Chromosome Choreography: The Meiotic Ballet

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Meiosis creates haploid daughters from a diploid parental cell in a manner that ensures each daughter cell a complete haploid genome. This is accomplished by first pairing homologous chromosomes to identify partners and then segregating these partners away from each other at the first meiotic division (meiosis I) in which homologs pair with each other, recombine, and then segregate from each other. The processes of chromosome alignment and pairing allow for homolog recognition. Reciprocal meiotic recombination ensures meiotic chromosome segregation by converting sister chromatid cohesion into mechanisms that hold homologous chromosomes together. Finally, the ability of sister kinetochores to orient to a single pole at metaphase I allows the separation of homologs to two different daughter cells. Failures to properly accomplish this elegant chromosome dance result in aneuploidy, a major cause of miscarriage and birth defects in human beings.

The separation of homologous chromosomes during meiosis in eukaryotes is the physical basis of Mendelian inheritance. The core of the meiotic process is a specialized nuclear division (meiosis I) in which homologs pair with each other, recombine, and then segregate from each other. The processes of chromosome alignment and pairing allow for homolog recognition. Reciprocal meiotic recombination ensures meiotic chromosome segregation by converting sister chromatid cohesion into mechanisms that hold homologous chromosomes together. Finally, the ability of sister kinetochores to orient to a single pole at metaphase I allows the separation of homologs to two different daughter cells. Failures to properly accomplish this elegant chromosome dance result in aneuploidy, a major cause of miscarriage and birth defects in human beings.

Meiosis creates haploid daughters from a diploid parental cell in a manner that ensures each daughter cell a complete haploid genome. This is accomplished by first pairing homologous chromosomes to identify partners and then segregating these partners away from each other at the first meiotic division (meiosis I). Meiosis I is followed by a second mitosis-like division in which sister chromatids separate from each other. Errors in meiosis occur in as many as one in four human oocytes, resulting in the production of aneuploid zygotes (i.e., zygotes with an incorrect chromosome number), and the frequency of errors in maternal meiosis I increases with maternal age (1). The consequences of such errors can be devastating: Aneuploidy is a factor in miscarriage and birth defects in human beings.

The degree to which DSB-dependent and DSB-independent pairing mechanisms are used in meiosis appears to vary among organisms. For example, along with DSB-based pairing, S. cerevisiae also uses at least some types of homolog-pairing interactions that occur in the absence of both DSBs and the SC, although additional mechanisms may be required to stabilize those interactions (2, 3). Indeed, substantial homolog pairing is detected in yeast expressing a catalytically inactive version of Spo11p, the protein responsible for generating meiotic DSBs (4). The finding that deletion of this protein abolishes pairing altogether suggests a structural role for Spo11p in chromosome pairing beyond that of initiating recombination by creating DSBs (5). Similarly, in the basidiomycete Coprinus cinereus, a substantial amount of homolog pairing occurs in the absence of meiotic DSBs (6), despite the fact that DSBs are essential for proper synopsis. At the end of the spectrum, homolog pairing occurs normally in the complete absence of DSBs in Caenorhabditis elegans and in both sexes of Drosophila (7–10).
Are there specific pairing elements or sites? A number of sites or regions have been identified that appear to facilitate pairing. The one commonality of these regions is that they all map near to or are comprised by repetitive sequences. The best characterized of these pairing sites is a 240-base-pair repeat sequence in the intergenic spacer found between ribosomal RNA genes clustered on the *Drosophila* X and Y chromosomes. When present in multiple copies, this sequence facilitates the pairing and subsequent segregation of the X and Y chromosomes during meiosis in *Drosophila* males (11). One can imagine that this represents a case where the aggregation of proteins that bind to specific sites (in this case, nucleolar proteins) serves both to pair chromosomes and to maintain the connection between them. Similarly, in *C. elegans*, genetic studies identified a region at one end of each chromosome, known as the homolog recognition region (HRR), which has the properties that might be expected of a pairing site (12). These sites are able to stabilize pairing in their vicinity even in the presence of mutants that block the formation of the SC (9). A number of repetitive DNA elements that are located within the genetically mapped HRRs were recently identified, but it is not yet known whether the repeats are required for HRR function (13). Several internal chromosomal sites that may play a role in facilitating pairing during *Drosophila* female meiosis have also been defined and mapped to regions of intercalary heterochromatin (14).

