Female Meiosis: Synapsis, Recombination, and Segregation in *Drosophila melanogaster*

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ABSTRACT A century of genetic studies of the meiotic process in *Drosophila melanogaster* females has been greatly augmented by both modern molecular biology and major advances in cytology. These approaches, and the findings they have allowed, are the subject of this review. Specifically, these efforts have revealed that meiotic pairing in *Drosophila* females is not an extension of somatic pairing, but rather occurs by a poorly understood process during premeiotic mitoses. This process of meiotic pairing requires the function of several components of the synaptonemal complex (SC). When fully assembled, the SC also plays a critical role in maintaining homolog synapsis and in facilitating the maturation of double-strand breaks (DSBs) into mature crossover (CO) events. Considerable progress has been made in elucidating not only the structure, function, and assembly of the SC, but also the proteins that facilitate the formation and repair of DSBs into both COs and noncrossovers (NCOs). The events that control the decision to mature a DSB as either a CO or an NCO, as well as determining which of the two CO pathways (class I or class II) might be employed, are also being characterized by genetic and genomic approaches. These advances allow a reconsideration of meiotic phenomena such as interference and the centromere effect, which were previously described only by genetic studies. In delineating the mechanisms by which the oocyte controls the number and position of COs, it becomes possible to understand the role of CO position in ensuring the proper orientation of homologs on the first meiotic spindle. Studies of bivalent orientation have occurred in the context of numerous investigations into the assembly, structure, and function of the first meiotic spindle. Additionally, studies have examined the mechanisms ensuring the segregation of chromosomes that have failed to undergo crossing over.

KEYWORDS meiosis; synapsis; synaptonemal complex; cohesion; double-strand break; meiotic recombination; crossing over; spindle assembly; chromosome segregation; FlyBook

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This review is dedicated to the memory of professor Robert C. King, whose early studies on the development and morphology of *Drosophila* oocytes were critical to the development of the field.
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THE process of meiosis is the physical basis of Mendelian genetics. Indeed, it was the analysis of meiotic **nondisjunction** (missegregation) that allowed Calvin Bridges (Bridges 1916) to validate the chromosome theory of inheritance (Ganetzky and Hawley 2016). Since that time, studies of meiosis in *Drosophila melanogaster* females have been a leading force in the genetic, genomic, and cytological analysis of the meiotic process.

The first part of our review will focus on the basic genetic characterization of recombination and segregation in *Drosophila* oocytes. Specifically, we will cover:

1. The number and position of crossovers (COs).
2. Processes that control crossing over and CO placement.
3. The two classes of COs in *Drosophila*.
4. The relationship of CO number and position with segregation.

The remainder of our review will focus on several key meiotic events, namely:

1. The current understanding of meiotic chromosome pairing.
2. The structure and function of the synaptonemal complex (SC), its assembly and disassembly, and its role in CO maturation.
3. The mechanisms that control the number and position of COs.
4. The assembly and function of the meiosis I spindle in terms of the segregation of both exchange and nonexchange chromosomes.

Part I: Recombination as a Read-Out of Meiosis

Over the last century, most genetic studies have used recombination as the primary tool to assess the fidelity of the meiotic process. Such studies have either directly measured changes in recombination frequency or relied upon the fact that genome-wide reductions in exchange can increase the level of nondisjunction, providing an easy read-out for recombination failure. Studies of the number and position of recombination events are also important to the discussion of meiotic cell biology, which comprises the majority of this review. [We would be remiss if we did not note several reviews that have covered many of these subjects in great detail as well; these include: Baker and Hall (1976), Lindsley and Sandler (1977), Hawley *et al.* (1993), McKim *et al.* (2002), and Sekelsky (2017).]

The number and position of COs

The standard assay of CO frequency and position in *Drosophila* involves the use of visible and typically regularly spaced **markers** along chromosomes [for overviews see: Lindsley and Sandler (1977) and Carpenter (1987, 1988)]. Both recessive and dominant markers can be used, and the resolution of events is limited only by the number of available markers. However, the genetic intervals examined are typically relatively large in terms of megabases of DNA and the exact position of exchanges cannot be determined at the nucleotide level. Moreover, with the exception of one study (Parry 1973), assays have typically been done for only a single chromosome or chromosome arm at a time.

A complementary method of evaluating crossing over by whole-genome sequencing (WGS) has been recently developed (Comeron *et al.* 2012; Miller *et al.* 2012, 2016c; Singh *et al.* 2013). Assaying recombination by WGS uses very large numbers of single-nucleotide polymorphisms (SNPs) to determine the parent of origin for a chromosome (Miller *et al.* 2012). In the simplest assays, COs are determined by identifying the sites where the SNP profile changes from one parental chromosome to another in females heterozygous for many SNPs. WGS has its own limitations (*e.g.*, cost and adequate SNP density between parents) and this strategy can presently only be applied to a relatively small number of flies. While it initially focused on wild-type flies, WGS provides a method for better understanding recombination-defective mutants, and WGS studies of meiotic mutants have already been initiated.

Both types of assays yield the number of progeny bearing chromosomes with zero, one, two, or more CO events. However, as first noted by Weinstein (1918), a parental or "noncrossover" (NCO) chromosome can result not only from a **bivalent** in which no COs occurred, but also from a bivalent with one, two, or more CO events (Figure 1). This realization forces us to consider our data in terms of the fraction of nonexchange tetrads (E_0), single-exchange tetrads (E_1), double-exchange tetrads (E_2), and so on (E_n), that produced the observed set of progeny. Discussions of methods for converting progeny counts into tetrad distributions may be found in Merriam and Frost (1964), Zwick *et al.* (1999), Hawley and Walker (2003), and Ashburner *et al.* (2005). The most important of these values, the fraction of $\boldsymbol{E_0}$ **tetrads**, provides us with the probability that a given pair of homologs (for example the *X* chromosomes) failed to undergo crossing over.

Based on both classical and WGS studies, we can conclude that in *D. melanogaster* most chromosomes undergo an average of ~1.2 CO events per chromosome arm, such that most oocytes experience five to six CO events per meiosis (Lindsley and Sandler 1977; Carpenter 1988; Miller *et al.* 2016c). The frequency of bivalents without a CO event (E_0 bivalents) is much lower than expected. While exchange distributions vary by chromosome arm (Miller *et al.* 2016c), generally the frequencies of E_0, E_1, and E_2 tetrads are ~5–10, 60–70, and 30–35%, respectively. E_3 and E_4 bivalents are quite rare. The relative paucity of bivalents with more than two exchange events reflects the phenomenon of CO **interference**, which is quite strong in *Drosophila* (see *Interference*) (Sturtevant 1913, 1915; Muller 1916).

As shown in Figure 2, COs are not distributed randomly, but rather most exchange occurs in the medial and distal euchromatic regions of each chromosome arm. Exchange in the proximal euchromatic regions is strongly suppressed by

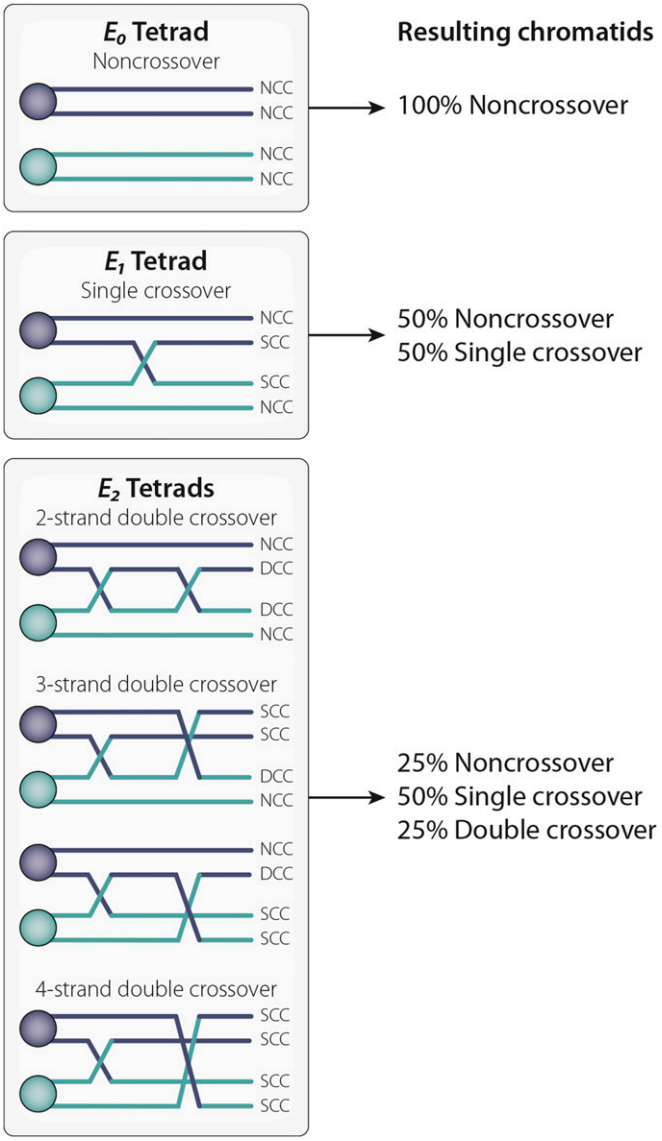


Figure 1 Tetrad analysis. Tetrad analysis allows us to determine the type and frequency of different kinds of exchange events. An E_0 tetrad did not undergo crossing over and thus results in four noncrossover chromatids (NCC). An E_1 tetrad experienced one crossover and results in two chromatids that each exhibit a single crossover (SCC) and two NCCs. An E_2 tetrad experienced two crossovers; E_2 tetrads result in some combination of NCCs, SCCs, or double-crossover chromatids (DCCs), depending on which chromatids were involved in the two crossover events. Although four chromatids are associated with an exchange (or nonexchange) event, only one chromatid will eventually make its way into the *Drosophila* oocyte nucleus; the remaining three are discarded. Because it is not currently possible in *Drosophila* to recover full tetrads, the frequency of E_0, E_1, E_2, ... E_n. tetrads must be calculated based on the number and type of exchange chromatids actually observed. This calculation is derived from classic Weinstein algebraic tetrad analysis [Weinstein 1918; Merriam and Frost 1964; adapted from Zwick *et al.* (1999)].

the **centromere effect**, a strong polar suppression of exchange emanating from the centromeres. Crossing over does not occur in the **heterochromatin** or on chromosome *4*. Extensive WGS- and population-based assays have failed to find evidence for **recombination hotspots** in flies (Chan *et al.* 2012; Comeron *et al.* 2012; Manzano-Winkler *et al.* 2013; Smukowski Heil *et al.* 2015; Miller *et al.* 2016c); however, additional studies may be required to fully address this absence of hotspots.

The centromere effect and the absence of crossing over in the pericentric heterochromatin

As noted above, recombination frequency is reduced in the proximal regions of each chromosome (Beadle 1932; Offermann and Muller 1932; Sturtevant and Beadle 1936; Hawley 1980; Miller *et al.* 2016c). This phenomenon is known as the centromere effect. It was initially unclear whether the centromere effect was caused by a polar suppression of exchange emanating from the centromeres or by the proximity of the proximal euchromatin to large blocks of **pericentromeric heterochromatin**. Yamamoto and Miklos (1977) examined this question by removing increasingly larger segments of proximal heterochromatin and measuring the effect that the amount of remaining heterochromatin had on the distribution of exchange. They found that such heterochromatic deletions strengthened the polar suppression of exchange, demonstrating that the observed reduction in exchange was due to the proximity of the centromere itself rather than simply the amount of pericentromeric heterochromatin. Alternatively, Sturtevant and Beadle (1936), used the recombinational properties of homozygous inversions to demonstrate that moving proximal regions of euchromatin to a more distal position relieved the exchange suppression generated by the centromere on these regions, while exchange was reduced when normally distal exchanges were moved to a more proximal position. Unfortunately, the mechanisms that mediate the centromere effect are still unclear.

The suppressive effect of the centromeres on crossing over is partially ameliorated by the presence of large blocks of pericentric heterochromatin that surround the centromeres. These blocks of heterochromatin are recombinationally inert. COs do not occur in the heterochromatic regions even when they are moved far away from the centromeres (Sturtevant and Beadle 1936; Szauter 1984). This is likely because **double-strand breaks** (DSBs), which are required for crossing over, do not typically form in heterochromatic regions. When examined in conjunction with a marker for heterochromatin, the DSB marker γH2AV (a modification to the histone H2A variant made in response to DSBs) failed to identify meiotically-induced DSBs within the labeled heterochromatic regions (Mehrotra and McKim 2006). However, DSBs could be identified in heterochromatin after the application of X-rays. Thus, the absence of heterochromatic γH2AV foci was due not to a failure to modify H2AV histones in response to DSBs in heterochromatin but, more likely, to the presence of mechanisms excluding programmed DSBs from highly repetitive regions (Mehrotra and McKim 2006).

Interference

CO placement is also affected by interference, the phenomenon that acts to ensure that when two COs occur on the same

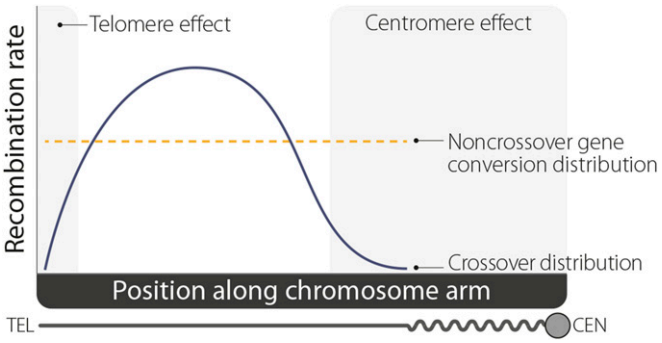


Figure 2 Recombination rates. The centromere effect strongly suppresses crossing over proximal to the centromere (CEN), while a much weaker telomere effect reduces crossovers at the distal tip of the chromosome (TEL). At the same time, interference reduces the likelihood of two crossovers occurring in close proximity. These forces result in the general crossover distribution (solid blue line) depicted here; this is fairly consistent among each arm of the *X*, second, and third chromosomes. Noncrossover gene conversions, on the other hand, are not subject to the centromere effect nor to interference, thus their distribution (dashed orange line) is more uniformly patterned.

pair of homologs they are widely spaced (Sturtevant 1913, 1915; Muller 1916; Berchowitz and Copenhaver 2010). Interference is a probabilistic rather than an absolute property, with the probability of a second DSB being resolved as a CO increasing the farther away one moves from an existing CO site. Indeed, although the average distance between double-CO events as assayed by WGS is 8–10 Mb, double COs can and do occur closer together, even as close as 1.5 Mb on occasion (Miller *et al.* 2016c). It appears that the strength of interference may vary among chromosome arms, with the recovery of fewer double COs on the arms of chromosome *2* compared to the *X* and third chromosomes (Miller *et al.* 2016c). How interference works remains one of the major unsolved problems of meiotic biology.

CO assurance: is there an obligate CO in Drosophila?

The fact that the observed number of E_0 tetrads in *Drosophila* females is less than expected based on the average number of CO events suggests the existence of a process known as **CO assurance**. In many organisms, CO assurance acts to ensure that at least one CO occurs per chromosome arm (Martini *et al.* 2006; Dumont 2017). Recently, Hatkevich *et al.* (2017) suggested that CO assurance does exist in *Drosophila* females, but that it is not as strong as in other organisms. However, the lack of COs on the fourth chromosome demonstrates that CO assurance is certainly not absolute. In addition, evidence against CO assurance in *Drosophila* has been presented by Mehrotra and McKim (2006). This question clearly remains open. As noted by J. J. Sekelsky, "*Drosophila* females do not have an obligate CO, but more of a highly recommended CO" (J. J. Sekelsky, personal communication). In *Drosophila* males, COs are completely absent during meiosis, demonstrating the presence of other mechanisms to segregate chromosomes lacking a CO.

Are there two pathways for crossing over in Drosophila?

In many organisms, COs can be produced by two separate pathways, **class I** and **class II** (Zalevsky *et al.* 1999; de los Santos *et al.* 2003; Hollingsworth and Brill 2004). These two CO pathways are defined both by the different enzymatic functions required to execute them and by how the CO events they produce respond to CO interference. Class I exchanges are highly sensitive to interference, while class II exchanges appear to be placed without regard to the proximity of other CO events. Recent work in *Drosophila* females indicates that there may be two classes of COs in this organism as well. While the vast majority of COs observed under normal conditions are class I, and thus are highly sensitive to interference, there may be a smaller set of class II COs that are not affected by interference (Miller *et al.* 2016c). Moreover, Sekelsky and his collaborators have provided strong evidence that the residual COs observed in at least some recombination-deficient mutants are class II COs (Kohl *et al.* 2012; Hatkevich *et al.* 2017). It is not yet clear if class II COs in *Drosophila* are processed by the same enzymatic pathway that produces class II COs in other organisms.

