily cultured during prophase I [Chan and Cande, 2000]. Meiocytes at later stages, metaphase I and anaphase I, are more amenable to culturing, a feature that was used by Yu and colleagues [1997] to examine the dynamics of chromosome movements during their segregation in anaphase I in maize. To circumvent the problem of culturing prophase I meiocytes, Sheehan and Pawlowski [2009] developed a system to image meiocytes inside intact anthers of maize with *multiphoton excitation microscopy* which is capable of visualizing cells up to 200 μm deep inside the tissue. In contrast to meiocytes, intact anthers can be readily cultured in vitro throughout meiotic prophase [Cowan and Cande, 2002; Sheehan and Pawlowski, 2009]. Using this approach, Sheehan and Pawlowski [2009] demonstrated that maize chromosomes in early meiotic prophase exhibit extremely complex and dynamic motility patterns (fig. 1E).

The traditional squashing and spreading techniques have a firm place in the repertoire of tools to study chromosome behavior as they are simpler, and in some types of studies more appropriate, than the 3D, high-resolution, or live imaging techniques. While not replacing the traditional approaches, rapid evolution of new imaging tools in the past few years has greatly contributed to improved understanding of meiotic prophase chromosome dynamics. This is particularly true in areas where the traditional imaging tools showed clear limitations such as examining the spatial arrangement of chromosomes in the nucleus or rapid chromosome movements.

Patterns of Chromosome Behavior during Meiotic Prophase I

Chromosome Movements in Zygotene and Pachytene

While reconstructions of meiotic prophase progression using fixed meiocytes provided information on the general patterns of chromosome behavior, live imaging

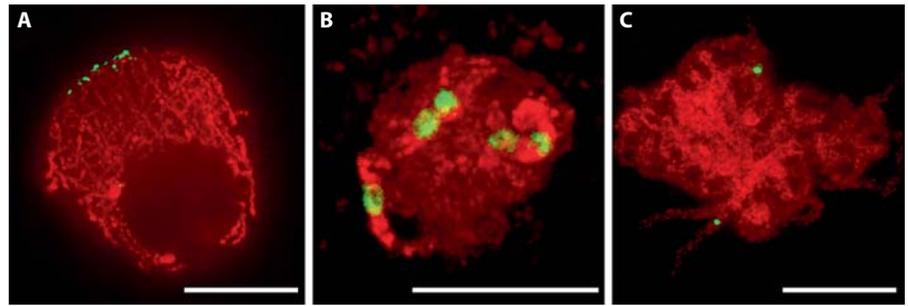


Fig. 2. Behavior of telomeres, centromeres, and interstitial chromosome regions. **A** Telomere bouquet in a wild-type maize nucleus in zygotene. Red = chromatin, green = telomeric FISH probe. Bar = 5 μm . Image courtesy of M. Sheehan. **B** Coupling of centromeres seen in a nucleus of the *Arabidopsis Atphs1* mutant in pachytene. Centromeric regions in the mutant only associate with

other centromeric regions even though interstitial chromosome regions exhibit mostly non-homologous associations. Red = chromatin, green = centromeric FISH probe. Bar = 5 μm . Modified from Ronceret et al. [2009]. **C** Heterosynapsis in the *phs1* mutant in maize in pachytene. Red = chromatin, green = 5S rRNA FISH probe. Bar = 5 μm . Modified from Pawlowski et al. [2004].

studies showed that observations of fixed cells were not able to convey the dynamics and complexity of chromosome behavior in live meiocytes [Chikashige et al., 1994; Conrad et al., 2008; Koszul et al., 2008; Sheehan and Pawlowski, 2009]. Using a live imaging technique, Sheehan and Pawlowski [2009] found that maize chromosomes during zygotene show movements with velocities of up to 400 nm/s which are similar to the velocities of the rapid prophase movements observed in yeast [Chikashige et al., 1994; Conrad et al., 2008; Koszul et al., 2008; Sheehan and Pawlowski, 2009]. The movements slow down to about 150 nm/s in pachytene and cease by diplotene. The patterns of chromosome motility are stage-specific. In zygotene small chromosome regions, mostly chromosome ends, exhibit fast, short-range movements. After a period of quiescence at the end of zygotene and the beginning of pachytene these movements are replaced by slower, sweeping movements of large chromosome segments. In addition to the movements of individual chromosome segments, the entire chromatin in the nucleus is subject to oscillating rotational motions. The rotational movements persist through zygotene and pachytene, including the quiescence period.