The nearly universal observation of a moderate to tight clustering of telomeres at the nuclear envelope during the leptotene-to-zygotene transition, a configuration known as the “bouquet,” has driven speculation that the processes involved in forming the telomere bouquet may be involved in pairing (15). Pairing and synapsis appear to coincide with bouquet formation in some systems (16), whereas in others pairing interactions occur well before the bouquet arises and in these cases the bouquet may serve as a gateway to synapsis (15). Indeed, in both *S. cerevisiae* and *S. pombe*, mutants have been identified that disrupt telomere clustering and/or separation and various aspects of pairing, synapsis, or recombination (17, 18). Perhaps the ability of telomeric regions to function as pairing elements may also reflect the aggregation of proteins that bind specifically to these sequences.

**Pas de Quatre: Synaptomere Formation**

In most organisms, pairing events appear to be stabilized by a tight axial association called synapsis, in which the four chromatids synapsis requires DSB formation in most organisms. In a large number of organisms, the initiation of synapsis requires the creation of DSBs by Spo11p (3, 5). DSBs are essential for synapsis in *S. cerevisiae*, and similar observations have been made in *Arabidopsis* and in mammalian spermatocytes (5). A specific requirement for DSB formation to execute synapsis is suggested by the observation in *Coprinus* and in mice that the synapsis defect observed in the absence of a functional Spo11 protein can be rescued by experimentally induced DSBs. Induction of DSBs by gamma irradiation rescues the synapsis defect in the *Coprinus* spo11 mutant (6), and cisplatin-induced DSBs cause a partial restoration of synapsis in *spo11Δ* mutant male mice (19). SC forms to a varying degree in yeast mutants defective for the processing of meiotic DSBs, indicating that further steps in recombination may also be required for correct partner choice during synapsis or timing of SC formation (3, 20).

The requirement for DSB formation in synapsis is not universal. In *Drosophila* females and *C. elegans*, synapsis occurs normally even in the absence of DSB formation (7, 8). *C. elegans* chromosomes enter meiosis unpaired and then undergo a rapid alignment. This alignment requires neither the initiation of recombination nor the function of proteins that will later facilitate synapsis (9). Similarly, in *Drosophila* females the existence of prior somatic pairing associations may well circumvent the need for DSB-dependent homology searches.

It seems likely that the lack of a requirement for the creation of recombination intermediates for synapsis in these organisms may reflect the ability of flies and worms to use other, DSB-independent means to mediate homolog recognition. However, despite using a different mechanism to achieve synapsis, the assembly and structure of the SC in these organisms are indistinguishable from what is observed in DSB-dependent synapsis.

The LEs are derived primarily from the cohesin complex. The cohesin complex,
which mediates sister chromatid cohesion, plays a major role in the assembly of the LEs (21, 22). In yeast, the cohesin proteins Smc3p and Rec8p are associated with SCs during pachytene, and formation of AE fragments or the SC is abolished in mutants that fail to produce these proteins (21). As meiotic prophase progresses in mammalian spermatocytes, REC8 is initially present on short axial structures in the absence of other cohesin components (22). Other cohesin complex proteins, SMC1/9, and SMC3, appear to associate with these REC8-containing AE fragments simultaneously during leptotene (22–24). Presumably, the remaining cohesin component, STAG3, also associates with the complex at this time, because this protein also localizes to AEs during prophase I (25, 26). Cohesin remains associated with these AE fibers as they coalesce to run along the entire length of the chromosomes at pachytene, suggesting that the cohesins form part of the LEs of the SC.

