Contribution of Structural Chromosome Mutants to the Study of Meiosis in Plants

Tomás Naranjo

Departamento de Genética, Facultad de Biología, Universidad Complutense, Madrid, Spain

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Chromosome segregation · Deletion · Inversion · Pairing · Plants · Recombination · Synapsis · Translocation

Abstract

Dissection of the molecular mechanisms underlying the transition through the complex events of the meiotic process requires the use of gene mutants or RNAi-mediated gene silencing. A considerable number of meiotic mutants have been isolated in plant species such as *Arabidopsis thaliana*, maize or rice. However, structural chromosome mutants are also important for the identification of the role developed by different chromosome domains in the meiotic process. This review summarizes the contribution of studies carried out in plants using structural chromosome variations. Meiotic events concerning the search of the homologous partner, the control of number and distribution of chiasmata, the mechanism of pairing correction, and chromosome segregation are considered.

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An Overview of Meiosis

Meiosis is a specialized cell division that is required to halve the chromosome complement, in organisms with sexual reproduction, in order to avoid the occurrence of

chromosome doubling in each generation. This reduction of the chromosome number actually gave its name to meiosis, a term that comes from the Greek μείωσις, meaning ‘to lessen’. In addition to this important biological feature, meiosis is also responsible for the formation of new allele combinations by the process of recombination. The first meiotic division is crucial to achieve both outcomes. During prophase I, homologous chromosomes find each other and undergo pairing, synapsis and recombination between non-sister chromatids. Suppression of sister chromatids separation during prophase I gives rise to the formation of bivalents, which split off into recombinant homologues that migrate to opposite poles in anaphase I.

During meiotic prophase I, homologous chromosomes, previously distributed throughout the nucleus [Bass et al., 2000; Maestra et al., 2002], align in close contact and form bivalents. In many species, all chromosome ends initiate a nonrandom movement at the entrance of meiosis that causes their progressive grouping to culminate in a tight cluster. This suprachromosomal configuration, the so-called bouquet, is consolidated concomitant with the initiation of alignment, pairing and synapsis, particularly at regions close to the telomeres; hence, it is currently considered as a meiotic-specific structure involved in homologous chromosome interactions [Bass et al., 1997, 2000; Niwa et al., 2000; Trelles-Sticken et al., 2000; Cowan et al., 2001; Scherthan, 2001]. Chromosome inter-

actions leading to homologous recognition initiate with the formation of programmed double-strand DNA breaks (DSBs) catalyzed by the topoisomerase-like conserved protein, Spo11, in conjunction with a number of additional proteins [Keeney et al., 1997; Neale and Keeney, 2006]. DSBs preferentially occur in short DNA regions, termed recombination hotspots, which are distributed along the chromosomes but with significantly reduced frequency in the centromeric and telomeric regions [Paigen and Petkov, 2010]. In budding yeast, hotspots are associated with regions of low nucleosome density predominantly located at gene promoters [Pan et al., 2011]. In mammals, hotspots do not correlate with transcription promoters and are located both in genic and intergenic regions [Paigen et al., 2008; Paigen and Petkov, 2010]. In mice and humans, DSBs occur at discrete hotspots defined by the DNA-binding specificity of the PRDM9 protein, a zinc-finger protein containing a histone H3 lysine 4 methyltransferase [Baudat et al., 2010; Myers et al., 2010; Parvanov et al., 2010]. The PRDM9 zinc finger domains that bind DNA are highly polymorphic, and different variants have different DNA-binding preferences. Genome-wide recombination initiation maps in individual human males have shown different sets of DSB hotspots defined by different alleles of the PRDM9 protein [Pratto et al., 2014].

In *Arabidopsis thaliana*, which lacks PRMD9, hotspots overlap gene promoters and are associated with active chromatin modifications, including histone H2A.Z, histone H3 Lys4 trimethylation (H3K4me3), low nucleosome density, and low DNA methylation [Choi et al., 2013]. A high-resolution map of recombination in maize revealed that crossovers (COs) are more likely to occur in the genic than intergenic regions, especially frequent in the 5' and 3' end regions of annotated genes [Li et al., 2015]. Repair of DSBs is controlled to ensure at least one CO per chromosome pair, referred to as the obligate CO. In budding yeast, ~160 DSBs are generated per meiosis [Pan et al., 2011]. On average, ~90 DSBs are repaired as COs, and the remainders are repaired as either interhomologous noncrossovers (NCOs) or intersister recombinants [Chen et al., 2008; Mancera et al., 2008]. However, in plants and animals, most of DSBs are repaired by a NCO pathway; only a minor fraction (5%) is repaired as COs [Higgins et al., 2014]. In most species, COs are more evenly spaced than would be expected from a random distribution. This phenomenon, termed interference, was first reported by *Drosophila* geneticists and has been subsequently observed in a vast range of organisms from yeasts to humans [Jones and Franklin, 2006; Berchowitz and Copenhaver, 2010].