Telomere Bouquet

The dramatic chromosome dynamics in meiotic prophase I start with a repositioning of the nucleolus during the leptotene-zygotene transition [Dawe et al., 1994; Armstrong et al., 2001]. In leptotene the nucleolus is located in the center of the nucleus and surrounded by chromatin. At the end of leptotene it moves to a peripheral position. At the same time telomeres in many species,

including plants such as rye, wheat, and maize, attach to the nuclear envelope and subsequently cluster, forming the ‘telomere bouquet’ (fig. 2A) [Harper et al., 2004]. The bouquet forms at the beginning of zygotene, and the telomeres remain clustered throughout zygotene.

In contrast to most other species *Arabidopsis* does not form a canonical bouquet although its telomeres are clustered at the edge of the nucleolus from G2 to early leptotene [Armstrong et al., 2001; Roberts et al., 2009]. Later during zygotene *Arabidopsis* telomeres also show a transient loose association within one hemisphere of the nucleus [Roberts et al., 2009]. Both these observations suggest that the general principle of chromosome end congression is conserved in *Arabidopsis*, although the mechanism and dynamics of this process might be different from most other species.

The role of the bouquet has not been firmly established in any species. Mutants that do not form the telomere bouquet exhibit defects in chromosome pairing, synapsis, and recombination, implying that bouquet formation facilitates multiple meiotic processes [Niwa et al., 2000; Trelles-Sticken et al., 2000; Golubovskaya et al., 2002; Harper et al., 2004]. The *pam1* mutant in maize, the best studied of the bouquet mutants in plants, in addition to pairing defects exhibits problems with timely progression through meiotic prophase and unresolved entanglements (interlocks) of chromosomes in pachytene [Golubovskaya et al., 2002]. Based on studies of bouquet mutants in a number of species it has been hypothesized that telomere clustering facilitates proper chromosome pairing by bringing chromosome ends together and pre-aligning chromosomes [Harper et al., 2004]. Indeed,

chromosome pairing in maize and wheat has been shown to first take place at the telomeres [Bass et al., 2000; Maestra et al., 2002]. Moreover, chromosome ends have been proposed to be the sites where discrimination occurs between non-homologous and homologous partners in wheat [Corredor et al., 2007]. In *Arabidopsis*, FISH experiments using specific subtelomeric sequences have shown that chromosome ends are effectively homologously paired as early as the leptotene-zygotene transition, i.e. before pairing commences along chromosome arms [Roberts et al., 2009]. In many plant species telomeric regions are also sites where synapsis first initiates [Stack and Anderson, 2002; Lopez et al., 2008].

Recent studies on chromosome motility in meiotic prophase point, however, to another possible role for telomere clustering. Evidence from yeast as well as maize indicates that the main source of chromosome movements in prophase are forces generated in the cytoplasm and conveyed onto the nuclear envelope and the telomeres which in zygotene and early pachytene are attached to the nuclear envelope [Koszul et al., 2008; Koszul and Kleckner, 2009; Sheehan and Pawlowski, 2009]. Meiotic chromosome movements in yeast, and at least some of the movements in maize, are telomere-led. Analyses of chromosome motility patterns in maize meiocytes suggest that chromosome movements in zygotene may aid homologous pairing [Sheehan and Pawlowski, 2009]. These observations imply that the main role of the bouquet may be facilitating chromosome movements rather than bringing chromosomes together.