**Formation of the transverse filaments.** Proteins that form the transverse filaments, which stretch between LEs, have now been identified in several species. These include Zip1p in *S. cerevisiae* (27), SCP1 in mammalian species (28), C(3)G in *Drosophila melanogaster* (29), and SYP-1 in *C. elegans* (9). These proteins play similar roles in the construction of the SC, and, although their primary amino acid sequences differ greatly, certain structural characteristics are shared among these proteins (Fig. 2).

The most notable common characteristic of these proteins is the presence of an extended coiled-coil rich segment located in the center of the protein, flanked by largely globular domains (9, 27–29).

Immunolocalization of SCP1 and Zip1p by electron microscopy has elucidated the organization of these proteins within the SC (30–33). These results support a model for transverse filaments in which the proteins form parallel dimers through the coiled-coil regions and then align between the chromosomes, with the C termini along the lateral elements. The N termini from opposing dimers interact in an antiparallel fashion down the center of the SC (Fig. 2) (31–34).

The roles played by the transverse filament proteins remain unclear. One obvious possibility is that they simply connect the LEs. However, several lines of evidence suggest an additional role of this structure in mediating the recombination process (see below).

**Formation of the central element.** Electron micrographs from numerous species suggest the presence of a structural element running down the center of the SC. To date, no proteins have been identified that localize exclusively to the central element. The electron micrograph–defined central element may be a distinct structure composed of proteins yet to be identified, or it could be a region of increased electron density resulting from the overlapping of transverse filament proteins in the center of the SC (Fig. 2).

**Possible functions for SC.** In at least some organisms, the SC serves to hold homologs together during the processes of chromosome compaction and condensation that occur during the transition from leptotene to zygotene (15, 20). In addition, elaborate SC-like structures have been implicated in preserving attachments between homologs that do not undergo exchange, such as in *Bombyx mori* oocytes (35) and the sex chromosomes of *Thyamys elegans* (36). We can imagine that modifications of the SC play an important role in other chromosome interactions as well. For example, in *Drosophila* oocytes the SC breaks down at the end of meiotic prophase and the euchromatin desynapses. Nonetheless, heterochromatic regions remain tightly associated with metaphase and undergo the process of achiasmate segregation (37–39). It might not be unreasonable to imagine that this maintenance of pairing reflects some modification of SC or SC remnants to ensure heterochromatic cohesion.

Second, components of the transverse filaments and/or central element may well play an important role in promoting interhomolog exchanges. For example, mutants in the *zip1* gene in *S. cerevisiae* reduce the frequency of recombination by 50 to 70% (27). Similarly, mutants in transverse filament protein–encoding genes c(3)G in *Drosophila* and *syp-1* in *C. elegans* abolish exchange altogether (9, 29). Indeed, a recent study supports the view that DSB formation is reduced by at least fourfold in the absence of the C(3)G protein (40).

Several lines of evidence suggest that transverse filament proteins may be capable of interacting with chromatin and influence recombination in an LE-independent manner (23, 41, 42). A second *Drosophila* SC component, C(2)M, may define a component of the SC required to direct the recombination process into an SC-dependent pathway (43).

One mechanism by which the SC may mediate interhomolog exchange is through its association with structures referred to as recombination nodules, or RNs. In organisms that possess SCs, sites of recombination are marked along the meiotic chromosomes during pachytene by RNs sitting on top of the SC (Fig. 3) (44, 45). RNs exist in two forms: early and late. Considerable evidence supports the view that early nodules may mark sites of nonreciprocal meiotic exchange (gene conversion), whereas late RNs mark sites of flanking marker exchange. Indeed, the number and distribution of late RNs parallels that of reciprocal exchange events [for review, see (45)]. A detailed study of protein localization within RNs has demonstrated that these structures contain recombination proteins and has provided support for the existence of two distinct types of nodules associated with crossover and noncrossover products (46). Finally, it is also possible that the SC may serve to mediate the processes by which exchanges interact to control their own distribution, called genetic interference. Interference is reduced in a c(3)G hypomorph and reduced or eliminated in *zip1* mutants, consistent with such a possibility (29, 34, 47).