DSBs are resected to generate 3' single-strand DNA overhangs that are initially bound by RPA, which is subsequently displaced by the recombinase RAD51 and/or the meiosis-specific recombinase DMC1 to form nucleoprotein filaments. These filaments serve to find a complementary sequence within a homologous chromosome, at which they instigate single-end strand invasions [Hunter and Kleckner, 2001]. During leptotene, a protein-rich backbone forms between sister chromatids, known as the axial element, that will keep them together until the second meiotic division. During this stage, chromosomes are relatively decondensed and long. Proteins that make up the axis, e.g. Hop1-Red1 in budding yeast, ASY1-ASY3 in *Arabidopsis* and PAIR2-PAIR3 in rice, promote the search of a repair template in one homologous chromatid [Hollingsworth and Byers, 1989; Thompson and Roeder, 1989; Caryl et al., 2000; Nonomura et al., 2006; Wang et al., 2011; Ferdous et al., 2012]. Thus, the initial steps of the DSBs-repairing process facilitate the homology recognition and are required for homologous pairing and normal synapsis in yeast and mice [Roeder, 1997; Baudat et al., 2000; Romanienko and Camerini-Otero, 2000], whereas in *Caenorhabditis elegans* and *Drosophila melanogaster*, homologous pairing and synaptonemal complex (SC) formation can occur independent of meiotic recombination [Dernburg et al., 1998; McKim et al., 1998; Liu et al., 2002; Colaiácovo et al., 2003]. The homologous pairs become increasingly aligned in the transition from leptotene to zygotene and undergo synapsis during zygotene [Page and Hawley, 2004]. Completion of tripartite SC along the entire length of chromosome axes is the hallmark of pachytene. SC supports and maintains homologues in close juxtaposition and serves as a scaffold for CO-promoting recombination factors. At the end of pachytene, homologues are fully synapsed and chromosomes are short and condensed. The SC disassembles during diplotene once COs formation is completed. Then homologues appear physically connected by one or more chiasmata in each bivalent. Chiasmata are the cytological expressions of COs and are formed in a recombinational process that runs in synchrony with synapsis. The chromatin condensation degree increases in the course of diplotene and diakinesis. After the nuclear envelope disorganization, the bivalents align on the equator at metaphase I before homologous disjunction. Maternal and paternal components of each chromosome pair segregate to daughter cells in the first division. Chiasmata are essential for correct orientation of homologous centromeres in the spindle during metaphase I. Chromosomes that fail to form chiasmata frequently fail to disjoin properly, leading

to aneuploidy. In the second meiotic division, sister chromatids are separated, culminating in the generation of haploid gametes.

Different Approaches in the Study of Meiosis

Different strategies have been used for the identification of meiotic genes in different organisms. Forward genetic screening involving the induction of random mutations, identification of a desired meiotic mutant phenotype, and isolation of the causal allele have been developed in different model organisms. Extensive screening carried out in budding yeast [Neiman, 2005] provided a large list of genes, which is available in the *Saccharomyces* Genome Database (www.yeastgenome.org). Because the basic principles of chromosome behavior and recombination are relatively conserved among fungi, plants and animals, yeast orthologues represent a powerful tool to identify meiotic genes in higher eukaryotes by using genetic reverse approaches. Meiotic transcriptome studies carried out in different organisms show the expression of a large number of genes during meiosis [Zhou and Pawlowski, 2014, and references therein]. However, large-scale proteome studies have shown that translation repression is important in gametogenesis. Nevertheless, proteins involved in the development of meiosis have been identified in different organisms [Sánchez-Morán et al., 2005; Scaife et al., 2010; Wang et al., 2012; Gan et al., 2013; Barakate et al., 2014; Kronjaa et al., 2014]. Although proteome profiles of meiotic cells reveal the number and types of proteins present in different stages of meiosis, dissection of the molecular mechanisms underlying the transition through the complex events of the meiotic process requires the use of gene mutants or RNAi-mediated gene silencing [Roeder, 1997; Cnudde and Gerats, 2005; Namekawa et al., 2005; Neale and Keeney, 2006; San Filippo et al., 2008; Bhalla and Dernburg 2008; Osman et al., 2011; Bolcun-Filas and Schimenti, 2012; Luo et al., 2014; Mercier et al., 2015]. Among plants, about 80 meiotic genes have been identified in *A. thaliana* [Osman et al., 2011; Mercier et al., 2015], 35 in maize [Golubovskaya et al., 2011], 28 in rice [Luo et al., 2014], and lower numbers in species such as wheat or barley, and the list will grow in the coming years.

Plants were in the forefront of the study of meiosis in the first half of the 20th century partially because of the many extant species, especially among monocotyledons, which possess large chromosomes that facilitate the observation of the different meiotic stages, and also because

meiosis generates new allele combinations and is related to fertility, which has implication in breeding-related programs. It is remarkable that the pioneering work of Creighton and McClintock [1931] showed convincing proof that genes are physically aligned along the chromosomes. Using a combination of 2 genes and 2 chromosome mutations located in the same chromosome, these authors demonstrated that an exchange between genes is accompanied by an exchange of a physical part of homologous chromosomes. This work is a representative example of the importance that cytological studies have in our knowledge of the meiotic process. Approaches concerning the analysis of meiosis focused on the use of structural chromosome mutants have revealed that different domains play different roles in the meiotic chromosome behavior. In this review, I will summarize the main contributions to the current knowledge of the meiotic process of studies based on the use of structural chromosome mutants in plants.

Isolation and Characterization of Structural Chromosome Mutants

Many structural chromosome mutants used to study meiosis in plants, which are considered in this review, were detected in routine controls of mitotic chromosomes as well as by the formation of diagnostic meiotic configurations in heterozygotes. New genomic tools have also been used to detect deletions and inversions in the model species *Arabidopsis* [Ederveen et al., 2015]. Chromosome rearrangements such as duplications, inversions or translocations can usually be obtained in homozygous or heterozygous condition because they generally do not cause an irreparable loss of genetic material. However, homozygosity for deletions is lethal in diploid species. Polyploids with more than 2 copies of the same genome, autopolyploids, or several genomes derived from related species, allopolyploids, can tolerate the loss of a given segment in 2 chromosomes. Extra copies of this segment present in the remaining intact homologous or homoeologous chromosomes compensate for the deletion. A representative example is the deletion stock of bread wheat, with 436 terminal deletions involving all chromosomes [Endo and Gill, 1996].

Polyplids have an additional advantage over diploids. Many of them tolerate the addition of chromosomes derived from related species, which can be identified at any stage of the cell cycle by FISH protocols using repeated DNA sequences or total genomic DNA as a probe (GISH).