Centromere Coupling

Telomere clustering in a bouquet leads to a telomere-centromere polarization of the nucleus, with the centromeres generally located at the opposite side of the nucleus than the telomere cluster. In wheat and *Arabidopsis* evidence exists for presynaptic coupling (association in pairs) of centromeres [Martinez-Perez et al., 2001; Ronceret et al., 2009]. In polyploid wheat centromeres associate in meiotic cells as well as in somatic cells that do not become meiocytes [Martinez-Perez et al., 2000, 2001]. This phenomenon is regulated by the *Ph1* locus which ensures that the centromere associations are restricted to homologs. In the absence of *Ph1*, centromeres of non-homologous but homoeologous chromosomes can associate which in meiosis leads to defective chromosome pairing [Martinez-Perez et al., 2001]. In *Arabidopsis* all centromeres agglomerate together in early zygotene [Armstrong et al., 2001] before separating into pairs concomitantly with pairing of homologous chromosomes. Ron-

ceret et al. [2009] have noticed that in the *Arabidopsis Atphs1* mutant, which is severely defective in homolog pairing, centromeres always couple and never associate with non-centromeric chromosome regions (fig. 2B). This observation suggests that also in *Arabidopsis* centromere coupling precedes pairing along chromosome arms. In contrast to the persistent centromere coupling in polyploid wheat, centromere coupling in *Arabidopsis* is very ephemeral. The mechanisms of centromere coupling in the 2 species may, however, be similar, which could also suggest that coupling may be a more common phenomenon that is present also in other species. Transient centromere coupling that precedes homologous pairing and synapsis has also been discovered in budding yeast [Tsubouchi and Roeder, 2005]. Centromere coupling in *Arabidopsis* resembles the yeast phenomenon, because in both species coupling is independent from the progression of recombination. However, coupling in yeast requires installation of the central element of the synaptonemal complex [Tsubouchi and Roeder, 2005; Tsubouchi et al., 2008], while in *Arabidopsis* it most likely does not [Ronceret et al., 2009].

Behavior of Interstitial Chromosome Segments in Early Meiotic Prophase

In comparison to the telomere and centromere behavior the dynamics of interstitial segments of chromosomes in meiotic prophase I are poorly understood. During zygotene loci along chromosome arms on homologous chromosomes find each other and pair. In plants, as in most eukaryotes, chromosome pairing is tightly linked to the progression of meiotic recombination [Pawlowski and Cande, 2005]. Mutants in genes responsible for the formation of meiotic DSBs and their subsequent processing and repair often show severe chromosome pairing defects (fig. 2C) [Grelon et al., 2001; Bleuyard et al., 2004; Li et al., 2004; Puizina et al., 2004; Stacey et al., 2006; Li et al., 2007; De Muyt et al., 2009; Ronceret et al., 2009]. The single-end invasion step of meiotic recombination has been directly implicated in facilitating homology recognition and pairing of chromosomes [Bozza and Pawlowski, 2008]. During this step single-stranded DNA overhangs, created by resection of meiotic DSBs, are coated by the 2 recombination proteins RAD51 and DMC1. The nucleoprotein filament formed in this way identifies and invades a double-stranded DNA region on the homologous chromosome [Neale and Keeney, 2006; San Filippo et al., 2008]. However, it is not clear how this very localized process is coordinated along an entire chromosome arm. Although pairing is thought to start at the

telomeres in plants [Bass et al., 2000; Maestra et al., 2002; Corredor, 2007; Roberts et al., 2009], it is not known whether it subsequently spreads along the entire arm by ‘zipping-up’ or if there are additional interstitial pairing initiation sites. The observations of vigorous chromosome movements in maize zygotene meiocytes [Sheehan and Pawlowski, 2009] suggest a ‘probing mode’ of pairing in which many different pairing combinations between individual chromosome segments can be tried before a correct homologous interaction is found rather than the unidirectional ‘zipping-up’ mode. The view postulating interstitial pairing sites is also supported by observations of Burnham et al. [1972] who studied pairing in maize plant heterozygous for chromosome translocations. Additional evidence comes from observations of ‘partner switches’ in meiotic mutants defective in pairing in which one chromosome associates with several different partners in different regions along its length [Pawlowski et al., 2004; Osman et al., 2006].