*Miosis is still possible even in the absence of synopsis.* Even though synopsis is
observed in most meiotic systems, it is not seen in others (e.g., *S. pombe* and *Aspergillus*). Thus, synopsis can be viewed as only one possible outcome of the pairing process. In *S. pombe*, chromosome pairing proceeds in cells undergoing meiosis, but a canonical SC does not assemble. Instead, discontinuous structures called linear elements form along paired homologs. The linear elements seem to play a role analogous to SC by maintaining pairing interactions and promoting interhomolog recombination (17, 18). *S. pombe* can nonetheless perform meiotic recombination and reductional segregation of homologous chromosomes in the absence of synopsis. In what is perhaps a similar fashion, mouse oocytes lacking the SC protein SCP3 are often able to complete oogenesis and support levels of meiotic recombination close to those of the wild type without the formation of a normal SC (42).

**Turnout: Chromosome Segregation**

At the end of meiotic prophase, chromosomes begin the process of aligning themselves on or, in the case of most female meiotic systems, constructing the spindle on which the first meiotic division will take place. There are three basic components to this process: (i) congression of chromosomes to the metaphase plate, (ii) alignment of all chromosomes on that plate, and (iii) the separation of homologous chromosomes to opposite poles at the first meiotic division.

Moving the chromosomes to the metaphase plate. The term “congression” refers to the processes that collect the bivalents to the metaphase plate such that the two kinetochores of each bivalent are attached to opposite poles of the bivalent (48). At the start of prometaphase, the two homologous centromeres are usually oriented in opposite directions. Thus, most bivalents immediately obtain a bipolar orientation that balances the bivalent on the metaphase plate by virtue of the fact that both kinetochores are being pulled toward opposite poles with an equal force (49). However, some bivalents fail to orient properly, either with both kinetochores attached to the same pole or with only one kinetochore attached to a pole. In this case, the kinetochores are able to go through successive cycles of microtubule release and reattachment until stable bipolar orientation is established. Errors in the progress of chromosome congression are known to be more common in older oocytes (50), and the first true mammalian aneuploidy-inducing compound to be discovered, bisphenol A, acts by impeding this process (51).

Many meiotic systems possess checkpoint or surveillance mechanisms to ensure that chromosomes are attached to the proper poles, as assessed by the presence of tension on the kinetochores. An improperly attached kinetochore, or pair of kinetochores, will not be under tension and thus trigger a meiotic arrest, or even apoptosis. Moreover, mutations in the spindle check-point genes lead to high frequencies of chromosome nondisjunction at anaphase I (52). There is a much stronger system for error detection in male meiosis that detects misaligned or unpaired chromosomes before the first meiotic division and, having done so, directs the cell toward death (53). Although oocytes appear to lack such a tension-sensitive checkpoint (54), at least some workers have proposed that age-dependent defects in the ability of oocytes to monitor at least some aspects of spindle assembly and/or chromosome position may well be a factor in the maternal effect on meiotic nondisjunction in humans (52).

Connecting two sister kinetochores to one spindle pole: monopolar attachment.

To guide homologous chromosomes to opposite poles during meiosis I, each pair of sister kinetochores must function as a single unit, establishing an attachment to the same pole. Recent work has identified a group of *S. cerevisiae* proteins, termed “monopolins,” that are required for monopolar attachment at meiosis I and has begun to elucidate how this process is regulated (55, 56). These proteins—Mam1p, Csm1p, and Lrs4—are required for the first meiotic division. In meiotic cells depleted for monopolins, homologs fail to segregate, despite normal recombination and regulation of sister chromatid cohesion, because sister kinetochores attain bipolar microtubule attachments that are resisted by Rec8p-mediated cohesion between sister chromatids. In the wild type, the monopolins are essential for ensuring that each pair of sister kinetochores establishes a monopolar spindle attachment, resulting in the reductional division of homologs at anaphase I (55, 56).