Fig. 1. Painting normal and telocentric rye chromosomes in mitotic metaphases of wheat-rye disomic addition lines. **A** Chromosome pair 1R labeled with probes pUCM600 (red) and pSc74 (green). **B** Telocentric chromosomes 1RS labeled with the centromeric probes pAWRC1 (green) and pSc74 (red).

These materials are called addition lines and contain one chromosome or chromosome pair from a diploid or polyploid species added to the full chromosome complement of the recipient species. Any chromosome rearrangement involving the alien chromosome can be obtained in heterozygous or homozygous condition without affecting the plant viability, since all of the genetic information of the recipient species is present.

Addition lines are often produced in breeding-related programs that aim to transfer desirable genes between species through hybridization and backcrossing. Although recombination between alien and recipient-species chromosomes is normally absent in these lines, their genetic and cytogenetic properties make them powerful tools for fundamental research concerning meiotic chromosome behavior, localization of chromosome-specific markers or heterologous gene expression. Complete sets of additions have been obtained in different plants [Chang and de Jong, 2005], but the number is especially high in hexaploid bread wheat, *Triticum aestivum* ($2n = 6x = 42$, genome formula AABBDD), where additions from more than 20 related species have been obtained [Shepherd and Islam, 1988]. In many of these addition lines, telocentrics for any of the arms of the added chromosomes (fig. 1) were recovered after spontaneous centromere misdivision, followed by de novo telomere addition at the broken centromeres. Other structural modifications of the added chromosomes such as deletions, inversions or translocations have been recovered.

Arrangement of Homologous Chromosomes in Somatic and Meiotic Cells in Addition Lines

Aspects of the chromosome organization in the nucleus as the relative position of homologues have been studied in disomic additions for 2-armed or telocentric chromosomes. For example, Corredor et al. [2005] analyzed the arrangement of the rye homologous pair in somatic cells of 2 wheat-rye disomic addition lines, wheat-7R and wheat-5RL (5RL is the telocentric of the long arm of chromosome 5R). In nuclei at interphase, painted chromosomes appear as discrete domains that show the Rabl orientation. Homologues are predominantly nonassociated, and their positioning seems to be unaffected by the *Ph1* locus, which suppresses meiotic pairing between homoeologous chromosomes. When chromosome duplication is induced by colchicine, sister chromosomes show a high level of somatic pairing, which persists in the following interphase, but homologous chromosomes of the same cell remain separated. This indicates that, if there is an order in the disposition of chromosomes into the somatic nucleus of hexaploid wheat, somatic association is not a relevant feature of this order.

The relationships of somatic association to premeiotic and meiotic pairing have been debated for nearly a century. An ordered premeiotic interphase nucleus with presynaptic homologous chromosome alignment as part of that order was proposed [Brown and Stack, 1968; Comings, 1968]. The association of homologues in allopolyploid species such as wheat implies the discrimination between homologues and homoeologues and the suppression of homoeologous interactions. This just happens in meiosis, where only bivalents containing homologous chromosomes are formed at diakinesis and metaphase I. The exclusive homologous chiasmatic association observed at metaphase I in hexaploid wheat, which is under the control of the *Ph1* locus [reviewed by Sears, 1976], was postulated to be a result of the existence of premeiotic association between homologues [Feldman, 1966]. However, the relative position of homologues in premeiotic cells, both in the presence and the absence of *Ph1*, is similar to that of somatic cells, which is contradictory to the Feldman's hypothesis. The premeiotic chromosome arrangement is modified by chromosome movements leading to the bouquet organization as well as by chromatin decondensation and homologous synapsis initiation [Maestra et al., 2002; Corredor et al., 2005; Naranjo and Corredor, 2008].

Addition lines are also used in the study of the chromosome dynamics in the course of prophase I. The be-

havior of the alien homologous pair can be scrutinized throughout the prophase I substages revealing features of the pairing-partner search as well as of synapsis, which can be completed with inferences on COs formation by analysis of metaphase I configurations [Maestra et al., 2002; Corredor and Naranjo, 2007; Corredor et al., 2007; Naranjo et al., 2010; Valenzuela et al., 2012].

Unraveling the Mechanisms Underlying the Search of the Homologous Partner

A feature of the nuclear architecture at premeiotic interphase that has been related to meiotic pairing is the distribution and orientation of centromeres and telomeres. In mitotically active cells of many species, chromosomes retain their arrangement of the previous anaphase during interphase, i.e. the Rabl configuration – with centromeres concentrated at one side of the nucleus and telomeres fanned out on the opposite side. This chromosome polarization extends to premeiotic cells, especially in species with large genomes [Fussell, 1987] and ensures the codirectional arrangement of homologous chromosome segments. Such a nuclear organization most likely minimizes the movements of chromosomes so that chromosomes located in separated nuclear territories can undergo homologous pairing. In species with non-Rabl orientation, clustering of telomeres at the beginning of meiosis creates an oriented organization of the prophase I nucleus [Dong and Jiang, 1998]. Functionally equivalent chromosome domains such as centromeres or telomeres that exist in all chromosomes represent the appropriate structure to initiate interactions leading to the identification of the homologous partner. Centromeres associate at leptotene and telomeres cluster to form the meiotic bouquet in many organisms [Naranjo and Corredor, 2008, and references therein]. However, the presynaptic formation of multicentromere structures is not based on homology; therefore, it plays no relevant role on chromosome sorting. Multicentromere complexes have been suggested to be involved in the maintenance of the nuclear stability while telomeres migrate to form a tight cluster in a small region of the nuclear envelope [Corredor et al., 2007]. The coincidence of telomere clustering and synapsis initiation has led to the hypothesis that bouquet configuration facilitates homologous pairing and synapsis by bringing chromosome ends into close proximity [Bass et al., 1997, 2000; Niwa et al., 2000; Trelles-Sticken et al., 2000; Cowan et al., 2001; Scherthan, 2001; Harper et al., 2004].