Gene conversion

DSBs created during meiosis can also be repaired as **gene conversions**, which involve the nonreciprocal transfer of genetic information from one homolog to the other. In other organisms, gene conversions can occur on their own or in association with COs, but it is currently unknown whether CO-associated gene conversion occurs in *Drosophila*. Gene conversions that are not associated with COs are also called **NCO events**. Such events are difficult to detect by standard marker-based recombination tests and therefore require large and complex assays to detect them. Early work on identifying NCO events in *Drosophila* involved assays in which only those progeny that had experienced an NCO within two deleterious alleles of the genes *maroon-like* or *rosy* could survive. This worked because an NCO event here would generate a wild-type copy of the gene, allowing the offspring to survive on selective media (Smith *et al.* 1970; Hilliker and Chovnick 1981; Hilliker *et al.* 1991). More recent work has used WGS to identify events genome-wide by finding polymorphisms that were copied from one homologous chromosome to another (Comeron *et al.* 2012; Miller *et al.* 2012, 2016c). Using both of these approaches, the frequency of NCO in *D. melanogaster* has been found to be $\sim 2.1 \times 10^{-8}$ per bp (Hilliker *et al.* 1994; Blanton *et al.* 2005; Miller *et al.* 2016c). WGS-based assays have also revealed that NCO events are evenly distributed along chromosome arms and are not sensitive to either interference or the centromere effect, nor do they generate interference (Figure 2) (Comeron *et al.* 2012; Miller *et al.* 2016c).

Exchange and the fourth chromosome

Under normal laboratory conditions, the fourth chromosome does not undergo crossing over. However, rare CO events may be recovered on the fourth chromosome by placing females at

an elevated temperature or by analyzing **triploids** [reviewed in Hartmann and Sekelsky (2017)]. The fourth chromosome is extremely small, consisting of a large block of centromere-proximal heterochromatin followed by ~1.5 Mb of euchromatin. Indeed, the centromere effect may largely explain the absence of recombination on the fourth chromosome (Hartmann and Sekelsky 2017).

It remains unclear whether NCOs occur on the fourth chromosome. However, in a study that looked at pooled NCO events after either a single or multiple rounds of meiosis, Comeron *et al.* (2012) reported several NCO events on the fourth chromosome. Curiously, these authors found both a higher frequency of gene conversion and a longer tract length on the fourth chromosome than was observed for NCO events on the other five chromosome arms.

Balancer chromosomes and the interchromosomal effect

Balancer chromosomes are multiply inverted and rearranged chromosomes that are used in *Drosophila* either to prevent exchange between a given chromosome and its balancer homolog or, if exchange does occur, to prevent the recovery of recombinant products (Sturtevant and Beadle 1936; Roberts 1976; Ashburner *et al.* 2005; Hawley and Ganetzky 2016; Miller *et al.* 2016a,b; Kaufman 2017). Balancers are available in *Drosophila* for the *X*, second, and third chromosomes. Each balancer is marked with a dominant visible mutation for easy identification and typically carries a recessive lethal or sterile mutation.

As demonstrated by both genetic and cytological studies, balancers are highly effective at preventing exchange between homologs. This is because, when heterozygous, the multiple inversion breakpoints that form the balancer function as powerful polar suppressors of exchange (Novitski and Braver 1954; Miller *et al.* 2016a). Exchange events do occasionally occur between a balancer and its normal-sequence homolog, but in these cases the recovery of recombinant products is prevented (Sturtevant and Beadle 1936). In the case of the *X* chromosome balancers, which are composed of overlapping **paracentric inversions** (inversions that do not involve the centromere), a single-exchange event within an inverted segment will result in an **acentric fragment** and a **dicentric bridge**, neither of which will segregate properly at the first meiotic division (Figure 3). Alternatively, many of the inversions that make up autosomal balancers do involve the centromere and are thus known as **pericentric inversions**. Single-exchange events within pericentric inversions will result in two chromosomes carrying large deletions and duplications that are lethal to the developing embryo (Figure 3).

Although heterozygosity for a balancer (or inversions) suppresses exchange between those homologs, it increases recombination on the remaining unbalanced chromosomes (Schultz and Redfield 1951; Lucchesi and Suzuki 1968; Ashburner *et al.* 2005). This phenomenon, known as the **interchromosomal effect**, can increase the level of exchange by as much as twofold in some intervals, and even in the context of total map length (Schultz and Redfield 1951; Ramel 1962; Ramel and Valentin 1966; Lucchesi and Suzuki 1968; Carpenter and Sandler 1974). The extent of the observed increase appears to intensify with the number of inversion breakpoints, perhaps resulting from a substantial weakening of both interference and the centromere effect.

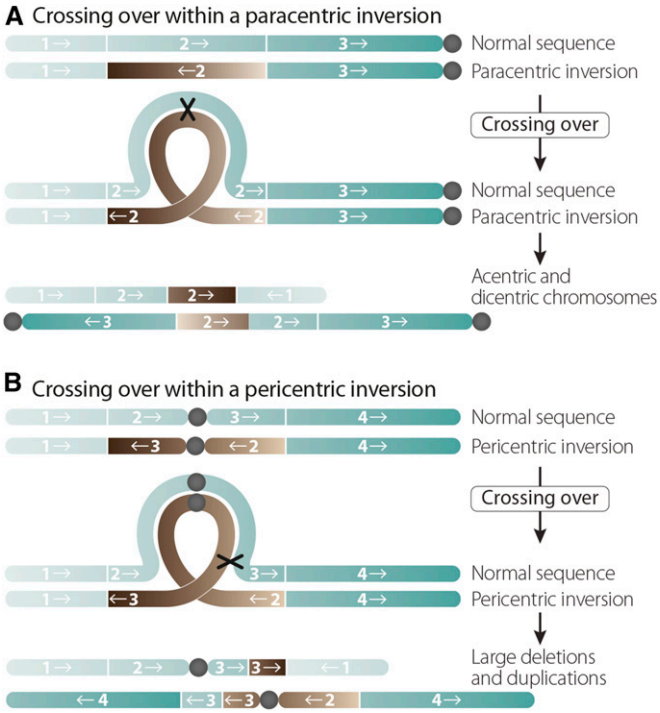


Figure 3 How balancers work. Balancers both suppress recombination and prevent the recovery of crossover events that occur within an inversion. (A) A single crossover within a paracentric inversion (one that does not include the centromere) will result in an acentric fragment, which the cell discards, and a dicentric chromosome, which cannot segregate properly. (B) A single crossover within a paracentric inversion (one that encompasses the centromere) will produce chromosomes with large deletions and duplications, which are inviable to the cell. Balancers that incorporate more inversions are thus more effective [adapted from Miller *et al.* (2016b)].

Exchange and segregation

As meiosis proceeds, COs are matured into **chiasmata**. The role of chiasmata in ensuring segregation is well documented (and equally well reviewed; see Nicklas (1974, 1977) and Hawley (1988)]. By physically linking two nonsister chromatids, one from each homolog, the bivalent uses sister chromatid cohesion both proximal and distal to the CO event to lock homologs together until anaphase I, at which point sister chromatid cohesion is released along the arms of the chromosomes. It is perhaps no surprise, then, that most of the *Drosophila* **meiotic mutants** known to induce the nondisjunction of exchange chromosomes either ablate sister chromatid cohesion or disrupt spindle structure and function [reviewed in Hawley *et al.* (1993)].

Exchange and nondisjunction in wild-type oocytes: Wild-type *Drosophila* females segregate their chromosomes with extremely high fidelity, but spontaneous chromosome

nondisjunction does occur at a low level. Spontaneous nondisjunction of the *X* chromosome is most commonly studied in *Drosophila* oocytes by assaying the appearance of exceptional progeny derived from females free of both structural aberrations or meiotic mutants. The observed exceptional progeny are referred to as **patroclinous males** (derived from oocytes lacking an *X* chromosome) and **matroclinous females** (derived from oocytes providing two *X* chromosomes). In some studies, patroclinous and matroclinous exceptions are also known as nullo-*X* and diplo-*X* exceptions, respectively. The frequency of spontaneous nondisjunction varies substantially among wild-type stocks in *Drosophila* (Zeng *et al.* 2010). However, most estimates are in the range of 0.001–0.0001 exceptional *X* chromosome progeny per generation. Provided that the centromeres are properly marked on the maternal *X* chromosomes (usually by appending a wild-type copy of the *yellow* gene to the right arm of one of the *X* chromosomes), it is possible to distinguish between those diplo-*X* exceptions resulting from nondisjunction at meiosis I (**reductional exceptions**) and those that appear to have occurred at meiosis II (**equational exceptions**).

The original analysis of spontaneous nondisjunction was performed by Bridges (1916); reviewed in Ganetzky and Hawley (2016). A subsequent study by Koehler *et al.* (1996) observed that > 76% of reductional exceptions involved nonexchange (E_0) *X* chromosome bivalents. Among the exceptions that were produced by exchange bivalents, all arose from E_1 tetrads and most were distal exchanges.* These findings are consistent with similar studies in yeast (Ross *et al.* 1996) and humans (Lamb *et al.* 1996), as well as with studies of nondisjunction in *Drosophila* meiotic mutants (Lindsley and Sandler 1977; Koehler *et al.* 1996).

Exchange and nondisjunction in meiotic mutants that suppress exchange: The rules that cover the disjunctional behavior of recombination-defective mutants have been covered in detail by Baker and Hall (1976). Briefly, the vast majority of nondisjunction in such mutants is due to nonhomologous segregation events involving two pairs of nonexchange major chromosomes. For example, *X* nondisjunction can result from both *X* chromosomes segregating away from a single second chromosome (with the remaining second chromosome segregating at random) in oocytes in which *X*, *2L*, and *2R* are all nonexchange. Because both arms of a major autosome must fail to CO for an E_0 *X* bivalent to nondisjoin, the frequency of *X* nondisjunction is proportional to E_0 cubed (Baker and Hall 1976).

For reasons that remain poorly understood, the frequency of nondisjunction for the obligately nonexchange fourth chromosome rises with the frequency of *X* chromosome nondisjunction in these mutants as well, usually to approximately one-third to one-half the frequency of *X* chromosome nondisjunction. This increase in the frequency of fourth chromosome nondisjunction cannot be accounted for by $XX \leftrightarrow 44$ nonhomologous segregations.

Summary

Studies of recombination have taught us a number of critical lessons about CO number and placement:

1. The average number of COs per chromosome arm is slightly greater than one, such that most oocytes experience 5–6 CO events per meiosis.
2. The frequency of chromosome arms without at least one CO is much lower than expected.
3. COs are not distributed randomly, but rather most exchange occurs in the medial and distal regions of each chromosome arm.
4. Crossing over does not occur in the heterochromatin or on chromosome *4*.
5. CO interference is strong.
6. NCOs are not sensitive to, nor do they generate, interference, leading to a random distribution along chromosomes.
7. COs are almost always sufficient to ensure segregation.

Part II: Overview of Drosophila Female Meiosis

Much of our ability to understand the cell biology of meiosis in *Drosophila* females reflects the major advances in cytology that have developed over the last 20–30 years. These studies have allowed us to reframe the formalisms created by genetic studies in terms of specific cellular events. To provide a context for those cytological studies, we begin our discussion with a summary of the cell biology of meiosis (Lake and Hawley 2012).

In females, the steps of meiosis can be visualized as a progression of stages in the several **ovarioles** that make up each ovary (Figure 4). Meiosis begins in the **germarium**, which is subdivided into regions based on developmental stage (Figure 5). At the tip of the germarium, in region 1, are two to three germline stem cells (GSCs) surrounded by support cells. After a GSC divides, the resulting cystoblast daughter cell and its progenitors undergo a total of four synchronized mitotic cell divisions with incomplete cytokinesis. These divisions generate a 16-cell interconnected cyst that will eventually yield the oocyte. For a review of germarium biology see Kirilly and Xie (2007), and for a review of oogenesis see McLaughlin and Bratu (2015).

Pairing

Surprisingly, the events that appear to facilitate the pairing of homologous chromosomes are initiated in region 1, while nuclei are still undergoing premeiotic mitotic divisions. Centromeric regions in *Drosophila* can be identified based on

* Curiously, a study published by Merriam and Frost (1964) attributed only 25% of reductional exceptions to the failure of exchange. Indeed, Merriam and Frost observed that nearly two-thirds of the exchange bivalents doomed to nondisjunction were E_2 tetrads (asterisks). Moreover, they did not observe a difference in the positioning of CO events between bivalents that segregated properly and those undergoing reductional nondisjunction. There does not appear to be a straightforward way to reconcile these findings with those of Koehler *et al.* (1996)].

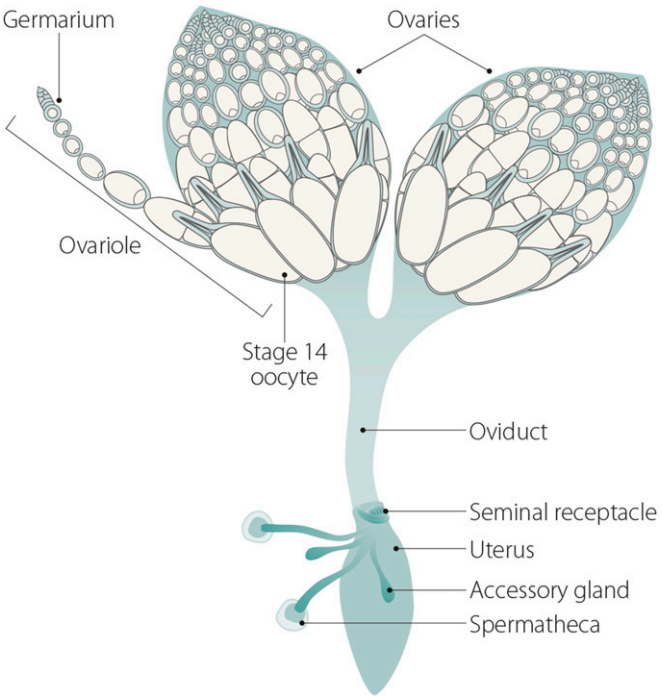


Figure 4 Ovaries. Each *Drosophila* ovary contains 12–16 ovarioles. Each ovariole in turn consists of a progression of distinct follicles, starting with the germarium at the anterior tip of the ovariole and progressing to a fully developed stage 14 oocyte at the posterior end.

antibodies to Centromere identifier (Cid), a centromere-specific H3 variant (Blower and Karpen 2001). The centromeres of homologous chromosomes first begin to pair in region 1 and are fully paired by the eight-cell cyst (Christophorou *et al.* 2013; Joyce *et al.* 2013). By the completion of the last mitotic divisions, the paired centromeres have clustered into an average of two groups. These clusters of paired centromeres persist throughout meiotic prophase (Takeo *et al.* 2011; Tanneti *et al.* 2011; Christophorou *et al.* 2013). **Centromere clustering**, as well as homologous **centromere pairing**, requires functional centromeres, as evidenced by the observation that decreased levels of the centromere assembly factors Centromeric protein-C (Cenp-C) and Chromosome alignment defect 1 (Cal1) lead to defects in meiotic centromere pairing and clustering (Unhavaithaya and Orr-Weaver 2013).

During the period of centromere clustering, the nucleus rotates and the centromeres undergo dynamic movements (Christophorou *et al.* 2015). The dynamic movements of centromeres, and centromere pairing and clustering, are dependent on microtubules as well as the minus-directed motor Dynein, centrosome components, the SUN (Sad1p, UNC-84)-domain protein Klaroid, and the KASH (Klarsicht, ANC-1, Syne homology)-domain protein Klarsicht. Perhaps more surprisingly, centromere clustering (but not homologous centromere pairing) requires components of the SC, the proteinaceous structure that holds homologous chromosomes together (Takeo *et al.* 2011; Tanneti *et al.* 2011; Christophorou *et al.* 2013).

The SC

The first visible cytological sign of meiosis is seen in germarium region 2A, visualized as the loading of the SC along the arms of the chromosomes in up to four nuclei within the 16-cell cyst (Figure 5). As the cyst progresses through the germarium, two of the four nuclei that initially formed euchromatic SC will disassemble their SC and back out of the meiotic program, followed by disassembly of the SC within an additional nucleus in region 2B. This results in a single pro-oocyte by region 3 that maintains full-length SC along the arms of the chromosomes. The remaining 15 cells assume a supporting role as **nurse cells**.

DSBs and recombination

Studies in multiple organisms have revealed that programmed DSBs are initiated during meiosis. In *Drosophila* oocytes, DSBs are first visualized in region 2A as the modification of the histone H2A variant γH2AV in response to DSB formation, shortly after the initiation of SC formation along the chromosome arms (Mehrotra and McKim 2006; Lake *et al.* 2013). A second cellular response to DSB formation is p53 activation, which, based on a p53 reporter construct, is activated in regions 2A and 2B and is dependent on DSB formation (Lu *et al.* 2010).

A subset of DSBs will result in the formation of around six COs, for an average of 1.2 COs per chromosome arm (Lindsley and Sandler 1977). CO formation is thought to occur at the sites of recombination nodules (RNs), electron-dense structures identified initially by Carpenter in electron microscopy (EM) studies (Carpenter 1975a,b). Those DSBs that do not become COs are repaired as NCO events (Figure 6).

DSBs are repaired progressively as the cyst moves through the germarium, and although few γH2AV foci are visible in the oocyte by region 3, it is unknown when the final stages of repair are complete (Mehrotra and McKim 2006). COs are matured into chiasmata that physically interlock homologous chromosomes together. These chiasmata are crucial for proper **biorientation** of homologs on the meiotic spindle, thus ensuring that they segregate away from each other at the first meiotic division.

If DSBs are not repaired when the cyst buds off from the germarium and enters the **vitellarium** at stage 2, as occurs in mutants such as *spindle-B* (*XRCC3* homolog), *spindle-D* (*Rad51C* homolog), and *okra* (*Rad54L* homolog), a checkpoint is initiated [reviewed in Ghabrial *et al.* (1998) and Sekelsky (2017)]. This checkpoint, which is dependent on Mei-41 (ATR homolog) and Loki (Chk2 homolog, also known as Mnk) (Abdu *et al.* 2002), initiates a cascade that leads to defects in chromosome condensation, abnormal egg chamber development, and ultimately sterility (Ghabrial *et al.* 1998).