Sister chromatid cohesion ensures chiasma function. Chiasmata, the physical manifestation of reciprocal meiotic exchange, lock the homologs together (57). Once the two homologous centromeres are attached to opposite poles of the meiotic spindle, the chiasmata prevent premature progression of the centromeres to the poles by balancing the poleward forces localized mainly at the kinetochore (Fig. 3). Sister chromatid cohesion distal to crossovers maintains chiasmata at their initial positions until anaphase I (58). For example, mutants of the *desynaptic* gene in maize display both precocious sister chromatid...
separation, suggesting a defect in sister centromere cohesion, and the early separation of chiasmata bivalents (39). In *Drosophila*, chiasmata fail to conjoin homologous chromosomes in the presence of mutations at the *ord* locus, which disrupt sister chromatid cohesion during meiotic prophase (60). Similarly, in yeast the cleavage of Rec8p, a protein required for mitotic sister chromatid cohesion, is required for chiasma resolution, and homolog separation is blocked in the absence of this cleavage (61).

Chiasmata are not positioned randomly along chromosome arms, but rather tend to cluster in the middle of the arms. Exchanges too distal will not provide sufficient sister chromatid cohesion near the centromeres until anaphase II (62). The release of sister chromatid cohesion distal to chiasmata allows homologs to separate (22). In mitotic cells, separase is necessary for the proteolytic cleavage of cohesin around the centromere (and both centromeres and arms in yeast), allowing chromosome segregation at anaphase I (63, 64). In a similar manner, cleavage of cohesin by separase is also necessary for chromosome segregation during anaphase I (61, 65). Although arm cohesion must be maintained through prophase in order to maintain chiasmata, the release of cohesion is essential to allow the segregation of homologs to opposite poles at anaphase I. However, this creates a “Catch-22” situation: Dissolution of sister chromatid cohesion in the arms would release the chiasmata that hold the homologs together, yet the centromeres of sister chromatids have to remain together to ensure that the homologs segregate correctly and prevent sister chromatid missegregation.

Maintenance of cohesion at the centromeres and release of sister chromatid cohesion along the arms is achieved through the differential removal of cohesins from the arms and centromeres of the chromosomes. Immunolocalization of cohesin components during mammalian meiosis indicates that the cohesin complex is removed from chromosome arms at metaphase II but retained at the centromere until the second meiotic division (22, 24, 26). Maintenance of cohesion at the centromeres depends on the presence of the meiosis-specific Scc1p homolog Rec8p, which replaces Scc1p in the meiotic cohesin complex in most systems (21, 22, 66, 67). Scc1p is sufficient for sister chromatid cohesion in yeast meiosis in the absence of Rec8p but is not protected from cleavage at the first meiotic division (55). Yeast Spo13p is necessary and sufficient to protect centromeric Rec8p from separase-mediated cleavage but cannot protect other separase substrates (21, 68, 69).

Pulling the chromosomes apart. The final step in executing the first meiotic division is the actual movement of homologous chromosomes to opposite poles, both by mechanisms in which motor molecules at the kinetochore pull the chromosomes to the poles and by mechanisms that actually push the two poles apart from each other. Although mechanisms of spindle elongation and kinetochore-based movement lie outside the scope of this review, these processes have been thoroughly discussed in several recent reviews (70–72).

Summary

Although many aspects of the meiotic process are now much better understood than they were even a decade ago, many aspects of the process remain obscure. Perhaps the most notable of these is the mechanism(s) by which initial pairing is achieved. This process, which embodies the fundamental biological problem of telling “self from non-self,” remains enigmatic. We are similarly uncertain with respect to the mechanisms by which exchange distributions are established or how it is determined whether or not a given DSB will result in flanking marker exchange. Finally, despite its intriguing and conserved structure, the SC remains the “elephant in the meiotic living room.”

We know it is there, we know something of its dimensions, but its “reason for being” remains a matter of some conjecture. It seems likely, though, that better techniques and better mutants will soon shed light on even these most vexing questions.

References and Notes

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789

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