Molecular interactions responsible for the attachment of chromosome ends to the nuclear envelope, which precedes their migration and clustering, specifically require the presence of functional telomere repeats. Telomere shortening in telomerase-deficient mice impairs synapsis and decreases the recombination frequency [Liu et al., 2004]. In telocentric chromosomes of maize and rye, the *cis*-dominant effect of the telomere adjacent to the centromere allows the centromere to enter at the bouquet, whereas the centromere of bi-armed chromosomes remain at the centromere pole [Carlton and Cande, 2002; Maestra et al., 2002; Corredor and Naranjo, 2007; Corredor et al., 2007]. When the telomeric sequences are located intercalarily, as it happens in a maize ring chromosome, they also associate with other telomeres allowing the chromosome to enter the bouquet [Carlton and Cande, 2002]. This demonstrates that the terminal position on a chromosome is not necessary for telomere sequences to localize to the bouquet.

Telomeres attached to the nuclear envelope associate first in a number of small aggregates, which undergo active movements to become associated in a tight cluster. Colchicine inhibits telomere migration, but does not affect telomere association [Carlton et al., 2003; Corredor and Naranjo, 2007]. This suggests that telomere association and telomere migration are 2 different steps in the bouquet organization. In lines of wheat carrying added telocentrics of rye, application of colchicine in presynaptic stages impedes the migration of the centromeric end of telocentric chromosomes, which remain associated with centromeres of bi-armed chromosomes at the centromere pole. The lack of telomere migration is accompanied by failure in homologous pairing and synapsis, denoting that the homologous chromosome ends are brought close to one another during bouquet organization [Corredor and Naranjo, 2007]. Some degree of homologous synapsis and recombination is however reached when telomere migration is inhibited by colchicine. Such a synapsis most likely occurs between chromosomes with telomeres included in the same mini-cluster. This arrangement is comparable to that of the homologous arms of an isochromosome, which lie close to one another after the last premeiotic mitosis and display a normal level of pairing and chiasma formation even in the presence of colchicine [Driscoll and Darvey, 1970]. But synapsis produced in colchicine-treated meiocytes may also involve homologous chromosomes initially separated that approach each other by forces non-exerted on the telomeres.

Fig. 2. Arrangement of the 2 telocentric chromosomes of rye at the leptotene-zygotene transition in the wheat-5RL disomic addition. **A** Nucleus with the 2 decondensed rye chromosomes (green). The centromeric ends of the 2 telocentric chromosomes occupy separated domains in the nuclear pole where wheat centromeres (dark red) are concentrated, while the other 2 chromosome ends converge in the telomere cluster (bright red). Reprinted with permission of Springer Verlag from Maestra et al. [2002]. **B** Diagrammatic representation of A. Scale bar = 10 μ m.

Telomere-led chromosome movements mainly bring together the distal region of homologous chromosomes, especially in large chromosomes with lengths at zygotene of the order of tens or even one hundred μ m. Large chromosomes of *Allium* species showing extensive chromosome pairing suggest that attraction forces between homologues operate on different points distributed evenly along the chromosome [Loidl and Jones, 1986; Albin and Jones, 1987]. Chromosome movements that make encounters between intercalary regions possible are likely derived of chromatin conformational changes leading to chromosome elongation, which is produced concomitantly with telomere clustering at the leptotene-zygotene transition [Maestra et al., 2002; Corredor and Naranjo, 2007]. Wheat and rye chromosomes multiply their length fivefold in leptotene relative to premeiotic interphase. Because the size of the nucleus remains the same at the leptotene-zygotene transition, or is even reduced, chromatin unfolding obliges chromosomes to move and span the entire nucleus (fig. 2). This chromatin unfolding is not inhibited by colchicine [Corredor and Naranjo, 2007] and is therefore independent of telomere migration. The movement that accompanies chromosome elongation can facilitate the occurrence of chance collisions between homologous chromosomes. The reduction of long distances between homologues during zygotene is particularly apparent in the case of heterozygotes for an inversion covering almost 95% of a rye chromosome arm. Observations were made in a line of wheat with the disomic addition of chromosome 1R [Valenzuela et al., 2012] and, therefore, in nuclei with more than 20 μ m in diameter. During bouquet formation, the subdistal part of the normal arm locates at the telomere pole, while its homologous counterpart in the inverted arm is situated near the centromere pole. However, such regions pair and synapse in many meiocytes.

Telomere mobility can be affected by factors such as chromosome conformation. This effect became apparent when the migration of the individual chromosome ends of 2 bi-armed rye homologues added to wheat was monitored for 2 different conformations: a submetacentric chromosome, with arms of very different length, and a metacentric chromosome, with arms of similar length. The unequal arm situation corresponds to the standard chromosome 5R, and the similar-sized arm situation corresponds to the truncated chromosome del5R. Chromosome del5R is the result of a large terminal deletion produced in the long arm (5RL). The 5RS arm is the same in both chromosome conformations. This arm shows a large subtelomeric heterochromatin chromomere that facilitates the location of its telomere into the nucleus of meiocytes at leptotene. The 5RS telomere fails to be included in the telomere cluster more often in the submetacentric than in the metacentric conformation, and this disturbed telomere migration is accompanied by the reduction of the level of synapsis and chiasmata [Naranjo et al., 2010]. The chromosomal conformation effect on telomere mobility is a common feature of all rye chromosomes. Two other chromosomes, 1R and 6R, with apparent differences in the arm ratio also show differences in the mobility of the telomeres of their short arms when they are in a wheat background. Disturbed telomere migration (fig. 3) occurs more often in the short arm of the submetacentric chromosome 6R than in the short arm of the almost metacentric chromosome 1R [Naranjo, 2014]. Incomplete telomere clustering of chromosome 6R is followed by failure of synapsis and chiasma formation.