Segregation

In terms of meiotic progression, only a few notable events have been documented during stages 2–12 of oocyte development, namely **karyosome** formation and SC disassembly. Around

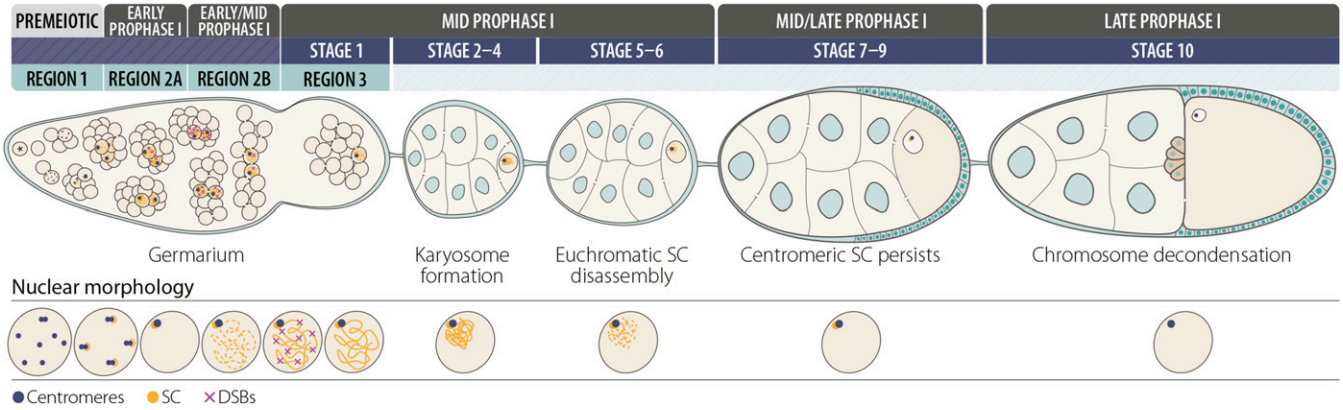


Figure 5 Oocyte development: stages 1–10. In the premeiotic region 1 of the germarium, a germline stem cell (asterisk) divides to produce a cystoblast, which then undergoes four incomplete mitotic divisions to produce a 16-cell interconnected cyst. Synaptonemal complex (SC) components (orange) begin loading next to unpaired centromeres (blue) in two-cell cysts, and centromeres begin pairing in four-cell cysts and clustering in eight-cell cysts. Prophase I of meiosis begins in 16-cell cysts in region 2A with the initiation of the euchromatic SC in up to four nuclei, followed by double-strand break (DSB) formation (purple). DSB formation and repair are dynamic processes that occur throughout early and early/midprophase. DSBs marked by γ-H2AV are mostly absent by midprophase in region 3 (stage 1), at which time one nucleus has been chosen as the oocyte nucleus. The other cells in the cyst serve as nurse cells. Around stages 2–3, chromosomes are reorganized and condense to form the karyosome. Euchromatic SC begins to disassemble in midprophase around stage 5 and will be completely absent by stages 7–9 during the transition from mid to late prophase (mid/late prophase). Centromeres remain clustered and centromeric SC persists through at least stage 9. In late prophase, chromosomes briefly decondense in stage 10 oocytes and transcription is upregulated before chromosomes recondense in stage 11.

stages 12–13, **germinal vesicle breakdown** (GVBD) and spindle assembly occur (Figure 7). After homologous chromosomes have bioriented on the meiosis I spindle, they are then segregated to opposite spindle poles at anaphase I. Sister chromatids are subsequently segregated at meiosis II to form four haploid products.

Part III: Chromosome Pairing and Synapsis

Pairing, SC assembly, DSB formation/resolution, and the segregation of homologous chromosomes at meiosis I will serve both as our central areas of concentration and as our landmarks in the discussion of meiotic biology that follow. This discussion will primarily be in the context of genes (and their protein products) that were identified in numerous screens for meiotic mutants (Box 1).

Homolog pairing

Genetic studies over the last century have attempted to provide insight into the pairing process by analyzing the effects of structural aberrations on meiotic recombination (Dobzhansky 1930, 1931; E. H. Grell 1964, R. F. Grell 1964, 1976; Roberts 1972; Hawley 1980; Craymer 1984). Unfortunately, in the absence of cytology it proved difficult—indeed, impossible—to make inferences about pairing based on a readout of CO frequency and distribution. The process of understanding meiotic pairing was further complicated (if not entirely stymied) by repeated assertions that meiotic pairing in *Drosophila* was simply an extension of the somatic pairing that is so ubiquitously observed in this organism [for review, see Joyce *et al.* (2013)]. However, this long-lived notion has recently been dispelled by advances in cytology that have allowed the direct observation of the process of meiotic pairing in the female germline (Box 2).

We now know that in GSCs, both euchromatic and heterochromatic regions of autosomes are unpaired, and thus meiotic pairing is not simply an extension of somatic pairing (Christophorou *et al.* 2013; Joyce *et al.* 2013; Rubin *et al.* 2016). Euchromatic regions begin to show low levels of pairing in cystoblasts, but heterochromatic regions remain mostly unpaired (Christophorou *et al.* 2013; Joyce *et al.* 2013). During the premeiotic mitotic divisions, both euchromatic and heterochromatic regions progressively pair to maximum levels by the 8–16-cell cyst stages, and this pairing is maintained until SC disassembly (Sherizen *et al.* 2005; Joyce *et al.* 2013). Homologous chromosome pairing thus initiates during the mitotic divisions that precede meiosis. At the end of **pachytene**, heterochromatic regions remain paired while euchromatic regions begin to unpair, with over half of oocytes showing two distinct foci for the euchromatic histone locus (Dernburg *et al.* 1996).

The timing of *X* chromosomal euchromatic pairing is similar to the pairing of autosomal euchromatic regions (Joyce *et al.* 2013). Euchromatic regions of the *X* chromosome remain highly paired in **zygotene** and in early pachytene, even in oocytes heterozygous for a highly rearranged balancer chromosome, which would require the pairing of homologous loci despite structural rearrangement of one of the two *X* chromosomes. (Gong *et al.* 2005; Sherizen *et al.* 2005). However, pairing of the heterochromatic 359-bp repeat region of the *X* chromosome is unusual. For reasons that remain unclear, the 359-bp repeat region on the *X* chromosome, which lies adjacent to the X chromosomal rDNA, is already paired in GSCs. Heterochromatic regions on the *X*

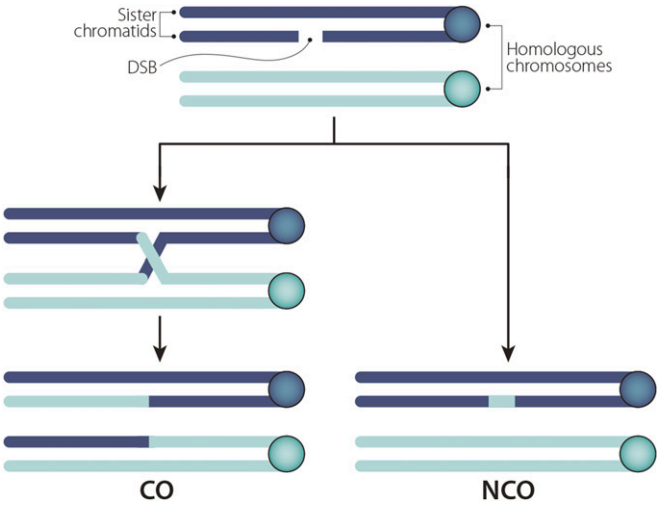


Figure 6 Homologous recombination. Repair of a double-strand break (DSB) by homologous recombination typically yields either a crossover (CO) or a noncrossover gene conversion (NCO). COs exhibit a Mendelian, or 2:2, segregation pattern of alleles, while NCOs exhibit a 1:3 segregation pattern of the alleles covered by the NCO. In flies, NCOs may be formed by synthesis-dependent strand annealing (SDSA) or by a modified version of SDSA in which both ends of the break engage with the homolog. Other, more complex outcomes of repair by gene conversion have also been observed in *Drosophila* (Crown *et al.* 2014).

chromosome remain highly paired throughout prophase, even in oocytes heterozygous for a multiply inverted balancer chromosome (Dernburg *et al.* 1996; Joyce *et al.* 2013).

The SC

The SC runs the full length of each set of paired homologous chromosomes. At least within the euchromatin, the tripartite structure of the SC can be compared to a railroad track or a zipper. The basic structure of the *Drosophila* SC was explored in detail by serial reconstruction EM performed by Carpenter and others (Carpenter 1975b; von Wettstein *et al.* 1984; Schmekel *et al.* 1993; Schmekel and Daneholt 1995). These studies have been reviewed previously (Carpenter 1988; Hawley *et al.* 1993; Ashburner *et al.* 2005).

Composition and structure of the SC: As shown in Figure 8A, the SC comprises three components:

1. Two **lateral elements** (LEs), derived from the axial cores of each of the two homologs, run along the chromosome arms.
2. The **central region** (CR) is composed of proteins extending between and connecting the LEs and, ultimately, homologous chromosomes.
3. The **central element** (CE) runs down the middle of the CR.

Although multiple LE proteins have been identified (Table 1), the internal architecture of the LE remains poorly defined in *Drosophila*, but has been explored in other organisms (Kohler *et al.* 2017). The LE links the chromosome cores to CR components of the SC and appears to be composed primarily of cohesin and cohesin-related complexes. Cohesin complexes are multiunit complexes that contain the core components Smc1 and Smc3 (Smc1/3) that hold sister chromatids together during critical stages of both mitosis and meiosis (Losada and Hirano 2005). In *Drosophila*, Smc1/3 are thought to form at least two different complexes in the female germline (Khetani and Bickel 2007; Gyuricza *et al.* 2016). These complexes are defined based on shared protein localization patterns and mutant phenotypes. The first of these complexes includes three proteins: Orientation disrupter (Ord), a protein required for proper cohesion but whose exact function in cohesion remains elusive (Khetani and Bickel 2007); the stromalin-related protein Sisters unbound (Sunn); and Sisters on the loose (Solo) (Yan and McKee 2013; Krishnan *et al.* 2014). These proteins localize to both the centromeres and the chromosome arms (Yan and McKee 2013; Krishnan *et al.* 2014). For simplicity, we will refer to this complex as the Ord complex.

The second proposed cohesin complex, referred to here as the C(2)M complex, consists of the cohesin component Stromalin (SA), the SCC2 homolog Nipped-B, and the kleisin-like protein C(2)M (Heidmann *et al.* 2004; Gyuricza *et al.* 2016). C(2)M, which has homology to kleisin-family proteins and has been to shown to interact with Smc3 (Manheim and McKim 2003; Heidmann *et al.* 2004), is loaded along the chromosome arms but is absent from the centromeres (Manheim and McKim 2003; Gyuricza *et al.* 2016). The mitotic α-kleisin Rad21 also interacts with C(2)M, and targeted cleavage of Rad21 in the ovary leads to an early disassembly of the SC (Urban *et al.* 2014).

The CR of the SC appears to be composed primarily of **transverse filament** proteins (TFs), which are characterized by their central coiled-coil domains flanked by globular domains at their N- and C-termini. In flies, the primary TF protein is encoded by the *c(3)G* gene. C(3)G forms a homodimer (Page and Hawley 2001) and, based on immuno-EM, the C-terminus of the dimer is located within the LE adjacent to C(2)M, while the N-terminus of the dimer overlaps with a second C(3)G dimer at the middle of the CR (Anderson *et al.* 2005) (Figure 8A). It is the C(3)G protein, then, that forms the zipper-like component of the SC to span the distance between LEs (Page and Hawley 2001). Two additional CR proteins have been identified: Corolla, which forms two tracks through the CR, and Corona (Cona), which also appears to reside as two tracks in or beside the CE (Page *et al.* 2008; Collins *et al.* 2014; Cahoon *et al.* 2017).

Recent work using more advanced microscopy techniques has provided insight into the three-dimensional architecture of the *Drosophila* SC as well. Based on this study, the SC forms as two mirrored layers, with C(2)M, Corolla, and Cona all lying slightly above/below each layer of C(3)G (Figure 8B) (Cahoon *et al.* 2017).

Assembly and function of the SC at the centromeres: The SC shows a highly regulated pattern of assembly. During the

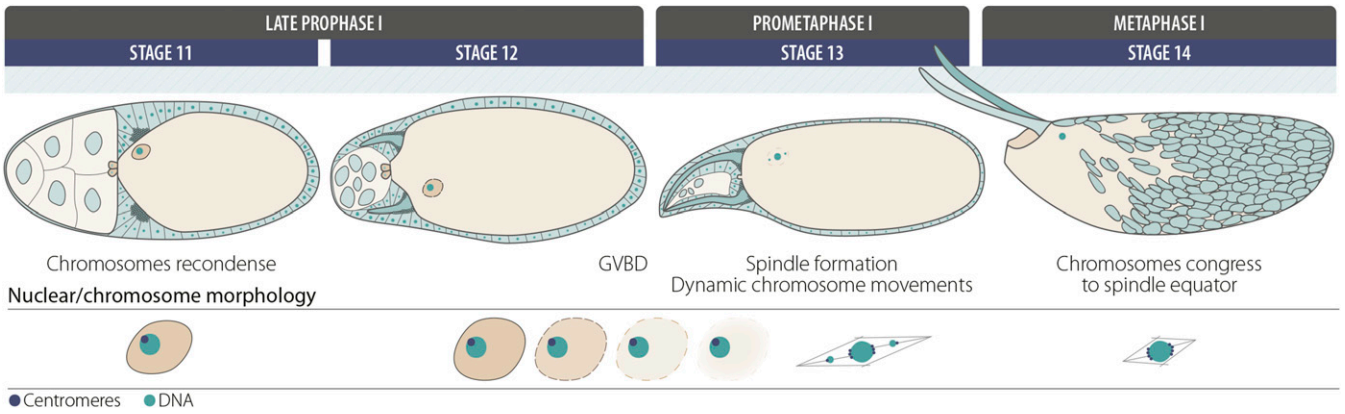


Figure 7 Oocyte development: stages 11–14. In stage 11, the karyosome recondenses and undergoes preparations during stage 12 for germinal vesicle breakdown (GVDB), which occurs at approximately the start of stage 13. After GVBD, tubulin is recruited to the chromosomes and then organized into a bipolar spindle. Achiasmate chromosomes undergo dynamic movements toward the spindle poles during prometaphase I, and by stage 14 the achiasmate chromosomes congress to join the chiasmate chromosomes. At this stage, homologous chromosomes have bioriented toward opposite spindles poles and formed a compact structure at the metaphase plate of the spindle. Oocytes will maintain this metaphase I arrest configuration until activation.

mitotic divisions to create the 16-cell cyst, the cohesin proteins Smc1/3 and the cohesin-related Ord complex proteins localize to the centromeres in all 16 nuclei of the cyst (Webber *et al.* 2004; Khetani and Bickel 2007; Yan and McKee 2013; Krishnan *et al.* 2014). The localization of Smc1/3 to the centromeres is dependent on Ord complex proteins (Khetani and Bickel 2007; Yan and McKee 2013; Krishnan *et al.* 2014), and the Ord complex proteins are mutually dependent on one another for loading to the centromeres (Webber *et al.* 2004; Khetani and Bickel 2007; Yan and McKee 2013; Krishnan *et al.* 2014; Gyuricza *et al.* 2016).

The CR proteins C(3)G and Corolla first load near the centromeres before the cyst has exited the mitotic divisions of region 1 (Christophorou *et al.* 2013). The loading of CR proteins to the centromeres has been shown to be dependent on Ord and Solo, and mutation of these cohesion proteins results in a failure to load centromeric SC (Yan and McKee 2013; Gyuricza *et al.* 2016). Loss of the CR proteins [either C(3)G or Cona] leads to defects in the pairing of pericentric heterochromatin during the mitotic divisions of the cyst (Christophorou *et al.* 2013). CR mutants also display greatly decreased levels of heterochromatic pairing in pachytene when compared to wild-type flies (Christophorou *et al.* 2013). The absence of any one of the three known CR proteins [Cona, Corolla, or C(3)G] also leads to a defect in centromere clustering (Takeo *et al.* 2011; Collins *et al.* 2014). This requirement for the CR proteins to cluster the centromeres is consistent with the role of the SC in mediating centromere clustering in several other organisms (Da Ines and White 2015; Kurdzo and Dawson 2015).

Loss of the Ord complex proteins also leads to defects in both centromere clustering and centromere pairing (Khetani and Bickel 2007; Takeo *et al.* 2011; Tanneti *et al.* 2011; Yan and McKee 2013; Krishnan *et al.* 2014; Gyuricza *et al.* 2016). These oocytes display up to eight centromeric foci in region 2A, suggesting that the centromeres of homologous chromosomes are unpaired, while the centromeres of sister chromatids remain associated (Takeo *et al.* 2011; Tanneti *et al.* 2011; Yan and McKee 2013; Krishnan *et al.* 2014). However, pro-oocytes with more than eight Cid foci can be observed at later stages (Takeo *et al.* 2011; Yan and McKee 2013; Krishnan *et al.* 2014; Gyuricza *et al.* 2016), indicating a progressive loss of sister chromatid cohesion at the centromeres. These observations demonstrate a critical role for the Ord complex in meiotic centromere clustering and sister chromatid cohesion.