Our understanding of chromosome dynamics after successful homology recognition and pairing is also far from clear. Coiling of chromosomes around each other was found to follow chromosome pairing [Moens, 1972; Zickler and Kleckner, 1999; Wang et al., 2009]. Wang et al. [2009] have recently characterized this phenomenon in detail using 3D-SIM. They found that maize chromosomes form a left-handed double helix along their entire lengths, including heterochromatic regions such as centromeres, knobs, and the nucleolus organizer regions. Changes in coil distribution during zygotene and pachytene indicated that chromosome twisting starts at the telomeres and is concomitant with the shortening and thickening of the bivalent.

Chromosome dynamics during pairing and synapsis may result in chromosome entanglements (interlocks) [Zickler and Kleckner, 1999; Wang et al., 2009]. Interlocks are detected in most chromosome regions that have remained unsynapsed at the end of zygotene, indicating that interlock resolution is a limiting step for the completion of synapsis [Wang et al., 2009]. Resolution of interlocks to achieve full pairing and synapsis takes place during pachytene, but mechanisms responsible for this process are unclear. Sheehan and Pawlowski [2009] observed in live maize meiocytes that chromosome movements during pachytene exhibit different patterns from the zygotene movements and suggested that the pachytene movements could play a role in interlock resolution.

Although studies on meiotic chromosome dynamics in plants are still in their infancy, the data available so far indicate that telomeres, centromeres, and interstitial

chromosome regions exhibit different dynamics patterns and are most likely controlled by different mechanisms. The most is known about the behavior of the telomeres, but even here our understanding is far from complete. The data already available also indicate substantial diversity of patterns of chromosome dynamics among species. A good example here may be the absence of the typical bouquet in *Arabidopsis*. There is also accumulating evidence that polyploid species, such as wheat, have evolved specialized patterns of chromosome dynamics, either completely new or modifications of existing meiotic mechanisms, to prevent ectopic pairing and recombination between non-homologous chromosomes [Griffiths et al., 2006; Cifuentes et al., 2009].

Factors Controlling Meiotic Prophase Chromosome Dynamics

Genetic studies on meiotic prophase I in plants have so far mostly focused on mutants that show general impairments in the progression of the major meiotic processes such as pairing, recombination, or synapsis [Ronceret et al., 2007; Mercier and Grelon, 2008]. The effects of these mutants on chromosome dynamics are therefore rather severe and static. Nevertheless, analyses of several meiotic mutants in *Arabidopsis*, rice, and maize have examined the relationship between the progression of the biochemical steps of meiosis (such as recombination) and the physical behavior of chromosomes. Many mutants defective in the initiation of recombination and early steps of DSB repair are also asynaptic, i.e. exhibit presence of mostly univalent chromosomes in pachytene [Grelon et al., 2001; Bleuyard et al., 2004; Puizina et al., 2004; Stacey et al., 2006; De Muylt et al., 2009]. These observations indicate that homologous chromosome pairing is tightly linked to the progression of recombination. Members of a small group of meiotic mutants defective in early recombination, and possibly other early prophase processes, lead to heterosynapsis, i.e. a situation in which pairing-like associations of non-homologous chromosomes replace homologous pairing. Maize and *Arabidopsis* *phs1* mutants are defective in resection of meiotic DSBs and exhibit high frequencies of heterosynapsis (fig. 2C) [Pawlowski et al., 2004; Ronceret et al., 2009]. Interestingly, in these mutants telomere clustering as well as the behavior of centromeres remain largely unaffected, indicating that different mechanisms control dynamics of telomeres, centromeres, and interstitial chromosome regions. Whether or not the *phs1* mutation has also a di-

rect impact on movements of prophase chromosomes remains to be determined.