The bouquet formation implies that all telomeres, regardless of their position in the nuclear periphery, migrate to a small region of the nuclear envelope. This movement is most likely polarized by the position of the

Fig. 3. Idiogram of rye chromosomes 1R and 6R and 3D analysis of their arrangement at the bouquet in wheat-rye addition lines. **A** Chromosome 1R showing the subteleric marks of probe pSc74 (green) in both arms and the centromere mark (red) of probe pAWRC1. **B** Arrangement of the subteleric rye heterochromatin chromomeres (green), rye centromeres (red c), and telomere clusters (red). Asterisks and arrowheads indicate the position of the subteleric chromomeres of the short and long arm, respectively. The end of one 1RS arm is separated from the telomere pole. **C** Chromosome 6R showing the marks of probes pSc74 (green) and pAWRC1 (red). **D** Arrangement of the subteleric rye heterochromatin chromomeres (green), rye centromeres (red c), and telomere clusters (red). Asterisks and arrowheads indicate the position of the subteleric chromomeres of the short and long arm, respectively. The two 6RL ends and one 6RS end are in the telomere cluster. Scale bars = 10 μ m.

spindle-pole body in fission yeast. However, in organisms such as plants, without a defined microtubule-organizing center, it is difficult to envisage how the movement of telomeres is oriented to converge in a region opposite the centromere pole. One can argue that the Rab1 disposition facilitate the polarized movement. However, the convergent cytoskeleton forces operate throughout the complete nuclear surface, since they are capable of clustering telomeres of telocentrics located in the centromere pole and those of 2-armed chromosomes located in the opposite hemisphere [Maestra et al., 2002]. The presence of subteleric heterochromatin chromomeres in both arms of chromosomes 1R and 6R revealed the position of their 2 chromosome ends in the bouquet. The end of the long arm of both chromosomes was, with some exceptions, always included in the telomere cluster, while the end of the short arms failed to migrate to the telomere pole [Naranjo, 2014]. This behavior suggests that the longer arms of the chromosome complement condition the location of the telomere cluster site and, therefore, the oriented movement of telomeres. The arrangement of submetacentric chromosomes probably conditions a more

distant positioning of their short arm telomere relative to the telomere cluster site (fig. 4) and the delay or failure in their migration to the telomere pole.

Chromosome Structure and Chiasma Distribution

Chiasmata are visible at diplotene once the SC is disassembled and homologues start to be separated. For many decades of the 20th century, it was assumed that the location of genetic COs in eukaryotes is not established until the chromosomes are completely synapsed. Maguire [1972] studied a heterozygote for a paracentric inversion in maize. As based on the synapsis and CO produced into the inversion region, she postulated that CO location establishment precedes, accompanies or closely follows initial formation of SC stretches. A further study of meiotic events in synchronous cultures in yeast demonstrated that DSBs, which can be repaired by the CO or NCO pathways, occur before SC formation [Padmore et al., 1991].

In some plant species, chiasma localization is imposed by a restriction of synapsis to specific chromosome re-

Fig. 4. Arrangement of a metacentric chromosome and a submetacentric chromosome at the bouquet stage before chromosome elongation.

gions, as in the proximal chiasma localization of *Fritillaria* [Newton and Darlington, 1930; Darlington, 1935; Fogwill, 1958]. However, many species undergo complete chromosome synapsis and also show uneven chiasma distribution. Among *Allium* species, which have large chromosomes that synapse completely along their length, *Allium fistulosum* bears most chiasmata proximally located, while *A. cepa* exhibits distal to unrestricted chiasma localization [Albini and Jones, 1988]. Other plant species, including important crops such as maize, wheat, barley, or rye, show a pronounced distal chiasma localization [Lukaszewski and Curtis, 1993; Künzel et al., 2000; Akhunov et al., 2003a; Anderson et al., 2004; Lukaszewski, 2008; Higgins et al., 2012]. In plants and animals, most of DSBs are repaired by an NCO pathway, only a minor fraction (5%) is repaired as COs [Higgins et al., 2014]. This low CO ratio suggests strict controls that ensure the pronounced preferential chiasma localization observed in many plant species.

The pattern of chiasma distribution and, therefore, the pattern of COs have been of great interest for decades, but the control mechanisms responsible for the CO distribution are poorly understood. Two aspects of the CO control, the obligate CO and CO interference are

considered to play a relevant role in the decisions performing the fate, CO or NCO, of each DSB repair event [Jones and Franklin, 2006]. At least one CO is required to ensure the physical connection between 2 homologues until their segregation. This is termed the obligate CO, and its occurrence involves the probability of forming a single exchange between homologues. Positive CO interference is a mechanism that prevents, or reduces, the probability of additional COs in the vicinity of an already established CO. As a result of interference, COs are well spaced along the chromosomes, with often only a single CO per chromosome arm. Most likely, decisions concerning the site of the first CO are mediated by the restricted location of initiation of homologous interactions, which are confined to a distal region imposed by the formation of the meiotic bouquet [Scherthan, 2001; Harper et al., 2004].

The ability of a given chromosome region to form chiasmata has been studied using structural chromosome mutants in wheat, rye and *Arabidopsis*. The distal fourth part of chromosome arms 1BS and 2BL of wheat and 1RS and 2RL of rye was partitioned using wheat-rye translocations of variable size. The distal 12% of 1BS form one CO in 50% of the cells, while the distal 6.7% of 2RL and the distal 10.5% of 2BL account for 94 and 81% of chiasmata formed in these arms [Valenzuela et al., 2013]. Other approaches have used deletions to assess the ability of the deleted segment to form chiasmata. The introduction of large deletions in heterozygous condition in *Arabidopsis* causes no major effect on the total recombination frequency. The loss of COs at the sites of structural change is compensated by increases in recombination frequencies elsewhere on the same chromosome. Thus, changes in the physical structure of a given chromosome affect the positioning of COs within that chromosome [Ederveen et al., 2015]. Heterozygosity for the loss of long terminal segments of wheat chromosome arms 4AL, 2BL and 5BL show a considerable reduction of chiasma frequency in the truncated arm. However, the amount of chiasma associations at metaphase I is restored to a normal level in the deficient chromosome homozygotes [Curtis et al., 1991]. The construction of genetic maps demonstrated a considerable increase of the chiasma frequency in the middle region, but not in the proximity of the centromere, in truncated chromosome arms 1BL or 5BL relative to the intact chromosomes [Jones et al., 2002; Qi et al., 2002]. These results suggest that the recombinogenic capacity of a given segment is mainly determined by their position along the telomere-centromere axis. However, the infrequent occurrence of chias-