Unlike the Ord Complex, C(2)M complex proteins are not essential for centromere clustering. Defects in centromere clustering are not seen in ovaries with decreased levels of the C(2)M complex proteins, consistent with C(2)M's failure to show localization to the centromeres (Takeo *et al.* 2011; Tanneti *et al.* 2011; Gyuricza *et al.* 2016). The loss of C(2)M, SA, or Nipped-B also has no effect on Smc1/3 retention at the centromeres (Gyuricza *et al.* 2016).

Assembly of the SC along the arms: After centromeres have clustered, the zipper-like SC will then assemble along the euchromatic arms in up to four nuclei of the 16-cell cyst in region 2A (Figure 5). As the cyst matures, this euchromatic SC then quickly disassembles in all but the pro-oocyte, leaving two nuclei with mostly full-length tracts of the SC at region 2B and only the single pro-oocyte with full-length SC at region 3. The cyst will exit the germarium and enter the vitellarium at stage 2, when the oocyte nucleus is reorganized into the karyosome. Full-length euchromatic SC persists in the oocyte nucleus until approximately stage 5, when it is progressively disassembled from along the chromosome arms. By approximately stage 7–8, the SC is completely disassembled along the chromosome arms but remains at the centromeres for additional stages.

Functional dependencies for LE assembly: The initial loading of Smc1/3 to the chromosome arms is dependent on the

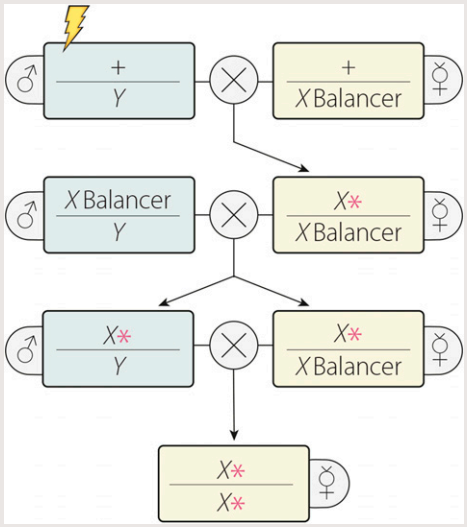
Box 1 Screening for Meiotic Mutants

Much of our knowledge of the proteins involved in female *Drosophila* meiosis has come through the analysis of mutations that disrupt various aspects of meiosis, many of which were isolated through forward genetic screens for mutations that cause chromosome nondisjunction. The screens have varied in their choice of mutagen and utilized different strategies for recovering and identifying the mutation of interest. These efforts have identified not only mutations that decrease recombination, which leads to chromosome nondisjunction, but also mutations that directly disrupt chromosome segregation during the two meiotic divisions.

The first meiotic mutations identified were found fortuitously: a mutation in *crossover suppressor on 3 of Gowen* (*c(3)G*) (Gowen and Gowen 1922; Gowen 1933), and a mutation in *D. simulans* that later was found to affect the homolog of the *D. melanogaster* meiotic gene *nonclaret disjunctional* (*ncd*) as well as the gene *claret* (Davis 1969; Sequeira *et al.* 1989). The *c(3)G* mutant provided the first insights into the genetic control of female meiosis. Later, Sandler *et al.* (1968) undertook a groundbreaking effort to systematically screen for genetic mutations that alter meiosis in natural fly populations by looking for lines with increased chromosome nondisjunction in females and males, which yielded several strong meiotic mutants. Baker and Carpenter (1972) used a similar approach to identify new female meiotic mutants induced by EMS (see figure).

Genetic screens continue to be used to identify new genes that play a role in meiotic processes, using sex chromosome nondisjunction as the assay. Traditional screens included using *P*-elements as the mutagen (Sekelsky *et al.* 1999), an EMS screen of the *X* chromosomes (Liu *et al.* 2000), and the examination of already existing highly EMS-mutagenized fly lines (Giunta *et al.* 2002). More recently, advances in screening methods by two labs employed a FLP-FRT system to screen for recessive meiotic mutants in heterozygous F1 females, with a requirement for nondisjunction of an autosome to select for strong female meiotic mutants and to potentially allow for the recovery of recessive lethal mutations (Fedorova *et al.* 2001; Page *et al.* 2007; Collins *et al.* 2012).

Additional methods to identify genes that play roles in female meiosis have included further analyzing mutants that originally were identified for their effects on male meiosis (Yan and McKee 2013; Krishnan *et al.* 2014), analyzing female-sterile collections (Pearson *et al.* 2005), screening deficiencies (Harris *et al.* 2003; Sousa-Guimaraes *et al.* 2011; Von Stetina *et al.* 2011), and, more recently, candidate RNAi approaches (Radford *et al.* 2012b; Hughes and Hawley 2014). The genes defined by these mutations have provided us with insights into the mechanisms that control SC formation, DSB formation, CO maturation and distribution, spindle formation, and chromosome segregation events.



Screening for new meiotic mutants on the *X* chromosome. Shown is a simple example of a forward genetic screen for new meiotic mutations on the *X* chromosome. Most commonly, females homozygous for the mutagenized chromosome have been tested by scoring the level of *X* chromosome nondisjunction among the progeny, but autosomal nondisjunction, recombination, or cytological parameters can also be examined. For more detailed descriptions of forward genetic screens of the *X* chromosome, including more complicated selection-type screens, see Baker and Carpenter (1972), Liu *et al.* (2000), Collins *et al.* (2012).

Box 2 The Three Peculiarities of Drosophila Female Meiosis

We are often asked why meiosis is different in flies compared to in yeast or worms. It would be convenient if, as we presume is true for things like gene regulation, there were only one mechanism for executing meiosis—a mechanism that is absolutely conserved in all species. Unfortunately, meiosis appears to be soft clay for the evolutionary process, resulting in quite impressive variation in many aspects of the meiotic process. This variation does make the study of meiosis more challenging.

There are three significant ways in which meiosis in *Drosophila* differs from meiosis in other well-characterized systems (*i.e.*, budding yeast, mice, and humans).

First, meiotic pairing begins during the premeiotic mitoses (Christophorou *et al.* 2013; Joyce *et al.* 2013).

Second, although in most other organisms, DSBs are required to initiate SC formation, complete SC assembly can occur in flies even in mutants that fully block DSB formation (McKim *et al.* 1998). Studies of a mutant that makes only a partial SC suggest that, under normal conditions, SC assembly results both from the extension of pericentromeric SC and from extension of the SC initiated from additional sites along the arms (Manheim and McKim 2003).

Lastly, *Drosophila* oocytes do not have a canonical **diplotene–diakinesis** in which homologs fully repel each other. Rather, homolog pairing is maintained in the heterochromatin that surrounds the centromere until prometaphase I (Dernburg *et al.* 1996).

We find the unique aspects of the *Drosophila* meiotic process, most notably premeiotic pairing, to be among the most fascinating problems for further study. Our rationale for focusing on what others may dismiss as "some odd fly thing" is that regardless of the exact route, flies still achieve the fundamental goals of pairing, synapsis, CO control, and segregation. Insights into any process that facilitates an end such as pairing will provide critical understanding of what it means to be "paired" or to be selected as a DSB that will become a CO.

C(2)M complex, and the LE protein C(2)M localizes only to the arms of the chromosomes (Manheim and McKim 2003; Gyuricza *et al.* 2016). Loss of either SA or Nipped-B leads to a failure of C(2)M to load along the chromosome arms, and loss of any member of the complex also leads to a loss of Smc1/3 along chromosome arms (Gyuricza *et al.* 2016).

The initial loading of Smc1/3 along the chromosome arms does not appear to be dependent on the Ord complex (Webber *et al.* 2004; Khetani and Bickel 2007; Yan and McKee 2013; Krishnan *et al.* 2014; Gyuricza *et al.* 2016). However, although Smc1/3 is initially loaded to the chromosome arms in *solo* and *ord* mutants, it is prematurely lost from the chromosome cores in older cysts, resulting in the eventual separation of sister chromatids (Khetani and Bickel 2007; Yan and McKee 2013; Krishnan *et al.* 2014).

The two cohesion complexes, C(2)M and Ord, appear to be interdependent in terms of their localization. In *c(2)M* mutants expressing a GFP-tagged version of Ord, the localization of Ord-GFP was patchy along the chromosome arms but Ord-GFP was loaded to the centromeres (Khetani and Bickel 2007). In *ord* mutants, C(2)M initially loads to the chromosomes arms but, like Smc1/3, the C(2)M protein is then precociously lost from the chromosome arms (Webber *et al.* 2004).

Finally, although the loss of LE proteins strongly affects the localization of CR proteins either along the arms or to the centromeres, the loss of CR proteins does not appear to affect the localization of any of the LE proteins thus far tested, based on immunofluorescence analyses (Page *et al.* 2008).

Functional dependencies for CR assembly: Loss of the C(2)M complex proteins has strong effects on elongation of the CR along the chromosome arms (Manheim and McKim 2003; Gyuricza *et al.* 2016). Specifically, in *c(2)M* mutants, the CR protein C(3)G can still load near the centromeres but loads only to several small sites along the chromosome arms (Manheim and McKim 2003; Gyuricza *et al.* 2016). RNA interference (RNAi) knockdown of Nipped-B or SA leads to a similar phenotype, with C(3)G loading only to the centromeres and several discreet sites on the chromosomes, and a failure to extend these sites into full-length SC (Gyuricza *et al.* 2016). The loading of C(3)G only to several discreet sites suggests that C(2)M, Nipped-B, and SA are required not for the initiation of SC assembly, but for its elongation into a full-length SC.

Based on immunofluorescence, CR proteins of the SC are still initially loaded along the chromosome arms in region 2A in Ord complex mutants (Webber *et al.* 2004; Yan and McKee 2013; Krishnan *et al.* 2014). However, although the CR initially appears wild-type in *ord* mutants by immunofluorescence, EM studies reveal the CR to be abnormal (Webber *et al.* 2004). Additionally, the CR progressively fragments and disassembles much earlier than in wild-type flies and is completely disassembled before the cyst exits the germarium (Khetani and Bickel 2007; Yan and McKee 2013).

Cona, Corolla, and C(3)G are mutually dependent on one another for loading to the CR of the SC (Page *et al.* 2008; Collins *et al.* 2014), and loss of any of these proteins leads to a complete failure to form the CR of the SC. As a consequence

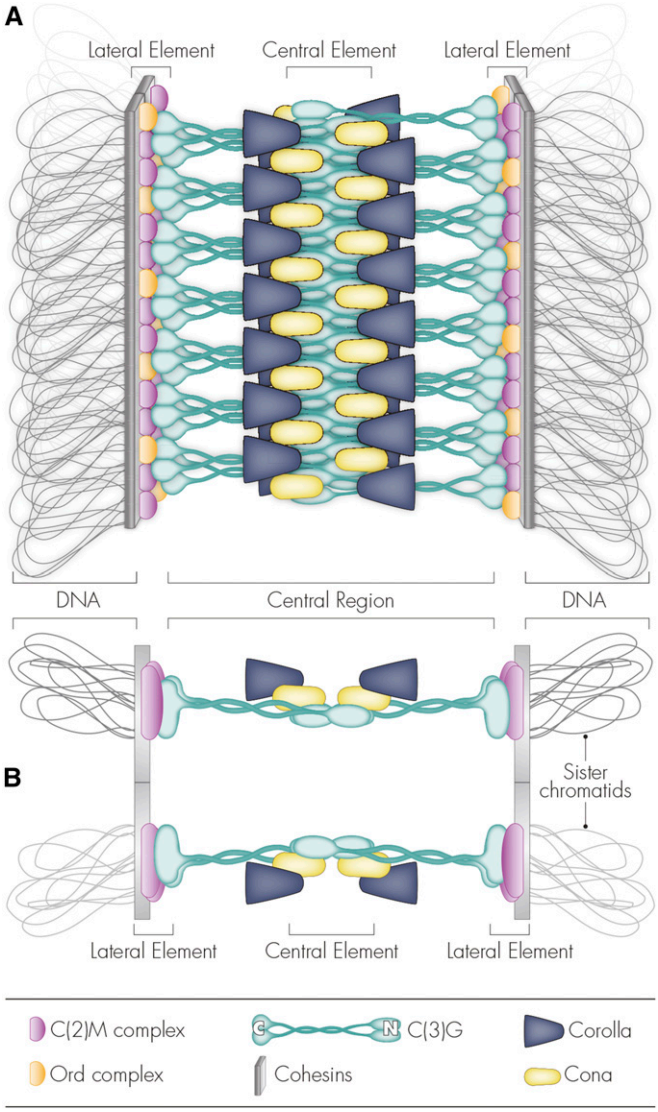


Figure 8 The synaptonemal complex (SC). (A) Along the chromosome arms, the SC consists of two axial/lateral elements and a central region that spans the distance between lateral elements. The axial elements serve as a scaffold that both connects sister chromatids and provides a link between chromatin and the SC in an unknown fashion. In *Drosophila*, axial element proteins include the cohesins Smc1/3, as well as Ord, Solo, Sunn, and the cohesin loader Nipped-B. These proteins localize along chromosome arms as well as at the centromere. C(2)M is a cohesin-like protein, generally described as a lateral element protein, that is found only along chromosome arms. The central region proteins include Corolla, the transverse filament C(3)G, and the central element protein Cona. (B) The SC forms in two distinct layers that mirror one another. These layers are known to contain C(2)M, C(3)G, Corolla, and Cona.

of a failure to assemble the SC, CR mutants also display greatly decreased levels of both heterochromatic and euchromatic pairing in pachytene when compared to wild-type flies (Sherizen *et al.* 2005; Page *et al.* 2008; Christophorou *et al.* 2013).

Role of SC proteins in facilitating meiotic recombination: All known null alleles of CR-encoding genes completely suppress recombination (Page and Hawley 2001; Page *et al.* 2008; Collins *et al.* 2014). CR mutants do still form DSBs, although at a reduced level (Mehrotra and McKim 2006; Collins *et al.* 2014), indicating that the CR of the SC is required for the maturation of DSBs into COs.

Null alleles of *ord* and *solo* cause large decreases in recombination, particularly in distal regions of the chromosomes, and a subset of the residual COs appear to be between sister chromatids (Mason 1976; Webber *et al.* 2004; Yan and McKee 2013). These studies indicate that the Ord complex may play a role in promoting exchange between homologs over **sister chromatid exchange** (Webber *et al.* 2004; Yan and McKee 2013). These defects in recombination lead to chromosome missegregation at both meiotic divisions for these mutants (Bickel *et al.* 1997; Yan and McKee 2013; Krishnan *et al.* 2014).

Mutants in *c(2)M* show a decrease in recombination to 25% of wild-type levels (Manheim and McKim 2003). Moreover, the residual COs show an altered distribution that is more proportional to physical distance, indicating a decrease in CO interference. Due to the strong decreases in recombination, *c(2)M* mutations cause increased chromosome missegregation. Approximately 10% of the *X* chromosome nondisjunction events observed by Manheim and McKim (2003) appeared to be sister chromatid nondisjunction, suggesting that C(2)M plays a moderate role in sister chromatid cohesion. The short stretches of residual SC observed in *c(2)M* mutant oocytes frequently colocalize with DSBs, suggesting that these short SC segments are sufficient for the maturation of COs in some instances (Tanneti *et al.* 2011).

Part IV: DSB Formation and Recombination

In *Drosophila* females, meiotic DSBs are initiated after the formation of the SC. DSBs can be visualized cytologically in the *Drosophila* ovary with an antibody recognizing γH2AV, a modification that occurs in response to DSBs (Mehrotra and McKim 2006; Lake *et al.* 2013). In the first 16-cell cyst of the germarium there are almost no γH2AV foci, and after SC formation, a few γH2AV foci are observed (Mehrotra and McKim 2006). The number of γH2AV foci steadily increases with each older cyst until it reaches a maximum of ~15 γH2AV foci and then steadily drops until few or no foci are observed by region 3 (Mehrotra and McKim 2006).