The overall structural properties of the chromosome, and particularly the chromosome axis, are known to affect homologous pairing. Control of sister chromatin cohesion and condensation involves cohesin and condensins which are required for proper pairing [Cai et al., 2003; Chelysheva et al., 2005; Golubovskaya et al., 2006; Sebastian et al., 2009]. Proteins involved in chromosome axis formation, such as ASY1 in *Arabidopsis* and its homolog PAIR2 in rice, have also direct roles in interhomolog interactions [Nonomura et al., 2006; Sanchez-Moran et al., 2007, 2008].

Chromosome dynamics are likely to be influenced by chromatin structure. Changes in chromatin organization are associated with the transition from leptotene to zygotene in maize [Dawe et al., 1994]. Studies in wheat suggest that telomere and centromere interactions in early meiosis induce a conformational change in the chromatin which makes it accessible to pairing interactions [Prieto et al., 2004, 2005]. Failure of chromosomes to undergo chromatin remodeling results in a loss of competency to pair [Colas et al., 2008]. Molecular mechanisms involved in meiotic chromatin reorganization are unclear. It is quite likely that epigenetic histone modification could play a role in this process. In budding yeast the presence of histone H3 lysine 4 trimethylation predisposes DNA sites to become hotspots for DSB formation in early meiotic prophase [Borde et al., 2009]. The *Arabidopsis ask1* mutant, deficient in homologous pairing and in the overall nuclear reorganization in meiosis, also shows defects in histone H3 acetylation, indicating a further link between chromosome dynamics and the patterns of chromatin modifications [Yang et al., 2006].

Relatively few genes have been shown to be directly and primarily involved in regulating chromosome dynamics in meiotic prophase. One of the best candidates for such a gene is *Pam1* in maize [Golubovskaya et al., 2002]. In the *pam1* mutant telomeres attach to the nuclear envelope but fail to cluster. Two other mutants with bouquet and chromosome dynamics defects have also been studied in maize, *dsy1* and *dy* [Bass et al., 2003]. The *dsy1* mutant exhibits incomplete bouquet formation, while *dy* shows premature release of the telomeres from the nuclear envelope. However, none of these 3 genes has been cloned yet or characterized at the molecular level.

Another class of factors that directly function in chromosome dynamics are factors controlling prophase chromosome movements. Studies of live maize meiocytes indicated that a major source of chromosome motility are

forces that act in the cytoplasm and involve both the actin and tubulin cytoskeletons [Sheehan and Pawlowski, 2009]. Moreover, these studies suggested that specific cytoskeleton regions are responsible for generating nuclear motility. However, genes encoding these specific cytoskeleton elements remain to be characterized.

A number of studies have suggested that protein modifications by ubiquitination and sumoylation may be involved in regulating meiotic chromosome dynamics. In the ubiquitination process a ubiquitin ligase targets proteins to the proteasome degradation complex by tagging them with a small protein ubiquitin [for review see Vierstra, 2009]. The *ASK1* gene in *Arabidopsis*, which encodes an essential component of the ubiquitination pathway, regulates meiotic chromosome remodeling, including alterations of homologous chromosome pairing and nucleolus migration [Yang et al., 2006]. It does so by controlling the detachment of telomeres from the nuclear envelope in leptotene. The exact molecular role of ubiquitination in meiosis in plants is still unclear since direct biochemical evidence that ubiquitin indeed labels meiotic proteins for degradation has not been found yet [Wang et al., 2006b]. Nevertheless, studies in mammals also imply a role for ubiquitination in meiosis. Mouse ubiquitin ligase Rad18 is known to be associated with unpaired and transcriptionally silenced chromosome regions in meiotic prophase [van der Laan et al., 2004], and ubiquitin ligase Hei10 is essential for crossover formation [Ward et al., 2007].