Deletion	2BS (%)	Chromosome 2B	2BL (%)
2BS-6	62		100
2BS-12	96		100
2BS-3	80		98
2BS-7	76		100
None	99		99
2BL-6	98		84
2BL-1	100		94
2BL-7	98		42

Fig. 5. C-banding idiogram of chromosome 2B and 7 of its deletions and frequency (%) of arm association at metaphase I.

mata in the completely synapsed proximal third of the 5RL chromosome arm of rye, both in normal homozygotes and homozygotes for truncated chromosomes lacking the distal 70%, contradicts this notion [Naranjo et al., 2010]. A similar conclusion is drawn from the strict proximal chiasma localization derived from the proximal repositioning of the recombining region caused by a paracentric inversion in the rye chromosome arm 1RL and in wheat chromosome arms 4AL and 2BS [Lukaszewski, 2008; Lukaszewski et al., 2012; Valenzuela et al., 2012]. Thus, the chiasma formation and distribution in the arms 5RL, 1RL, 4AL, and 2BS is not conditioned by the position, but depends on the DNA sequence, or the chromatin organization, present in each chromosome region.

Is It Possible to Change the Pattern of Chiasma Distribution?

In cereals such as wheat or barley, COs are mainly confined to gene-rich regions of chromosomes [Künzel and Waugh, 2002; Erayman et al., 2004; Mayer et al., 2011]. However, a substantial proportion (more than 30%) of the gene complement is outside of these regions. Elucidating the basis for chiasma localization, particularly in cereals, has an implication on the possibility of manipulating recombination in CO-poor regions, which is potentially important for breeding-related programs. With the aim of developing strategies that enable the manipulation of recombination in CO-poor regions, the site of the CO was forced to shift from distal to proximal re-

gions in chromosome 4A and the seven B-genome chromosomes of wheat by progressive shortening of their arms [Naranjo, 2015]. The chiasma frequency was estimated at metaphase I in different homozygotes for terminal deletions of variable size in each chromosome arm. The shortening of chromosome arms was accompanied by a gradual reduction of the chiasma frequency, which is consistent with the preferred distal localization of chiasmata in intact chromosomes. The normal chiasma frequency was not recuperated in most deletions. However, results support a considerable increase of chiasma frequency in intercalary regions (fig. 5). Truncated chromosome arms del2BS, del2BL and del6BS with breakpoints occupying subdistal positions show an increase of the chiasma frequency relative to the deleted arms with breakpoints more distally located. The *ph1b* intercalary deletion, which lacks *Ph1*, maps 0.9 cM from the centromere; that is, 0.018 COs are formed in this interval [Sears, 1984], while the truncated del5BL-5 arm, with a breakpoint more proximally located than *Ph1*, forms at least one chiasma in 62% of meiocytes. Most of the exchange events produced in the intact chromosome 3B occur in the distal 68 Mb of 3BS and the distal 59 Mb of 3BL [Choulet et al., 2014]. Such segments represent ~20% of the distal 3BS and ~14% of the distal 3BL, respectively. Therefore, they are smaller than the segments missing in deletions 3BS-7 and 3BL-11, respectively. Lines for these 2 deletions, especially 3BL-11, show very high recombination frequencies, suggesting that chromosome arm shortening increases the recombination in intercalary regions of chromosome 3B greatly.

Factors Affecting Chiasma Positioning

The decreasing recombination gradient along the wheat chromosome arms agrees, at least in part, with a model of chiasma distribution based on homologous recognition sites distally located and positive chiasma interference [Jones et al., 2002]. Once the first distal CO site is defined, interference reduces the chance of additional COs in each arm. The interference intensity is inversely related to the physical distance between adjacent CO sites [Jones et al., 2002; Saintenac et al., 2009]. In truncated chromosomes, a more proximal location of the pairing initiation site and absence of interference, since the distal recombinogenic region is lost, are likely conditioning the increase of the recombination rate in normal intercalary regions located distally in truncated chromosomes. Nevertheless, deletions lacking the terminal third of any chromosome arm do not recuperate the level of chiasma frequency reached by the intact chromosomes. On the other hand, truncated chromosome arms containing only the proximal 20% do not form chiasmata. This suggests that additional factors are involved in the control of chiasma distribution. The DNA sequence, or chromatin organization, present in each chromosome stretch also conditions the possibility of producing a first CO. Heterochromatin is considered to strongly inhibit CO formation [Gaut et al., 2007]. Such an effect would explain the lack of chiasmata in the pericentromeric region of wheat chromosome 4A and the 7 chromosomes of the B genome, which is especially rich in heterochromatin, as well as the null effect on chiasma frequency caused by the loss of the large subtelomeric heterochromatin C-bands of the chromosome arm 1BL [Naranjo, 2015]. The reduced recombination frequency observed in the distal region of the intact 3BS arm is also consistent with the high heterochromatin content present in this region [Saintenac et al., 2009]. On the other hand, the coincidence of the distal location of both chiasmata and euchromatin histone marks H3k9me3, H3K27me3, H3K4me3, and H4K16ac in barley chromosomes, during meiotic prophase I, supports that the euchromatin organization favors COs localization [Higgins et al., 2014]. The preferred distal location of euchromatin marks denotes uneven chromatin remodeling along the telomere-centromere axis. The high degree of packaging of heterochromatin might be a limiting factor in the accessibility to such chromatin regions of proteins either involved in the formation of DSBs or in the subsequent recombinational repairing process. It is also possible that the degree of compaction of chromatin affects the ability of the chromatin fiber to generate the chromosome movements required to

find the homologous partner and produce a CO. In fact, the highly recombinogenic distal region of the 1RL arm of rye find the homologous partner easier than the proximal CO-poor regions, even though their positions are inverted [Valenzuela et al., 2012]. This dissimilar behavior of CO-rich and CO-poor regions may be based on differences in the pattern of chromatin remodeling.