In mutants that are unable to complete repair of DSBs (Table 2), such as *spn-A* (*Rad51* homolog), *spn-B* (*Xrcc3* homolog), or *okra* (*Rad54L* homolog), the average number of γH2AV foci in region 3 is 21.3, 24.3, and 20.6, respectively (Mehrotra and McKim 2006). Since DSBs are not being repaired in these mutants, it is believed that these numbers would represent the upper limit of meiotic DSBs that occur. Observations in wild-type oocytes have shown that an average of 15 DSBs can be observed in a single region of the germarium, but DSBs may be induced and repaired in several regions so this number may be on the lower limit of the average number of meiotic DSBs created (Mehrotra and McKim 2006). The wild-type number of DSBs is two to three

Table 1 Genes involved in pairing and synapsis

Gene symbol	Common name (homolog)	Function[a]	References
klaroid	SUN domain protein	Inner nuclear membrane protein	Christophorou *et al.* (2015)
klarsicht	KASH domain	Outer nuclear member protein	Christophorou *et al.* (2015)
dhc	Dynein heavy chain	Minus-end-directed motor	Christophorou *et al.* (2015)
Chromosome axis			
smc1	Smc1	Core cohesion component	Khetani and Bickel (2007); Gyuricza *et al.* (2016)
smc3	Smc3	Core cohesion component	Khetani and Bickel (2007); Gyuricza *et al.* (2016)
ord		Cohesion: Ord complex	Bickel *et al.* (1996), (2002); Gyuricza *et al.* (2016)
sunn	Stromalin-related	Cohesion: Ord complex	Krishnan *et al.* (2014); Gyuricza *et al.* (2016)
solo		Cohesion: Ord complex	Yan and McKee (2013); Gyuricza *et al.* (2016)
SA	Stromalin	Cohesion: C(2)M complex	Gyuricza *et al.* (2016)
NipB	SCC2	Cohesion: C(2)M complex	Gyuricza *et al.* (2016)
rad21/vtd	SCC1	Cohesion	Urban *et al.* (2014)
SC lateral element			
c(2)M		Kleisin-like protein; cohesion: C(2)M complex	Manheim and McKim (2003); Heidmann *et al.* (2004); Gyuricza *et al.* (2016); Cahoon *et al.* (2017)
SC central region			
c(3)G	Zip1/SCP1	Transverse filament	Hall (1972); Page and Hawley (2001); Cahoon *et al.* (2017)
corolla		Transverse filament-like protein	Collins *et al.* (2014); Cahoon *et al.* (2017)
cona		Central element	Page *et al.* (2008); Lake and Hawley (2012); Cahoon *et al.* (2017)

SUN, Sad1p, UNC-84; KASH, Klarsicht, ANC-1, Syne homology; SC, synaptonemal complex.
[a] Most likely function based on genetic, biochemical, or cytological data.

times higher than the around six COs per genome, or 1.2 COs per arm, observed by genetic and WGS techniques (Lindsley and Sandler 1977; Miller *et al.* 2016c), indicating that the remaining DSBs should be recovered as NCOs. Indeed, a recent study using WGS to identify CO and NCO events observed an average of 11.2 NCOs during a single meiosis (Miller *et al.* 2016c).

Genes involved in the induction of meiotic DSBs

Four genes are known whose products are required for the induction of meiotic DSBs: *mei-W68*, *mei-P22*, *vilya*, and *trade embargo* (*trem*) (Figure 9 and Table 2). The Mei-W68 protein, a homolog of yeast SPO11, catalyzes meiotic DSBs (McKim and Hayashi-Hagihara 1998) and is related to a subunit of TopoVI DNA topoisomerase (TopoVIA) (Bergerat *et al.* 1997). In *mei-W68* mutants, recombination and gene conversion are absent and γH2AV foci fail to be detected (McKim and Hayashi-Hagihara 1998; Mehrotra and McKim 2006).

Like Mei-W68, Mei-P22 is also required for the induction of meiotic DSBs as assessed by γH2AV foci, and recombination is eliminated in these mutants (Liu *et al.* 2002). Recently, Mei-P22 was found to have homology to the TopoVIB family, which works with A subunits as a heterotetramer to catalyze DSBs (Robert *et al.* 2016), suggesting that Mei-P22 and Mei-W68 may work as a complex to initiate meiotic DSBs. Although no antibody is available for localizing Mei-W68, a tagged rescue construct of Mei-P22 shows that Mei-P22 foci first appear just prior to the appearance of γH2AV foci (DSBs). Moreover, the two types of foci are highly colocalized, consistent with Mei-P22's role in DSB formation (Liu *et al.* 2002; Mehrotra and McKim 2006).

The localization of Mei-P22 to discreet foci requires the protein Trem. The Trem protein contains a zinc finger-associated domain and five C2H2 zinc fingers. Mutation of *trem* leads to a strong decrease in the number of meiotic DSBs as based on γH2AV foci and recombination is strongly reduced, which results in high levels of chromosome missegregation (Lake *et al.* 2011). The Trem protein appears to be chromatin-associated, leading to the hypothesis that Trem may be required to alter chromatin structure to allow the binding of Mei-P22 or may assist directly in bringing Mei-P22 to DNA (Lake *et al.* 2011) (Figure 9).

Finally, Vilya is a protein with homology to the yeast E3 ligase Zip3. Mutations in *vilya* eliminate recombination, display a strong reduction in the number of γH2AV foci, and cause high levels of chromosome nondisjunction (Lake *et al.* 2015). The strong reduction in γH2AV foci indicates that one role of Vilya is to promote DSB formation. Indeed, Vilya strongly interacts with Mei-P22 in yeast two-hybrid assays. Prior to the appearance of γH2AV foci, an epitope-tagged version of Vilya localizes along the length of the SC. In the presence of DSBs, Vilya then concentrates into around six foci in early/midpachytene, believed to be the sites of COs. Vilya foci are not formed in mutants that do not make DSBs (Lake *et al.* 2015). This localization strongly suggests that Vilya may play an additional role after DSB formation (described in *RNs*).

Genes involved in DSB fate determination

Once formed, a meiotic DSB can be repaired either as a CO or as an NCO. Because only six COs are formed per meiosis and approximately three times as many DSBs are observed by cytological methods, the majority of DSBs are repaired as

Table 2 Genes involved in DSBs and recombination

Gene symbol	Common name (homolog)	Function[a]	References
spn-B	Rad51/Dmc1	Strand invasion	Ghabrial *et al.* (1998)
spn-D	Rad51C	Strand invasion	Ghabrial *et al.* (1998)
okr	Rad54	Strand invasion	Ghabrial *et al.* (1998)
rec	Mcm8	Crossover promotion	Grell and Generoso (1980); Blanton *et al.* (2005); Kohl *et al.* (2012); Hatkevich *et al.* (2017)
mei-218	MCM family-related	Crossover promotion	Carpenter and Sandler (1974); McKim *et al.* (1996); Kohl *et al.* (2012)
mei-217	MCM family-related	Crossover promotion	Liu *et al.* (2000); Kohl *et al.* (2012)
mcm5	Mcm5	Crossover promotion	Lake *et al.* (2007)
blm	Blm	Crossover promotion	McVey *et al.* (2007); Kohl *et al.* (2012); Hatkevich *et al.* (2017)
mre11	Mre11	DSB repair	Kusch (2015)
p53	p53	Stress response	Lu *et al.* (2010)
DSB formation			
mei-W68	Spo11	DSB formation	McKim and Hayashi-Hagihara (1998); Mehrotra and McKim (2006)
mei-P22	TopoVIB family	DSB formation	McKim *et al.* (1998); Liu *et al.* (2002); Mehrotra and McKim (2006)
vilya	E3 ligase	DSB formation; crossover maturation	Lake *et al.* (2015); Lake and Hawley (2016)
trem		DSB formation	Lake *et al.* (2011)
Resolvase			
mei-9	Rad1/XPF/ERCC4		Carpenter and Sandler (1974); Sekelsky *et al.* (1995)
mus312	Slx4		Green (1981); Yildiz *et al.* (2002); 2004
ercc1	ERCC1		Yildiz *et al.* (2002); Radford *et al.* (2007)
hdm	MEIOB		Joyce *et al.* (2009)

MCM, mini-chromosome maintenance; DSB, double-strand break.
[a] Most likely function based on genetic, biochemical, or cytological data.

NCOs (Miller *et al.* 2016c). We currently understand the process of DSB maturation and fate choice at two levels: (1) the enzymes that process the DSB itself to create an NCO or CO event, and (2) the formation and function of cytologically visible RNs that mark the physical sites of CO formation.

The molecular events that underlie the production of CO *vs.* NCO resolution events in *Drosophila* have been thoroughly reviewed by Kohl and Sekelsky (2013) and Sekelsky (2017). In other organisms, NCOs are thought to form through a process called synthesis-dependent strand annealing (SDSA) (Figure 6), as described in Haber (2013). In *Drosophila* females, many NCOs also appear to be formed via a modified version of the SDSA pathway. Evidence suggests that instead of only one end of the DSB engaging with the homolog to prime synthesis, about half of the time both ends of the break interact with the homolog in some manner (Figure 6) (Crown *et al.* 2014).

We will organize our further description of the enzymology of *Drosophila* meiotic recombination into three sets of functions: (1) a group of proteins known collectively as the Mei-MCM (mini-chromosome maintenance) proteins, (2) the Mei-9 resolvase complex, and (3) the Bloom syndrome helicase (Blm helicase) (Table 2). An oversimplified model that explains the roles of these proteins is presented in Figure 10. Briefly, the Mei-MCM proteins are thought to act to promote the resolution of DSBs along the interference-sensitive class I CO pathway. The Mei-9 resolvase complex then acts to facilitate the processing of recombination intermediates that have been matured in the presence of the Mei-MCM complex into class I COs. Blm helicase functions as an anti-CO protein that inhibits DSBs from being processed by the interference-insensitive class II pathway (see section *Are there two pathways for crossing over in Drosophila?* for a discussion of class I *vs.* class II COs).

The Mei-MCM proteins: The Mei-MCM proteins are related to the MCM family of proteins, and these proteins are hypothesized to work as a complex (Kohl *et al.* 2012). The Mei-MCM complex is thought to include Rec/Mcm8, Mei-217, Mei-218, and likely Mcm5 proteins, all of which appear to act in place of the Msh4 and Msh5 proteins [in other organisms Msh4 and Msh5 play critical roles in promoting the repair of DSBs into COs, but these proteins appear to have been lost in the evolution of *Drosophila* (Sekelsky *et al.* 1998; Sekelsky 2017)]. Each of these genes is briefly described below.

Null alleles of *rec* display high rates of chromosome nondisjunction (Grell 1984) and recombination is strongly decreased. The reduction in recombination is polar, with the reduction in COs strongest in centromere-distal regions and less severe nearer the centromeres (Grell 1984; Blanton *et al.* 2005). In addition, NCO frequencies are increased, but NCO tract length is shortened (Grell 1984; Blanton *et al.* 2005).

Mutations in *mei-217* and *mei-218* reduce exchange to $< 10\%$ of wild-type levels, with residual COs occurring more in proportion to physical distance (Sandler *et al.* 1968; Baker and Carpenter 1972; Carpenter and Sandler 1974; McKim *et al.* 1996; Liu *et al.* 2000). Mei-217 and Mei-218 proteins

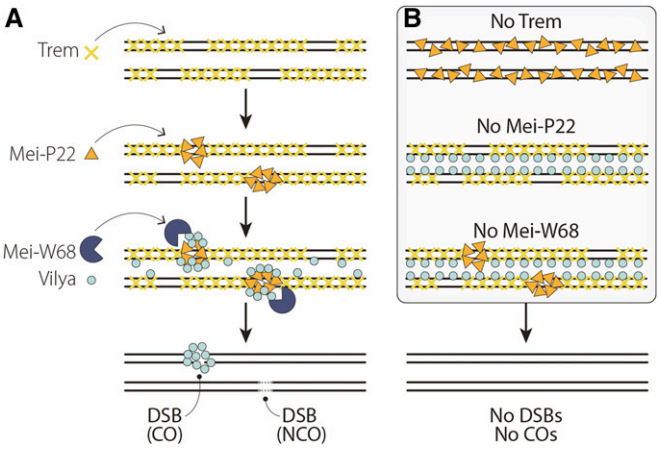


Figure 9 Double-strand break (DSB) formation. (A) Before DSB formation, Trem localizes to the chromosomes and is required for the recruitment of Mei-P22 to discreet foci. Mei-W68 and Vilya are recruited to sites of Mei-P22, leading to the formation of DSBs. (B) Without Trem, Mei-P22 localizes along chromosome arms rather than to discreet foci. Without either Mei-P22 or Mei-W68, Trem still localizes to chromosome arms, but Vilya does not form discreet foci. If any of these proteins are missing, DSBs are not made. Adapted from Lake *et al.* (2015). CO, crossover; NCO, noncrossover.

have been shown to interact by yeast two-hybrid assay, and sequence analysis of each protein led to the proposal that they both evolved from a single MCM-like protein (Kohl *et al.* 2012). Mei-217 also physically interacts with Rec (Kohl *et al.* 2012). The physical interaction of Mei-217 with both Mei-218 and Rec, as well as the similarity of the phenotypes of *mei-217/mei-218* mutants with *rec* mutants, led to the classification of Mei-217, Mei-218, and Rec as Mei-MCM proteins. Mei-218 and Rec may also have additional roles in blocking nonhomologous end-joining and in the DSB repair checkpoint during female meiosis (Joyce *et al.* 2012).

Lake *et al.* (2007) characterized a meiosis-specific allele of *mcm5* (null alleles are lethal) that caused increased chromosome missegregation and a 10-fold reduction in crossing over. The reduction in crossing over was not uniform, with CO reductions being strongest in intervals distal to the centromere. Since DSBs were both induced and repaired (based on γH2AV foci and a lack of checkpoint activation associated with failure to repair DSBs), it was concluded that loss of *mcm5* function results in the repair of DSBs by NCO or sister-chromatid exchange over COs (Lake *et al.* 2007). The similarity of the *mcm5* mutant phenotype with *mei-217*, *mei-218*, and *rec* mutants suggests that Mcm5 functions in the Mei-MCM complex as well.

The observation that the residual COs observed in Mei-MCM mutants are distributed more in proportion to physical distance suggests that one of the primary roles of the Mei-MCM complex is to promote class I COs. Based on the model proposed by Blanton *et al.* (2005), loss of Mei-MCM proteins leads to those DSBs designated to mature as class I COs being repaired instead by SDSA. Models suggest that the remaining COs in Mei-MCM mutants are class II COs that are not influenced by interference (Carpenter 1982; Liu *et al.* 2000; Blanton *et al.* 2005; McVey *et al.* 2007; Kohl *et al.* 2012).

The Mei-9 complex: Recombination intermediates must be fully resolved to complete repair. Evidence suggests that Mei-9 acts in concert with Mus312, Ercc1, and Hold'em (Hdm) to resolve such intermediates that have been directed down the class I CO pathway by the Mei-MCM complex (Hatkevich *et al.* 2017). Mei-9, the *Drosophila* homolog of the Rad1/XPF/ERCC4 single-strand endonuclease, is thought to play a critical role in resolving recombination intermediates into COs during meiotic recombination (Sekelsky *et al.* 1995). Although mutations in *mei-9* do not appear to reduce the frequency of NCOs (at least at the *rosy* locus), crossing over is reduced to $<$ 10% of wild-type (Baker and Carpenter 1972; Carpenter and Sandler 1974; Carpenter 1982). Importantly, the remaining COs display a wild-type distribution, unlike the mutations in Mei-MCM protein-coding genes, which show an abnormal distribution of COs (Yildiz *et al.* 2004).

Mei-9 physically interacts by yeast two-hybrid assay with Mus312, which is required for repair of interstrand cross-links, and *mus312* mutants display strong recombination and nondisjunction defects similar to *mei-9* mutants (Yildiz *et al.* 2002, 2004). A point mutation in *mei-9* that abrogates the physical interaction with Mus312 causes strong meiotic phenotypes, indicating that Mei-9 functions with Mus312 to facilitate resolution of recombination intermediates into class I COs (Yildiz *et al.* 2002). Mei-9 also physically interacts by yeast two-hybrid assay with the protein Ercc1, using a different part of the protein than the interaction with Mus312 (Yildiz *et al.* 2002; Radford *et al.* 2007). *Ercc1* mutants display only about half the levels of nondisjunction of *mei-9* mutants, indicating that Mei-9 is dependent on Ercc1 for only a portion of its role in resolving meiotic COs (Yildiz *et al.* 2002).

The Mei-9 complex likely utilizes the single-stranded binding protein Hdm to resolve the remaining COs. Hdm interacts with both Mei-9 and Ercc1 by yeast two-hybrid assay (Joyce *et al.* 2009), and mutations in *hdm* reduce recombination and increase chromosome nondisjunction to levels comparable to *ercc1* mutations (Joyce *et al.* 2009). More importantly, double mutants for *ercc1* and *hdm* affect recombination and nondisjunction as strongly as *mei-9* mutants, indicating that the Mei-9 complex may utilize Ercc1 and Hdm to resolve different COs (Joyce *et al.* 2009).

The Blm helicase: Studies of Blm (or Mus309) provide more insight into the designation of class I *vs.* class II COs. Both hypomorphic and null alleles of *blm* cause a moderate reduction in meiotic recombination (McVey *et al.* 2007; Kohl *et al.* 2012). The decrease in recombination is not uniform across the chromosome arms, with COs distributed in proportion to physical distance on the chromosomes (McVey *et al.* 2007; Hatkevich *et al.* 2017).

Mutants in *blm* produce enough COs to directly examine interference, which is strongly reduced in these mutants

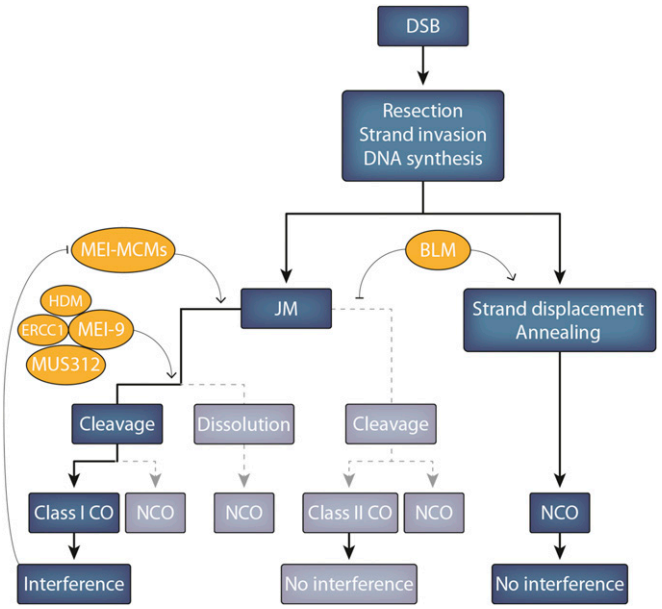


Figure 10 Double-strand break (DSB) repair. A DSB is typically repaired as either a crossover (CO) or a noncrossover gene conversion (NCO). Two classes of COs can occur, each of which follows the formation of a joint molecule (JM). Class I COs, which are sensitive to interference, are by far the most common, while noninterfering class II COs happen infrequently under normal circumstances (rarely, a JM may be dissolved into an NCO.) The Mei-MCM proteins (Rec, Mei-217, Mei-218, and MCM5) are thought to stabilize those JMs designated to become class I COs, and the Mei-9 resolvase likely functions to cleave double Holliday junctions into COs. Under wild-type conditions, the anticrossover helicase Bloom (BLM) both inhibits class II crossovers and promotes the formation of NCOs by synthesis-dependent strand annealing.