Studies on the role of protein modifications by SUMO (sumoylation), another small protein capable of modifying protein functions, have not yet been conducted in plants. However, sumoylation has been shown to play a major role in controlling chromosome dynamics during meiosis in budding and fission yeasts [Cheng et al., 2007]. Ubiquitination and sumoylation use similar mechanistic pathways [Gill, 2004]. They can also compete for the same amino acid residue on their target protein, although ubiquitination and sumoylation usually have different effects on protein regulation [Perry et al., 2008]. It is, therefore, possible that both ubiquitination and sumoylation may have roles in meiotic chromosome dynamics in plants.

Most factors controlling chromosome behavior in meiotic prophase I still remain to be discovered in plants. Processes controlling telomere bouquet formation or prophase chromosome movements are likely to have direct roles in regulating chromosome dynamics. The available data show that chromosome dynamics are strongly impacted by meiotic processes that take place at

the molecular level, such as recombination and homologous chromosome recognition. They also suggest involvement of general cellular control mechanisms such as ubiquitination and sumoylation in regulating meiotic chromosome dynamics.

Future Research

Although the progress toward elucidating chromosome dynamics in meiotic prophase has been greatly accelerated during the past decade, our understanding of these processes is still quite limited, particularly when it comes to the molecular mechanisms directing chromosome behavior. The pace of this progress in the future will depend on how successfully the new imaging technologies, particularly high-resolution 3D and live microscopy, can be integrated with genetic and molecular biology tools that can infer the mechanistic bases of chromosome behavior. Detailed investigations of the patterns of dynamics of different chromosome regions offer a substantial promise for elucidating general mechanisms that govern chromosome interactions. Studies in living cells are particularly exciting, but they will require development of new chromosome tracking tools, particularly transgenic lines expressing fluorescent tags that mark chromosome landmarks such as centromeres, telomeres, single-copy loci, or heterochromatic regions. Understanding the molecular forces responsible for meiotic chromosome movements, as well as mechanisms that regulate them, will be even more challenging than describing the movement patterns, as few mutants specifically defective in meiotic chromosome motility have been identified so far.

An area under intense investigations by several groups is the process of meiotic telomere clustering and its role in prophase I progression. Particularly, the role of the telomere cluster in generating chromosome movements by conveying forces from the cytoskeleton to the chromatin is intriguing, yet poorly understood. Several structural elements of the bouquet, i.e. proteins that mediate attachment of the telomeres to the nuclear envelope and link them to the cytoplasmic cytoskeleton, have been identified in yeasts [Scherthan, 2007]. However, because of poor sequence conservation of most of these proteins [Sheehan and Pawlowski, unpubl. data], their functional homologs in plants will have to be identified *de novo*. Once these proteins are identified, it will be interesting to see if they are also involved in the non-typical telomere clustering in *Arabidopsis*.

Investigating mechanisms that specifically govern polyploid chromosome interactions is particularly interesting to plant scientists, since most plants are either recent or ancient polyploids. Genetic systems that have similar roles in regulating chromosome behavior as the well-known *Ph1* locus in wheat are likely to exist in other polyploids and have major impacts on chromosome dynamics in these species [Jenczewski et al., 2003; Cifuentes et al., 2009; Nicolas et al., 2009]. We expect that now that the genetic bases of *Ph1* are being uncovered [Griffiths et al., 2006] similar systems in other species will also become subjects of investigation.

We are particularly excited about the prospects of developing mathematical models to describe chromosome dynamics. Such models would integrate imaging data with the understanding of molecular mechanisms regulating chromosome interactions and take into account the physical properties of chromosomes. As chromosome dynamics are affected by biochemical as well as physical processes, an integrated approach will undoubtedly be able to much better explain meiotic events than any single approach alone. Attempts to use integrated multidisciplinary approaches have already been made to model the distribution of recombination events on chromosomes [Kleckner et al., 2004; Falque et al., 2007], but they are still in their early beginnings. Plants with their large and conspicuous chromosomes and good genetic tools are an excellent system to test such models.

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