Advances in the knowledge of the cereals' genome organization achieved in the last decade have led to the proposal that the evolution of such genomes is related to the distribution and rate of recombination. Duplication-derived loci arisen in the evolution of wheat chromosomes accumulated mainly in distal high-recombination regions, whereas the ancestral copies remained proximally located [Akhunov et al., 2003a]. The decreasing gradient of the recombination along the telomere-centromere axis is assumed to promote a more rapid evolution rate in the distal highly recombinogenic region than in the proximal low-recombination regions of chromosomes. Synteny erosion produced in the differentiation of chromosomes of the 3 wheat genomes was caused by insertions and deletions of loci, which occur preferentially in high-recombination regions [Akhunov et al., 2003b]. A similar conclusion was obtained from comparative studies of wheat-rice or wheat-sorghum chromosome relationships; a number of chromosome rearrangements accumulated in the distal recombination-rich region of wheat chromosomes in the evolution [See et al., 2006; Luo et al., 2009]. The recombinogenic regions of the 2 arms of wheat chromosome 3B are enriched in gene categories related to adaptation such as resistance to pathogens, which is consistent with the assumption that meiotic recombination generates the variability in these important evolutionary chromosomal domains [Choulet et al., 2014].

In addition to the genotype, environmental factors such as temperature modulate the number and distribution of chiasmata [Stern, 1926]. COs in plants, fungi and most animals fall in 2 categories: type I COs, which are formed in the interference-sensitive pathway mediated by ZMM proteins [Shinohara et al., 2008] and type II COs, which are formed in the interference-insensitive pathway mediated by MUS81 [Berchowitz et al., 2007]. Higgins et al. [2012] reported changes in the number and distribution of chiasmata after the increase of temperature in barley. In this species, Phillips et al. [2015] have analyzed the distribution and number of type I COs using immunofluorescence localization of MLH1 foci in plants growing at control and elevated temperatures. They also quantify the total CO numbers using genetic mapping. The increase in temperature increases total COs without

modifying the frequency of type I COs, which shift towards more proximal placements. Thus, changes induced by temperature in barley are mainly conditioned by the increase in the number of type II COs. However, these changes occur only in male meiosis.

Pairing Correction

The searching chromosome movements across the surrounding space to find a partner generate interactions between homologues and also between nonhomologues as supported by nonhomologous synapsis produced in a number of meiotic mutants in different organisms [Leu et al., 1998; Pawlowski et al., 2004; Golubovskaya et al., 2011; Bolcun-Filas and Schimenti, 2012; Labrador et al., 2013]. This implies that the homology-identification mechanism should include some components with the function of destabilizing and dissociating nonhomologous collisions to reinitiate additional search attempts that culminate in homologous pairing. Little is known about the mode by which chromosomes detect and eliminate inappropriate interactions. Valenzuela et al. [2012] reported the timely concurrent disappearance of improper chromosome associations with the telomere cluster disorganization in heterozygotes for the nearly complete inversion of the rye chromosome arm 1RL. Improper and unstable associations involving terminal or proximal regions of the normal and inverted chromosomes disappear concomitant with telomere clustering dissolution. In contrast, interactions involving the distal and proximal homologous regions of the normal and inverted chromosomes undergo stable synapsis. This suggests that chromosome movements causing the dispersion of chromosome ends during bouquet dissolution are involved in the correction of pairing. Such movements may keep nonhomologous chromosomes apart, which are capable of initiating a new search to find the correct partner. The difference between stable and unstable associations, and, therefore, consolidation or correction of pairing, seems to depend on the ability of chromosome contacts to form a CO or not.

Dissolution of telomere clustering concerns most likely the correction of pairing and synapsis produced in allopolyploid wheats as well as in autotriploids and autotetraploids of the silkworm *Bombyx mori*. Chromosome interactions produced at early zygotene in allopolyploid wheats lead to pairing between both homologous and homoeologous chromosomes, which yields a synaptic pattern characterized by the presence of some multivalent

SCs formed by homologues and homoeologues, in addition to homologous bivalents [Hobolth, 1981; Holm, 1986; Martínez et al., 2001]. SC multivalents are reduced to homologous bivalents at late zygotene and pachytene. This pairing-correction mechanism is under the control of the homoeologous pairing suppressor *Ph1*. In the presence of *Ph1*, chiasmata are formed only between homologous chromosomes, but when *Ph1* is absent, chiasmata can also be formed between homoeologues, multivalents persisting until metaphase I [Holm and Wang, 1988; Luo et al., 1996]. The females of *B. mori* undergo achiasmatic meiosis, while males form chiasmata and display the standard meiotic pathway. In the triploid females, homologous chromosomes form trivalents in zygotene which are converted in bivalents plus univalents by the end of pachytene. A similar synaptic correction occurs in the tetraploid females; each quadrivalent formed at zygotene is transformed in 2 bivalents at pachytene. However, in autotriploid and autotetraploid males, multivalents are preserved until metaphase I [Rasmussen, 1977; Rasmussen and Holm, 1979; von Wettstein, 1984]. Thus, chiasmata also condition the dynamics of pairing and synapsis in polyploid wheats and silkworm.