(Hatkevich *et al.* 2017). Additionally, COs in *blm* mutants can be identified on the fourth chromosomes, which normally fail to cross over in wild-type flies (Hatkevich *et al.* 2017). The lack of interference suggests that the remaining COs in *blm* mutants are generated via the class II CO pathway. To further address this idea, mutations in *blm* were combined with either *mei-218* or *rec* mutations (Mei-MCM mutants) (Kohl *et al.* 2012). The strong reduction in recombination and high *X* chromosome nondisjunction that is characteristic of both *mei-218* and *rec* mutants was substantially alleviated, resulting in nearly wild-type levels of recombination in the *mei-MCM blm* double mutants (Kohl *et al.* 2012). *blm rec* double mutants also display even higher levels of crossing over on the fourth chromosome than *blm* mutants alone (Hatkevich *et al.* 2017). Moreover, the observed CO events display a distribution similar to that of *blm* mutants alone. These results suggest that, in the absence of Blm function, DSBs can be directed into a CO pathway (class II) that does not require Mei-218 and Rec (Kohl *et al.* 2012).

Double mutants of *blm* and *mei-9* display recombination levels similar to *blm* mutants, indicating that the COs remaining in *blm* mutants are not dependent on *mei-9* (Hatkevich *et al.* 2017). Since the Mei-9 complex is thought to be required for class I COs, these results lend further support to the hypothesis that the noninterfering COs observed in *blm* mutants must be class II COs. (Hatkevich *et al.* 2017).

Taken together, these studies indicate that the role of Blm helicase is to inhibit the repair of DSBs by the class II pathway and instead direct DSBs down the path to be repaired as class I COs (Hatkevich *et al.* 2017). There is still much to be learned about the mechanisms determining the designation of DSBs into NCOs *vs.* class I or II COs, but the intent of the model shown in Figure 10 is to provide a framework to drive future studies.

RNs

As DSBs are made and their fates designated, some DSB sites appear to be associated with large proteinaceous structures known as RNs. Using EM, Carpenter first observed these electron-dense spherical structures in a distribution consistent with the number of COs in *Drosophila* females (Carpenter 1975a,b, 1979). Thus, RNs, which associate with the SC, appear to mark the sites of crossing over. Despite their obvious association with sites of crossing over, very little is known about how RNs function. The most obvious possibility seems to lie in a role for RNs in the modification of chromosome axes to accommodate the DNA interchange associated with crossing over.

To date, only one RN component (Vilya) has been definitively identified. An epitope-tagged version of the Vilya protein was observed to first localize in tracts along the SC and then concentrate into discreet foci along the chromosome arms in midpachytene (Figure 9). The localization of the epitope-tagged Vilya foci showed a strong correlation with the number and distribution of the COs observed by genetic crosses (Lake *et al.* 2015). More importantly, immuno-EM demonstrated Vilya localization to the RNs. By immunofluorescence, Mre11 localizes to foci in a pattern indicating that it may also be a component of the RN, but Mre11 localization has not yet been examined by EM (Kusch 2015).

Part V: Stages 2–14; Oocyte Development, Spindle Assembly, and Segregation

The events detailed thus far all occur primarily within the germarium of oocyte development (Figure 5). Once DSB formation and resolution has concluded, a number of additional steps must still be completed for the successful production of a haploid oocyte. The remainder of this review will describe our current understanding of these later stages of meiosis in *Drosophila* (Table 3).

Karyosome formation

After the designation of a single pro-oocyte in region 3 or stage 1, the 16-cell cyst buds off from the germarium. In this stage 2–3 oocyte, the nucleus reorganizes to form a compact structure called the karyosome (King 1970; Spradling 1993). The histone H2A kinase Nhk-1 (Nucleosomal histone kinase-1) appears to mediate karyosome formation by phosphorylating the protein Baf (Barrier to autointegration factor), which links chromatin to the nuclear envelope (Cullen *et al.* 2005;

Table 3 Genes involved in oocyte development

Gene symbol	Common name (homolog)	Function[a]	References
nhk-1	Vrk family	Karysome formation	Cullen *et al.* (2005); Ivanovska *et al.* (2005)
baf	Banf	Karysome formation; links nuclear envelope and chromatin	Lancaster *et al.* (2007)
SRPK	SRPK	Karysome formation; kinase	Loh *et al.* (2012)
kdm5/lid	Kdm5	Karysome formation; trimethyl H3K4 histone demethylase	Zhaunova *et al.* (2016)
Su(var)205	HP1a	Heterochromatin-binding protein	Giauque and Bickel (2016)
piwi	Argonaute superfamily	piRNA binding	Giauque and Bickel (2016)
Su(var)3-9	SUV39	H3K9 methyltransferase	Giauque and Bickel (2016)
eggless	SetDB	H3K9 methyltransferase	Giauque and Bickel (2016)
polo	Plk1-4	Spindle assembly; chromosome alignment; GVBD	Xiang *et al.* (2007); Das *et al.* (2016)
mtrm		Inhibitor of Polo kinase	Harris *et al.* (2003); Xiang *et al.* (2007), Bonner *et al.* (2013); Whitfield *et al.* (2013)
endos	Ensa	GVDB; spindle formation; chromosome alignment	Von Stetina *et al.* (2008)
twe	Germline-specific version of CDC25	GVBD; chromosome alignment; spindle assembly; cell cycle	Alphey *et al.* (1992); Courtot *et al.* (1992), White-Cooper *et al.* (1993); Xiang *et al.* (2007)
gwl	MastL	Polo regulator	Archambault *et al.* (2007)
elgi	Nrdp1	GVDB; predicted E3 ubiquitin ligase	Von Stetina *et al.* (2008)
Wispy	Gld-2	Cytoplasmic poly(A) polymerase; mRNA regulation	Brent *et al.* (2000); Cui *et al.* (2008)

GVBD, germinal vesicle breakdown.
[a] Most likely function based on genetic, biochemical, or cytological data.

Lancaster *et al.* 2007). The conserved kinase SRPK (SR protein kinase) also appears to be required to establish and maintain the karyosome (Loh *et al.* 2012).

Mutants in *nhk-1* and *SRPK* show severe defects in maintaining a single-chromosome mass, and the multiple chromosome masses remain attached to the nuclear envelope (Cullen *et al.* 2005; Ivanovska *et al.* 2005; Loh *et al.* 2012). Heterochromatic regions are also disrupted in *SRPK* mutants (Loh *et al.* 2012). *nhk-1* mutants display both a delay in the disassembly of euchromatic SC and a failure to properly load condensin components to the chromosomes (Ivanovska *et al.* 2005). The dispersed chromosomes go on to nucleate separate spindles after meiotic spindle assembly in both *nhk-1* and *SRPK* mutants, indicating that the condensation of the chromosomes into a single karyosome is an important prerequisite for later meiotic events (Cullen *et al.* 2005; Ivanovska *et al.* 2005; Loh *et al.* 2012).

Defects in karyosome formation are also seen with the loss of the histone demethylase *Kdm5/Lid*, possibly through defects in chromatin architecture during meiotic prophase (Zhaunova *et al.* 2016). *Kdm5/Lid* mutants also cause defects in the maintenance of the SC along the chromosome arms, as well as in the maintenance of centromere pairing and clustering during midprophase (Zhaunova *et al.* 2016).

SC disassembly

Around stages 4–5 of oocyte development, the SC along the chromosome arms begins to progressively disassemble, and it is completely disassembled by stages 6–8. This loss of the SC along the arms coincides with the loading of condensin complex components (Resnick *et al.* 2009). Interestingly, at least some SC components remain associated with the centromeres beyond euchromatic SC disassembly (Takeo *et al.* 2011).

The maintenance of heterochromatic associations

After SC disassembly, chromosomes remain linked by heterochromatic associations throughout prophase (Dernburg *et al.* 1996). These associations occur regardless of whether chiasmata have formed. This was an important observation, as it revealed how those chromosomes that fail to undergo exchange during meiosis are still able to properly segregate during anaphase I (Grell 1976). The vital role that heterochromatic pairing plays in ensuring the proper segregation of nonexchange chromosomes became apparent through studies examining the consequences of heterochromatic duplications and deletions on both the *X* and fourth chromosomes (Hawley *et al.* 1992). Furthermore, experiments using a series of heterochromatic deletions have shown that the rate of missegregation is directly associated with the size of a heterochromatic deletion (Karpen *et al.* 1996). Heterochromatic associations are seen after SC disassembly between nonexchange chromosomes carrying whole-arm inversions (Dernburg *et al.* 1996), suggesting that it is not simply the maintenance of centromeric SC that facilitates or helps to maintain these associations. Decreasing the level of the heterochromatin-binding protein HP1a [Su(var)205], the H3K9

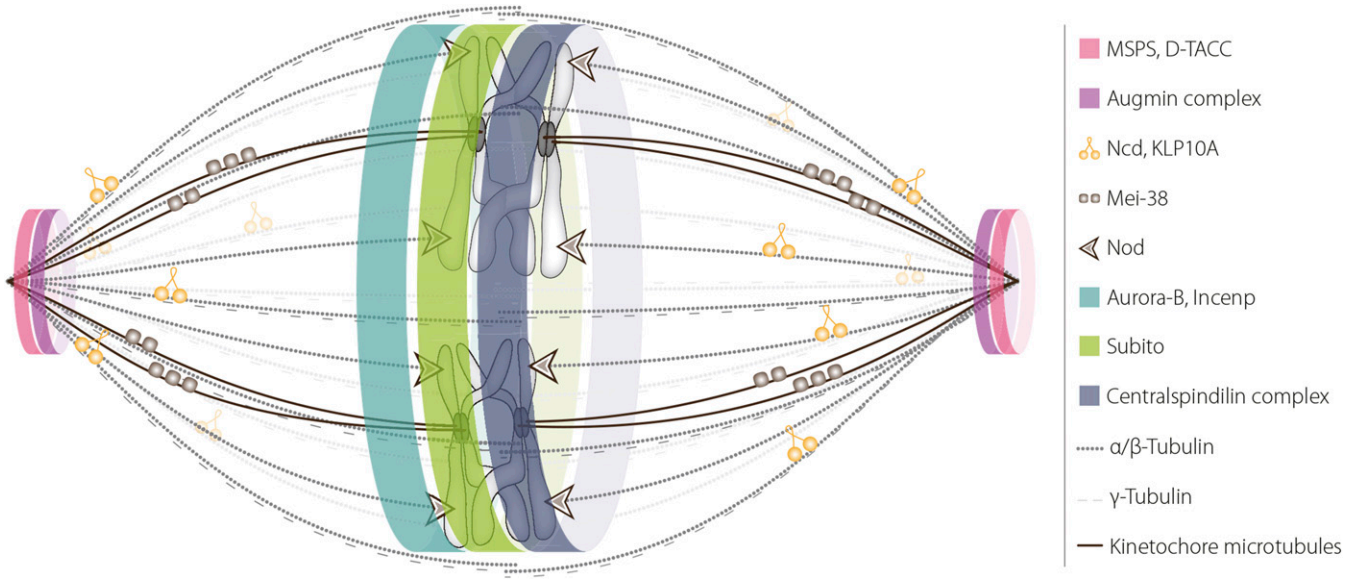


Figure 11 Spindle assembly. Because meiotic spindles in *Drosophila* are acentriolar, spindle assembly is organized by the chromosomes. The chromosomal passenger complex proteins Aurora-B and Incenp (teal), as well as Subito (green) and the Centralspindlin complex (blue), localize to the central spindle around the DNA and function in chromosome movement and/or spindle assembly/stabilization. Nod (brown arrowhead) acts as the polar ejection force to push chromosomes away from the spindle poles. The proteins MSPS and D-TACC (pink) function at the spindle poles to maintain spindle bipolarity. The Augmin complex (purple) is also located predominately at the spindle poles, where it recruits γ-tubulin and promotes spindle assembly. Both γ-tubulin (dashed lines) and α/β-tubulin (dotted lines) localize along the entire meiotic spindle. The kinesins Ncd and KLP10A, as well as additional kinesins, help regulate the assembly (Ncd) and disassembly (KLP10A) of the bipolar spindle. Mei-38 promotes the assembly or stabilization of kinetochore microtubules (solid lines).

methyltransferases Su(var)3-9 and Eggless, or the piRNA-binding protein PIWI all lead to defects in the association of heterochromatic regions of the *X* chromosomes when *X* chromosomes are heterozygous for the *FM7a* balancer (Giauque and Bickel 2016).

Chromosome decondensation

At approximately stage 9–10, the chromosomes undergo a temporary decondensation that is associated with an upregulation in transcription by the oocyte nucleus (Mahowald and Tiefert 1970). Whether this decondensation and transcription is important for meiotic progression is unknown since mutations that solely affect this process have not been identified.

GVBD

The chromatin in the oocyte nucleus recondenses after stage 10 in preparation for GVBD, which begins after the end of stage 12. Polo kinase is a protein shown to have roles in mitotic cell cycle entry (Archambault and Glover 2009), and research on two presumed regulators of Polo (Mtrm and Endos) suggests that Polo is likely a regulator of GVBD in *Drosophila* oocytes. Specifically, reducing the copy number of the Polo inhibitor Matrimony (Mtrm) causes precocious GVBD that is suppressed by a corresponding reduction in Polo levels (Xiang *et al.* 2007); GVBD is accelerated when Polo is overexpressed (Xiang *et al.* 2007). The loss of Endosulfine (Endos), a positive regulator of Polo, leads to a delay in GVBD (Von Stetina *et al.* 2008). Likewise, mutation of the Polo target Twine, a Cdc25 homolog, also leads to a delay in GVBD (Xiang *et al.* 2007).

Mutation of the Endos-binding protein Early girl (Elgi), a predicted E3 ubiquitin ligase, leads to precocious GVBD by a mechanism separate from Polo kinase regulation (Von Stetina *et al.* 2008). Additional mechanisms of regulating GVBD and the meiotic cell cycle in *Drosophila* oocytes likely exist, but because some GVBD regulators likely also play roles in the mitotic cell cycle, approaches that circumvent earlier lethality will be required to examine the function of these players.

Meiosis I spindle assembly

Upon GVBD, tubulin is recruited to the chromosomes (Matthies *et al.* 1996; Skold *et al.* 2005; Hughes *et al.* 2009). Meiotic spindles are **anastral** (**acentriolar**) in *Drosophila* oocytes, thus it is the chromosomes that recruit tubulin and organize the direction of the developing bipolar spindle (Theurkauf and Hawley 1992; Matthies *et al.* 1996). The spindle is composed of antiparallel microtubules at the central spindle that interact laterally with the chromosomes, kinetochore microtubules that connect kinetochores to the spindle poles, and additional interpolar microtubules that overlap in the central spindle region (Jang *et al.* 2005).

Multiple kinesins regulate spindle assembly: Several proteins that play a role in meiotic spindle formation in *Drosophila* have been identified (Figure 11 and Table 4). One of the best characterized is Nonclaret disjunctional (Ncd), a minus-directed kinesin protein required for proper bipolar spindle

Table 4 Genes involved in spindle assembly and segregation

Gene symbol	Common name (homolog)	Function[a]	References
ncd	Kinesin family member C1	Bundles microtubules	Hatsumi and Endow (1992a); Matthies *et al.* (1996)
asp	Aspm	Microtubule-associated protein	Saunders *et al.* (1997); Riparbelli *et al.* (2002)
Klp54D	Kinesin family member 12	Spindle symmetry	Radford *et al.* (2017)
Klp61F	Kinesin family member 11	Spindle symmetry	Radford *et al.* (2017)
Klp10A	Kinesin-13	Spindle assembly	Zou *et al.* (2008); Radford *et al.* (2012a); Do *et al.* (2014)
Eb1	Mapre family	Microtubule binding	Do *et al.* (2014)
aurB	Aurora Kinase B	Chromosomal passenger complex; spindle midzone	Radford *et al.* (2012b)
Incenp	Incenp	Chromosomal passenger complex; spindle midzone	Colombie *et al.* (2008); Radford *et al.* (2012b)
sub	MKLP-2/kinesin 6	Spindle midzone	Giunta *et al.* (2002); Jang *et al.* (2005); Radford *et al.* (2017)
tum	RacGAP50C	Centralspindlin complex; spindle midzone	Das *et al.* (2016)
mei-38	TPX2	Promotes kinetochore microtubules	Baker and Carpenter (1972); Wu *et al.* (2008)
msps	XMAP215/TOG	Maintains bipolarity	Cullen and Ohkura (2001)
cks30A	CKs/Suc1	Spindle assembly	Cullen *et al.* (2005)
d-tacc	D-TACC	Maintains bipolarity	Cullen and Ohkura (2001)
α-Tub67C	Maternally loaded α-tubulin	Microtubule nucleation	Matthies *et al.* (1999)
γ-Tub37C	γ-tubulin	Microtubule nucleation	Tavosanis *et al.* (1997); Hughes *et al.* (2011)
Augmin complex		Chromosome alignment and movement	Meireles *et al.* (2009); Colombie *et al.* (2013)
Grip71	γTurc complex	Microtubule nucleation	Reschen *et al.* (2012)
Sentin/ ssp2		Kinetochore attachment to microtubules	Gluszek *et al.* (2015)
rod	Kntc1	Rzz complex; spindle assembly checkpoint	Gluszek *et al.* (2015)
Top2	Topoisomerase 2	Chromosome orientation	Hughes and Hawley (2014)
nod	Nonmotile member of kinesin family	Polar ejection force; chromosome alignment	Baker and Carpenter (1972); Zhang and Hawley (1990); Zhang *et al.* (1990); Theurkauf and Hawley (1992); Hughes *et al.* (2009)
Axs	Ano family	Spindle width; achiasmate chromosome segregation	Zitron and Hawley (1989); Kramer and Hawley (2003a)
mps1/ald	Mps1	Meiotic spindle assembly checkpoint; chromosome segregation	O'Tousa (1982); Gilliland *et al.* (2005), (2007)
cnn	Centrosomin	Central aster of meiosis II spindles	Riparbelli and Callaini (2005)
Grip75	γTurc Complex	Meiosis II spindle assembly	Vogt *et al.* (2006)
Grip128	γTurc Complex	Meiosis II spindle assembly	Vogt *et al.* (2006)
Mud	NuMa	Meiosis II central aster	Yu *et al.* (2006)
sra	RCAN	Meiosis II entry; oocyte activation	Horner *et al.* (2006); Takeo *et al.* (2006); (2010)
canB2	calcineurin regulatory subunit B	Meiosis II entry; oocyte activation	Takeo *et al.* (2010)
mei-S332	Shugoshin	Protection of centromeric cohesion	Kerrebrock *et al.* (1992), (1995)
cort	Cortex	APC; Meiosis II progression; egg activation	Page and Orr-Weaver (1996); Whitfield *et al.* (2013)
grau	Grauzone	Meiosis II progression; egg activation; destruction of proteins	Page and Orr-Weaver (1996)
fzy	Cdc20 (APC)	Meiosis II progression; destruction of proteins	Swan and Schupbach (2007)

APC, anaphase-promoting complex.
[a] Most likely function based on genetic, biochemical, or cytological data.

assembly and chromosome movement (Endow *et al.* 1990; McDonald *et al.* 1990; Skold *et al.* 2005). It is thought that Ncd moves along microtubules toward the spindle poles to cross-link and bundle microtubules into focused spindle poles (Hatsumi and Endow 1992a,b; Matthies *et al.* 1996). Mutations in *ncd* cause misaligned chromosomes; **achiasmate** (nonrecombinant) **chromosomes** to be lost from the primary spindle; frayed, unfocused, or absent spindles poles; and chromosome missegregation (Kimble and Church 1983; Hatsumi and Endow 1992b). Live imaging of *ncd* mutant oocytes reveals a delay in spindle formation, a defect in microtubules interacting laterally with the chromosomes, and abnormal spindle conformations (Matthies *et al.* 1996; Skold *et al.* 2005).

The maintenance of both spindle and centromere symmetry at meiosis I requires the kinesin-5 protein KLP61F (Radford

et al. 2017). The meiosis I spindle develops asymmetrically in *klp61F* mutants and this spindle asymmetry of *klp61F* mutants depends on Ncd, the microtubule-associated Abnormal spindle protein, and the kinesin-12 KLP54D (Radford *et al.* 2017).

Another kinesin, the depolymerizing kinesin-13 KLP10A, has also been shown to have a role in meiotic spindle assembly (Zou *et al.* 2008; Radford *et al.* 2012a). KLP10A localizes to the meiotic spindle, and loss of KLP10A results in meiosis I spindles that are extremely elongated, with frayed or absent poles (Zou *et al.* 2008; Radford *et al.* 2012a; Do *et al.* 2014). *klp10A* mutant oocytes also display a failure of homologous chromosome centromeres to biorient and properly attach to spindle microtubules, and a dominant-negative form of KLP10A results in short meiosis I spindles (Zou *et al.* 2008). Evidence suggests that KLP10A regulates the rate of microtubule disassembly and the interaction of the microtubule-interacting protein EB1 with the ends of microtubules (Radford *et al.* 2012a; Do *et al.* 2014).

Central spindle proteins: As it does in mitosis, the chromosomal passenger complex (CPC) plays crucial roles in meiosis I spindle assembly. Both Aurora B kinase and Incenp, two components of the CPC, localize to the central region of the meiosis I spindle as a ring around the DNA (Jang *et al.* 2005; Radford *et al.* 2012b). This localization is dependent on Subito, a homolog of MKLP-2/kinesin 6 (Jang *et al.* 2005). Subito also localizes as a ring around the DNA on the meiosis I spindle, and is involved in the formation and maintenance of the central spindle (Jang *et al.* 2005; Radford *et al.* 2012b). Like KLP61F, Subito promotes spindle and chromosome symmetry (Radford *et al.* 2017). The Centralspindlin complex and some downstream effectors of this complex also localize to the central spindle and interact with Subito to regulate central spindle assembly and chromosome orientation (Jang *et al.* 2005; Das *et al.* 2016).

Mutations in central spindle proteins cause a variety of defects. Hypomorphic alleles of *incenp* result in the formation of ectopic or split spindle poles, as well as aberrant chromosome orientation and alignment (Colombie *et al.* 2008; Resnick *et al.* 2009). Live analysis of the hypomorphic *incenp* mutants reveals that spindles are unstable, and assembly is delayed, indicating that Incenp is required for spindle formation and stability (Colombie *et al.* 2008). Stronger knockdown of either *Aurora B* or *incenp* results in strong spindle assembly defects (Radford *et al.* 2012b). Meanwhile, *subito* mutations cause the formation of monopolar and tripolar spindles and increased chromosome nondisjunction (Giunta *et al.* 2002). Spindles in *subito* mutants are also unstable, although the timing of spindle formation is not delayed as it is in some *incenp* mutants (Colombie *et al.* 2008).

Spindle pole proteins: The Mini-spindles (Msps; XMAP215/TOG homolog) and Transforming acidic coiled-coil (D-TACC) proteins localize to the poles of the acentrosomal meiosis I spindle. Mutations in these proteins cause the formation of tripolar spindles, indicating that these proteins are important for maintaining spindle bipolarity (Cullen and Ohkura 2001). Mutants in *cks30A*, a homolog of Cks/Suc1 and a subunit of the Cdc2 (Cdk1)-cyclin B complex, cause chromosome misalignment and spindle defects in meiosis I (Cullen *et al.* 2005). These spindles display ectopic poles near the spindle equator, most likely due to mislocalization of Msps and D-TACC to the spindle equator.

Proteins involved in microtubule nucleation and stability: Another mutant that affects spindle formation, *mei-38*, still forms bipolar spindles, but the spindles have decreased microtubule density near the kinetochores (Wu *et al.* 2008). The localization of central spindle proteins is mostly unaffected in *mei-38* mutants, and a tagged version of Mei-38 localizes to microtubules other than those in the central spindle, suggesting that the decreased spindle density is due to loss of kinetochore microtubules (Wu *et al.* 2008). This indicates that Mei-38 is required for the stability of the kinetochore microtubules. Mutation of *mei-38* also leads to chromosomes that are disorganized on the aberrant spindles and causes chromosome nondisjunction, with especially high levels of achiasmate chromosome missegregation (Wu *et al.* 2008). This increased susceptibility of achiasmate chromosomes to missegregate is a common phenotype of mutations that affect spindle formation.

Not surprisingly, tubulin mutants also display meiotic spindle defects. For example, oocytes heterozygous for a mutation in the maternally loaded α-*Tubulin 67c* (α*Tub67C*) display shorter meiosis I spindles, and chromosomes fail to stretch toward the spindle poles (Matthies *et al.* 1999). Centromeric regions of the chromosomes do not orient toward opposite spindle poles and achiasmate chromosomes nondisjoin (Matthies *et al.* 1999). γ-tubulins are important in the nucleation of microtubules, and γTubulin37C, the female germline version of γ-Tubulin in *Drosophila*, localizes to the entire meiotic spindle (Hughes *et al.* 2011). Mutations in γ*Tub37c* lead to abnormal spindle formation, with highly disorganized **prometaphase I** spindles exhibiting untapered poles, chromosomes that fail to align properly on the spindle, and centromeres that fail to biorient toward opposite spindle poles (Tavosanis *et al.* 1997; Hughes *et al.* 2011). Additionally, D-TACC localization to the spindle is aberrant in γ*Tub37c* mutants, and live imaging of mutant γ*Tub37c* oocytes revealed spindles that underwent dynamic movements and changes in shapes, as well as chromosome fluctuations (Hughes *et al.* 2011).

It is believed that γ-Tubulin functions in a complex called the γ-Tubulin ring complex (γTuRC), which is thought to be recruited to some microtubules by the protein complex Augmin (Meireles *et al.* 2009; Reschen *et al.* 2012). In meiosis I, Augmin components localize predominantly to the spindle poles, where they likely promote microtubule assembly (Meireles *et al.* 2009; Colombie *et al.* 2013). Loss of the Augmin complex component *wee Augmin* (*wac*) in oocytes results in increased chromosome movement on the meiosis I spindle and a failure of chromosomes to achieve proper alignment (Meireles *et al.* 2009; Colombie *et al.* 2013). Furthermore, mutation of the protein

Dgp71WD, which functions with the γTuRC complex, results in meiosis I spindles that are narrower than wild-type, with reduced or absent D-TACC localization (Reschen *et al.* 2012).

Mutations in many spindle assembly proteins lead to sterility due to failures in meiosis and the early mitotic divisions in the embryo. This sterility has made the study of many of these proteins during meiosis difficult until the recent use of stage-specific RNAi lines.

Chromosome movement, biorientation, and the polar ejection force

While a proper bipolar spindle is necessary for proper biorientation and segregation of homologous chromosomes, it is not sufficient to ensure that these processes occur correctly. For correct chromosome alignment and biorientation on the spindle, kinetochores must also properly attach to the kinetochore microtubules during acentrosomal spindle assembly, and inappropriate kinetochore–microtubule attachments must be destabilized. The EB1 effector protein Sentin appears to be important for this process in *Drosophila* oocytes (Gluszek *et al.* 2015). Mutation of Sentin results in a decrease in chromosome movement in prometaphase I and a failure of homologous centromeres to separate and biorient on the bipolar spindle (Gluszek *et al.* 2015). Although the meiotic spindle is mostly normal in *sentin* mutant oocytes, localization of the RZZ (Rod-Zw10-Zwilch) complex component Rough deal (Rod), which is part of the spindle assembly checkpoint, indicates that kinetochores become stably attached to spindle microtubules too quickly after spindle assembly, often to the same pole. Indeed, deficiencies in kinetochore components also cause a failure in chromosome orientation and movement (Unhavaithaya and Orr-Weaver 2013; Radford *et al.* 2015).

In addition, as the spindle elongates during prometaphase I, chiasmata hold exchange chromosomes together in the middle of the spindle while achiasmate chromosomes move toward the poles (Figure 7). The rate of movement of achiasmate chromosomes to the poles is size-dependent, as evidenced by the smaller fourth chromosome proceeding to the pole before the larger *X* chromosome. The size-dependent nature of migration location was confirmed by Sullivan and Karpen, who observed that a small *X* chromosome duplication—smaller in size than a fourth chromosome—migrated farther to the pole than the fourth chromosome itself (W. Sullivan and G. Karpen, personal communication). Chromosomes are pushed away from the poles and toward the spindle equator by the **polar ejection force**. No distributive disjunction (Nod), a kinesin-like protein (discussed in detail in *Distributive mutants*), helps to ensure that achiasmate chromosomes do not migrate off the spindle by acting as a brake on chromosome movement toward the spindle poles (Theurkauf and Hawley 1992; Afshar *et al.* 1995). The elimination of this brake is clearly seen in *nod* mutant oocytes, where the inherently achiasmate fourth chromosome is essentially always found in the cytoplasm (Theurkauf and Hawley 1992; Hughes *et al.* 2009).

Meiosis I segregation

In mitosis as well as meiosis II, sister chromatids segregate away from each other at anaphase, but in meiosis I, homologous chromosomes must biorient toward opposite spindle poles and segregate away from each other at anaphase I. While correct positioning and orientation on the meiotic spindle are essential for proper homologous chromosome segregation at the first meiotic division, crossing over and the formation of chiasmata provide the force needed to hold bivalents together. This force balances the tension on the kinetochores of homologous chromosomes from the opposing spindle poles to properly biorient homologous centromeres at the metaphase plate until chromosome segregation occurs at anaphase I. Indeed, chromosomes that fail to recombine often fail to properly segregate away from each other. Additionally, at least one pair of homologous chromosomes must form a chiasma to generate the tension required for oocytes to maintain metaphase I arrest (Jang *et al.* 1995). In the absence of chiasmata, oocytes precociously enter anaphase I. Finally, when chromosomes fail to become balanced at the middle of the meiosis I spindle, the forces of the microtubules, kinesins, and other proteins progressively move the chromosomes apart. The chromosomes can move so far apart, in fact, that they may nucleate multiple, separate spindles. Thus, although a byproduct of recombination is to increase genetic diversity, its primary function is to ensure proper chromosome segregation at anaphase I.

The distributive system

Although recombination is the primary system for ensuring segregation of the autosomes in *Drosophila* females, the fourth chromosomes never undergo recombination in wild-type females and the *X* chromosomes fail to recombine in 8–10% of oocytes (Hawley *et al.* 1993). Additionally, when a normal-sequence *X* chromosome is heterozygous to a balancer *X* chromosome, the *X*'s will fail to undergo recombination. Nonetheless, such achiasmate *X* and fourth homologs still segregate faithfully to opposite poles in $>$ 99% of wild-type oocytes using what has been termed the **distributive system** (Hawley *et al.* 1993). This mechanism of segregating achiasmate chromosomes differs from the segregation of achiasmate chromosomes in male meiosis, where recombination never occurs.

Heterochromatic sequences on the *X* and fourth chromosomes are found to be both necessary and sufficient for the distributive system to properly segregate achiasmate chromosomes (Hawley *et al.* 1992; Karpen *et al.* 1996). More importantly, heterochromatic associations have been shown to exist between *X* and fourth homologs during prophase and these associations persist until just prior to GVBD (Dernburg *et al.* 1996). Additionally, heterochromatic DNA threads have been shown to connect achiasmate chromosomes even after spindle assembly, as discussed in the next section (Hughes *et al.* 2009).

Achiasmate chromosomes undergo dynamic movements: Upon assembly of the bipolar spindle, the achiasmate chromosomes undergo dynamic movements toward the spindle

poles and back toward the center of the spindle (Figure 7). These dynamic movements, during which homologs may at times associate on the same side of the spindle, continue until all achiasmate homologs have properly bioriented and have congressed to join the chiasmate autosomes that are properly balanced at the central spindle (Gilliland *et al.* 2009; Hughes *et al.* 2009). After achiasmate chromosomes have congressed, the chromosomes form a compact lemon-shaped structure with the centromeres of homologous chromosomes bioriented toward opposite spindle poles (Figure 7) (Gilliland *et al.* 2009).

It is not fully clear why chromosomes undergo dynamic movements on the spindle during prometaphase I. One possibility is that chromosomes make improper centromere attachments to the kinetochore microtubules at spindle assembly, and these chromosome movements can facilitate the shedding of incorrect attachments and allow for new, correct attachments to form. This idea is supported by data from *sentin* mutants (described in *Chromosome movement, biorientation, and the polar ejection force*). Oocytes mutant for *sentin* display decreased chromosome movement, precocious stable attachment of kinetochore microtubules to centromeres (as based on Rod localization), and, ultimately, centromeres that remain maloriented (Gluszek *et al.* 2015). Chromosomes undergoing movements would need a mechanism to help ensure that achiasmate homologs end up on opposite half-spindles and biorient in the absence of chiasmata.

During the chromosome movements, DNA threads composed of heterochromatin connect achiasmate homologs (Hughes *et al.* 2009). These threads may allow achiasmate chromosomes to maintain contact with their homolog in the absence of chiasmata and thus facilitate proper orientation toward opposite spindle poles (Hughes *et al.* 2009). These DNA threads are likely resolved by Topoisomerase 2 (Top2). Knockdown of *Top2* by RNAi results in chromosomes that fail to properly separate and biorient on the meiosis I spindle, and causes the heterochromatic regions of chromosomes to become highly stretched out in many prometaphase I oocytes (Hughes and Hawley 2014). These oocytes fail to properly separate homologs at anaphase I when *Top2* is decreased, indicating that the heterochromatic connections must be resolved before homologous chromosome segregation.