The molecular basis of the bouquet disorganization is poorly understood. In budding yeast meiosis, the absence of the Rec8 cohesin mediates the association of the telomere cluster and the spindle-pole body and the exit from the bouquet stage [Trelles-Sticken et al., 2005]. Rapid telomere-led chromosome movements, which are considered to foster homologous pairing, have been proposed also as a stringency factor for eliminating unwanted connections [Koszul and Kleckner, 2009].

Chromosome Segregation

The first meiotic division is essentially different from a mitotic division. Instead of achieving sister chromatids disjunction, homologous chromosomes orientate and segregate to opposite poles. A special segregation machinery ensures the maintenance of centromeric cohesion between the 2 sister chromatids of a chromosome and the monopolar attachment of their kinetochores during anaphase I. Sister chromatid cohesion is completely lost only in anaphase II, once the sister kinetochores are attached to the opposite spindle microtubules. Entry and progression through meiosis are controlled by many of the same regulators as in mitosis, i.e. CDK-cyclin complexes and the anaphase-promoting complex/cyclosome. In yeast and animals, several meiotic checkpoints have been identified

that roughly correspond to mitotic checkpoints. Among them is the spindle checkpoint that detects unattached chromosomes in order to prevent aneuploidy. However, the metaphase I to anaphase I checkpoint does not appear to be very potent in plants [Wijnker and Schnittger, 2013].

Chromosome segregation has been largely studied in translocation heterozygotes. Complete homologous synapsis in a diploid heterozygote for a reciprocal translocation yields the formation of one quadrivalent SC and $n-2$ bivalents SC. The multivalent persists during metaphase I because of chiasmata formed both in interchanged and noninterchanged homologous segments of the chromosome involved in the translocation. In the absence of interstitial chiasmata within the quadrivalent, the balanced delivering of the chromosomes in anaphase I would only be accomplished by the occurrence of an alternate segregation (the 2 normal chromosomes to one pole and the derivatives to the other). Any other segregation mode, adjacent-1 (homologous centromeres oriented to opposite poles), adjacent-2 (homologous centromeres oriented to the same pole), 3:1 and 4:0 would lead to the production of unbalanced gametes. Reciprocal translocations are one of the most common structural chromosome rearrangements in humans, with an incidence of ~0.14% in newborn [Nielsen and Wohler, 1991]. Understanding the behavior of meiotic multivalent is important for the estimation on the reproductive risk of translocation carriers.

In human translocation heterozygotes, multivalent behavior has been mainly inferred from sperm segregation studies [Anton et al., 2008] or preimplantation chromosome screening in embryos from translocation carriers [Ko et al., 2010]. However, in other species, it is possible to monitor the dynamics of chromosomes involved in the translocation throughout meiosis. A number of translocations have been studied in heterozygosis in animal species such as cattle, sheep, pig, mouse, grasshoppers, cockroach, *Drosophila*, mosquito, Tipula, onion fly, termite, and in plant species such as maize, cotton, barley, rye, rice, pearl millet, and species of *Allium*, *Lilium*, *Oenothera*, *Rhoeo*, *Chrysanthemum* or *Tradescantia* [reviewed by Rickards, 1983]. The alternate orientation frequency varies between translocations. There are interchanges with 90% of alternate configurations in ring quadrivalents and others showing almost total adjacent orientation; chain quadrivalents produce more alternate orientation than ring quadrivalents in some translocations, but in others the frequency is the same [Rickards, 1983, and references therein]. Random centromere orientation takes place, however, when chromosomes involved in the translocation form 2 bivalents instead of one quadrivalent [Naranjo and Lacadena, 1979].

3:1 segregation, which generates aneuploidy, is relatively frequent in humans; average numbers of 6.8, 25, and 43% have been reported in studies concerning samples with different interchanges [Anton et al., 2008; Ko et al., 2010; Ye et al., 2012]. The frequency of 3:1 segregation increases when acrocentric chromosomes are involved in translocations [Ye et al., 2012]. In contrast, chiasmata formed in interstitial segments of cotton translocation heterozygotes reduce the frequency of 3:1 segregation [Ray, 1984]. 4:0 segregation is much less frequent than 3:1. Environmental changes, genotype, sex, chromosome size, quadrivalent symmetry, and chiasma number and position are factors that have been reported to affect multivalent orientation [Rickards, 1983; Ray, 1984; Ko et al., 2010; Ye et al., 2012]. Multiple combinations are possible with such a number of factors, which make it very difficult to predict the meiotic behavior of any multivalent. Rickards [1983] proposes that the orientation of centromeres in metaphase I and anaphase I is predetermined by the 3-dimensional conformation of quadrivalent during prophase I, which, of course, should be conditioned by the factors mentioned above.

Perspectives

Isolation of mutants for meiotic genes and RNAi-induced gene silencing are contributing to reveal many steps of the molecular mechanisms involved in the meiotic cell division. Advances are mainly based on studies carried out in model organisms, such as budding yeast, fission yeast, *C. elegans*, *Drosophila*, mouse, or *Arabidopsis*. Most of these species have very small genomes; early cytological studies on meiosis were carried out mainly in large genome species of plants and animals. Investigation on meiosis features inferred from cytological observations, such as restricted synapsis, chiasma number and distribution, suppression of homoeologous pairing in allopolyploids or chromosome segregation in multivalent, need additional approaches that confront the behavior of different chromosomal subregions. Structural chromosome rearrangements provide valuable information on different aspects of meiosis, and their use should be further promoted in the future. The possibility of manipulating steps of meiosis such as homologous recombination has implication on crop breeding programs, since recombination affects almost exclusively to the distal region in many species. Chromosome rearrangement could contribute to modify the pattern of chiasma distribution. New genomic tools can facilitate the isolation and identification of appropriate structural chromosome changes. Efforts should be also

devoted to the study of multivalent behavior in translocation heterozygotes in plant species, where their induction and isolation is relatively easy. Such studies have implications not only on medical genetics, but also on other disciplines such as biological control of insect pests, veterinary science, plant breeding or cell biology.

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