Heterologous segregation: While heterochromatic homology appears to be the primary mechanism for segregating achiasmate homologs, other mechanisms likely influence the segregation of chromosomes that do not have a homolog or **heterologous segregation** (Table 5). In *D. melanogaster*, flies with compound chromosomes carrying two arms of the same chromosome attached together are viable. Flies have been generated carrying two sets of compound chromosomes, such as an attached *X* chromosome and an attached fourth chromosome, or compound *2L* and compound *2R* chromosomes. In these instances, the compounds segregate away from each other with extremely high fidelity, based on both genetics and the orientation of chromosomes after spindle assembly (Grell 1963, 1970; Gilliland *et al.* 2014). Thus, a second mechanism may exist that orients chromosomes lacking homologs to ensure balanced chromosome segregation. Interestingly, reduced achiasmate chromosome movement is observed when an attached fourth chromosome is present without a normal fourth chromosome, indicating that the fourth chromosomes influence chromosome movement (Gilliland *et al.* 2014).

Another example where a chromosome must segregate in the absence of a homolog is in *XXY* females. Bridges (1916) [reviewed in Ganetzky and Hawley (2016)] observed that in *XXY* females where the X chromosomes recombine, the *X* chromosomes segregate away from each other with the *Y* segregating at random. When the *X* chromosomes are achiasmate, the *Y* chromosome associates with the *X* chromosomes, resulting in their aberrant coorientation after spindle assembly and ultimately in their missegregation (Bridges 1916; Xiang and Hawley 2006). This suggests that the segregation of both achiasmate chromosomes and chromosomes without homologs may be complex.

Distributive mutants: Some mutants display a stronger effect on the segregation of achiasmate chromosomes than on the segregation of chiasmate chromosomes. This can be viewed as a defect in the distributive system, thus these mutants are termed distributive mutants. As mentioned above, mutations that affect spindle assembly cause defects in proper chromosome biorientation during meiosis I. Chiasmata can partially compensate for a poor meiotic spindle or aberrant forces on the chromosomes. However, chromosomes that are achiasmate, and therefore not held together by chiasmata, are especially prone to biorientation errors when the spindle fails to properly form. Chromosomes that depend solely on heterochromatic associations for accurate segregation require tapered, bipolar spindles and balanced forces to maintain proper alignment. The genes most strongly associated with distributive segregation are *nod*, *Aberrant X segregation* (*Axs*), *monopolar spindle 1* [*mps1*, originally known as *altered disjunction* (*ald*)], and *mtrm*.

One of the first identified distributive mutants was *nod* (Baker and Carpenter 1972). The *nod* gene encodes a kinesin-like protein believed to act as the polar ejection force, which is the force that pushes chromosomes back to the metaphase plate (Theurkauf and Hawley 1992; Afshar *et al.* 1995; Cochran *et al.* 2009). The polar ejection force acts against the forces of the kinetochore microtubules that pull centromeres of chromosomes toward the spindle poles. Studies of Nod, including the determination of its crystal structure, reveal that while Nod has homology to kinesins, it is nonmotile (Afshar *et al.* 1995; Cochran *et al.* 2009). Instead, it binds to microtubules and chromosomes to act as a brake for chromosome movement (Afshar *et al.* 1995; Cochran *et al.* 2009). Analysis of fixed and live *nod* mutant oocytes shows that during the dynamic movements achiasmate chromosomes undergo on the meiosis I spindle, achiasmate chromosomes are often ejected from the primary spindle containing the

chiasmate chromosomes (Theurkauf and Hawley 1992; Hughes *et al.* 2009). Although these lost chromosomes can nucleate their own mini-spindles, they may not always participate in the normal segregation of chromosomes at anaphase of meiosis I and II. Because achiasmate chromosomes are lost from the primary spindle in *nod* mutant oocytes, mutations in *nod* result in achiasmate chromosome nondisjunction values > 50% (Carpenter 1973; Rasooly *et al.* 1994). In the absence of Nod, chiasmata are still able to hold chiasmate chromosomes at the metaphase plate, which allows for the normal segregation of these chromosomes (Carpenter 1973).

The meiosis I spindle is surrounded by a membranous sheath to which the protein Axs localizes (Kramer and Hawley 2003a). A dominant mutation in *Axs*, Axs^D, causes the formation of improperly tapered meiosis I spindles (Kramer and Hawley 2003a). Yet, unlike other spindle mutants, Axs^D appears to disrupt only the segregation of achiasmate chromosomes (Zitron and Hawley 1989; Kramer and Hawley 2003a). Spindles in Axs^D flies are still bipolar, but they are much broader than in wild-type flies (Kramer and Hawley 2003a). Additionally, overexpression of the Axs^D mutant protein leads to a failure to maintain metaphase I arrest in some oocytes (Kramer and Hawley 2003a). Axs has homology to a family of calcium-activated chloride channels (Kramer and Hawley 2003b; Hartzell *et al.* 2009), but further investigation is needed to fully understand the wild-type function of Axs, as well as the mutant phenotype of Axs^D, during meiosis.

Achiasmate chromosome segregation is strongly affected by the haplo-insufficient mutation *mtrm* (Harris *et al.* 2003). While heterozygosity for *mtrm* mutations leads to near random segregation of achiasmate chromosomes, homozygosity leads to sterility due to meiotic catastrophe after meiosis I spindle assembly (Xiang *et al.* 2007; Bonner *et al.* 2013). The Mtrm protein appears to be a physical inhibitor of Polo kinase (described in *GVBD*), which regulates a number of processes in mitosis in addition to cell cycle entry (Archambault and Glover 2009). Strong knockdown of *polo* leads to spindle and chromosome alignment defects (Das *et al.* 2016), while the probable increase in unregulated Polo in *mtrm* mutant oocytes likely leads to the failure of achiasmate chromosomes to properly biorient. A mutation of *greatwall* (*gwl*) that causes decreased Polo activity in meiosis can suppress the nondisjunction of *mtrm* heterozygotes, further illustrating the interaction of Mtrm and Polo (Archambault *et al.* 2007).

Another example of distributive mutants that undergo improper cell-cycle regulation and highly increased achiasmate chromosome nondisjunction are female germline-specific alleles of *mps1/ald*. Mutations in *mps1* appear to alter the timing of meiosis I events, with chromosomes undergoing anaphase I-like separation very quickly after meiosis I spindle assembly (Gilliland *et al.* 2005, 2007). Achiasmate chromosomes undergoing movements to biorient on the bipolar spindle likely become trapped on the same side of the meiotic spindle at the time of the precocious chromosome segregation, thus leading to achiasmate chromosome nondisjunction (Gilliland *et al.* 2007).

The mutants affecting achiasmate chromosome segregation disrupt proteins that appear to play diverse roles. This indicates that the distributive system may be complex, with multiple components.

Meiosis II

Drosophila oocytes can remain arrested at metaphase I for up to 2 days (King 1970). In *Drosophila* oocytes, it is passage through the oviduct, rather than fertilization, that leads to oocyte activation and the resumption of meiosis (Doane 1960; Mahowald *et al.* 1983; Page and Orr-Weaver 1997; Heifetz *et al.* 2001; Horner and Wolfner 2008). After activation, anaphase I and all of meiosis II can be completed within ~20 min, making analysis of the events after metaphase I difficult (Riparbelli and Callaini 1996). Upon activation, there is a wave of Ca^{2+} in the oocyte indicating that, like in other organisms, Ca^{2+} signaling plays an important role in activation (Horner and Wolfner 2008; Kaneuchi *et al.* 2015). Consistent with Ca^{2+} playing a role in activation, the progression from anaphase I to meiosis II is dependent on the calcineurin pathway (Horner *et al.* 2006; Takeo *et al.* 2006, 2010, 2012). Mutation in either *calcineurin B2* (*canB2*) or *sarah* (*sra*), which interacts with and regulates calcineurin activity, leads to an arrest at anaphase I, with chromosomes stopped before reaching the spindle poles (Horner *et al.* 2006; Takeo *et al.* 2006, 2010). Both failure to translate bicoid mRNA and elevated cyclin B levels, which are indicative of a failure to enter meiosis II, support that Sarah is required for full egg activation (Horner *et al.* 2006).

Upon activation, the spindle rotates to align at a right angle with the anterior–posterior axis of the oocyte and the CR of the spindle elongates (Endow and Komma 1998; Riparbelli and Callaini 1996). As the chromosomes move toward the spindle poles in anaphase I, the center of the spindle pinches in between the chromosomes and an aster of microtubules forms between the separating chromosomes (Endow and Komma 1998; Riparbelli and Callaini 1996). This central aster of microtubules progresses to form a central spindle pole body composed of centrosomal components (Riparbelli and Callaini 1996, 2005; Endow and Komma 1998; Llamazares *et al.* 1999). Loss of the centrosomal protein Centrosomin leads to an aberrant central aster between the developing prometaphase II spindles, illustrating the requirement for centrosomal proteins for the formation of the central aster (Riparbelli and Callaini 2005). After prometaphase II, the sister chromatids segregate away from each other on the twin spindles to rapidly complete the second meiotic division (Riparbelli and Callaini 1996; Endow and Komma 1998).

The γTuRC binds to microtubule minus ends and stimulates microtubule-nucleating activity *in vitro* (Zheng *et al.* 1995; Moritz and Agard 2001). In mutants affecting two components of the γTuRC (*Grip75* and *Grip128*), meiosis I spindle formation appears mostly normal, but meiosis II spindles are severely disrupted (Vogt *et al.* 2006). The central aster of microtubules between the two tandem spindles is absent, the two spindles fail to remain properly aligned with

Table 5 Glossary of terms

Term	Definition
Acentric fragment	A chromosome fragment that does not contain a centromere
Acentriolar spindle	A spindle lacking centrioles, most commonly found during meiosis I in oocytes; also known as an anastral spindle
Achiasmate chromosome	A chromosome that does not form a chiasma (CO)
Balancer chromosome	A chromosome that is multiply inverted and/or rearranged in comparison to the normal chromosome and that, when heterozygous with a normal-sequence chromosome, suppresses exchange and/or prevents the recovery of recombinant products; typically carries both a dominant visible marker and a homozygous lethal or sterile mutation
Biorientation	The alignment of homologous chromosomes toward opposite spindle poles during meiosis I
Bivalent	A pair of homologous chromosomes held together by at least one chiasma
Class I CO	The predominant type of exchange event whose placement is influenced by interference and the centromere effect
Class II CO	A CO that is placed in a manner independent of other COs or the centromere
Central element (CE)	As observed by electron microscopy, the electron-dense region of the SC that lies within the CR, midway between the lateral elements
Central region (CR)	The portion of the SC between the lateral elements that encompasses the space between two homologous chromosomes; includes the transverse filament and CE proteins
Centromere clustering	The association of the paired homologous centromeres of one chromosome with the paired centromeres of other, nonhomologous chromosomes
Centromere effect	The strong suppression of exchange events in proximity to the centromere
Centromere pairing	The association, mediated by SC proteins, of the centromeres from two homologous chromosomes
Chiasma (pl. chiasmata)	A mature CO that physically links two nonsister chromatids; required for accurate meiotic chromosome segregation
Crossing over	The process that exchanges genetic material between homologous chromosomes, leading to the formation of chiasmata and recombinant chromosomes
Crossover (CO)	Event marking the location in the DNA where two nonsister chromatids exchange their genetic material; the physical outcome of the process of crossing over
CO assurance	The observed phenomenon where each chromosome is guaranteed at least one CO during meiosis
Diakinesis	In most organisms, the last phase of meiotic prophase I, during which individual chromosomes further condense in preparation for germinal vesicle breakdown and spindle assembly; *Drosophila* lacks a canonical diakinesis
Dicentric bridge	An aberrant chromosome with two centromeres that is pulled apart or broken during segregation, resulting in two unstable chromosomes
Diplotene	In most organisms, the phase of meiotic prophase I in which the SC disassembles and chromosomes become individualized; *Drosophila* lacks a canonical diplotene
Distributive system	Term applied to the mechanism for properly segregating chromosomes that failed to undergo exchange; denotes two very separate processes: the mechanism that ensures the segregation of nonexchange homologs and a poorly understood process that can segregate nonhomologous chromosomes
Double-strand break (DSB)	A break that involves both of the Watson and Crick strands of a DNA molecule
E_0 tetrad	A tetrad having no CO events; the fraction of E_0 tetrads is used to calculate the likelihood that a pair of homologous chromosomes did not undergo crossing over
Equational exception	Exceptional progeny resulting from nondisjunction at meiosis II
Euchromatin	The more lightly-condensed portion of a chromosome that contains the majority of an organism's genes
Gene conversion	The nonreciprocal exchange of a small amount of genetic information from one homologous chromosome to the other, resulting in a 3:1 allele ratio at that locus; can occur in association with a CO or independently of crossing over
Germarium	The structure at the tip of the ovary where egg chambers are formed and meiosis is initiated
Germinal vesicle breakdown (GVBD)	The breakdown of the nuclear envelope at the end of stage 12 of oocyte development
Heterochromatin	Tightly condensed chromatin that contains few genes and often contains repetitive DNA sequences
Heterologous segregation	The segregation of nonhomologous chromosomes away from one another
Interchromosomal effect	The phenomenon whereby heterozygosity for chromosome aberrations, especially balancer chromosomes, suppresses crossing over between those homologs but increases crossing over on the other unbalanced chromosomes
Interference	The phenomenon in which a CO in one interval decreases the likelihood of a CO in an adjacent interval
Karyosome	The condensed oocyte nucleus that forms in midprophase
Lateral element (LE)	The outermost components of the SC that run along the axis of two homologous chromosomes
Marker	A mutation that produces an innocuous, easily identifiable visible phenotype, such as eye color, wing attribute, or bristle quality
Matroclinous females	Exceptional progeny derived from *XX* oocytes, also known as diplo-*X* exceptions
Meiotic mutant	A stock bearing a mutation in a gene involved in a meiotic process
Missegregation	See ***Nondisjunction***
Noncrossover (NCO) event	A gene conversion that is not associated with a CO event

(continued)

Table 5, *continued*

Term	Definition
Nondisjunction	The aberrant segregation of chromosomes during meiosis, such that both homologs (meiosis I) or sister chromatids (meiosis II) go to the same daughter nucleus
Nurse cells	Polyploid, interconnected support cells that produce proteins and RNAs needed by the developing oocyte
Ovariole	One of ~16 tubules in each *Drosophila* ovary that contains a germarium and developing egg chambers connected sequentially from stage 1 through stage 14
Paracentric inversion	A chromosome inversion that does not include the centromere
Pachytene	Phase of meiotic prophase I during which paired chromosomes recombine and full-length SC is established; in flies, this begins in region 2A in the germarium
Patroclinous males	Exceptional progeny derived from oocytes lacking an *X*; also known as nullo-*X* exceptions
Pericentric heterochromatin	Recombinationally inert portion of a chromosome located near the centromere
Pericentric inversion	A chromosome inversion that encompasses the centromere
Polar ejection force	A force that pushes chromosomes away from the spindle poles and toward the central spindle during prometaphase
Prometaphase I	An intermediate period between prophase and metaphase I during which the nuclear envelope breaks down, the bipolar spindle elongates, and chromosomes undergo dynamic movements on the spindle
Recombination hotspot	A location or particular DNA sequence in the genome that exhibits a higher rate of meiotic DSB formation and/or recombination than expected by chance
Recombination nodule (RN)	Protein structure associated with the SC that mediates CO/chiasma formation
Reductional exception	Exceptional progeny resulting from nondisjunction at meiosis I
Sister chromatid exchange	Meiotic exchange that occurs between sister chromatids rather than homologous chromosomes
Synapsis	The full-length alignment of homologous chromosomes by the SC in preparation for recombination
Transverse filament (TF)	Coiled-coil-containing SC protein that functions to span the distance between the two lateral elements
Triploid	An individual with three sets of each chromosome
Synaptonemal complex (SC)	The proteinaceous, zipper-like structure that connects homologous chromosomes during prophase
Vitellarium	The posterior portion of an ovariole that contains oocytes in developmental stages 2–14
Zygotene	Phase of meiotic prophase I during which homologous chromosomes begin to pair and synapse; characterized in flies by the observation of short patches of synaptonemal complex in early region 2A of the germarium

each other and/or with the cortex, and spindles are often disorganized. Similar phenotypes are observed at meiosis II in oocytes mutant for *mushroom body defect* (*mud*) (Yu *et al.* 2006). The Mud protein localizes to the region between the tandem meiosis II spindles.

A hypomorphic allele of polo kinase, *polo*^{1}, which can complete meiosis I, causes defects in meiosis II spindle formation (Riparbelli *et al.* 2000). The twin spindles are frequently misaligned, and the central array of microtubules improperly organized, and the chromosomes fail to stay properly aligned on the defective spindles (Riparbelli *et al.* 2000). Polo kinase appears to play roles in both meiotic divisions of *Drosophila* oocytes.

In meiosis I, cohesion between the arms of the homologs is cleaved to allow separation of homologs at anaphase I, but cohesion at sister centromeres is maintained until meiosis II by the Shugoshin homolog Mei-S332 (Kerrebrock *et al.* 1992). Mutation of *mei-S332* causes precocious loss of centromeric cohesion and the eventual random segregation of sister chromatids in meiosis II (Davis 1971; Kerrebrock *et al.* 1992). Mei-S332 localizes near centromeres until the metaphase II/anaphase II transition, consistent with its role in protecting centromeric cohesion (Kerrebrock *et al.* 1995).

Mutation of *cortex* or its transcriptional activator *grauzone* primarily results in meiotic arrest at anaphase II, but both mutants also display defects in anaphase I chromosome segregation (Page and Orr-Weaver 1996). A smaller proportion of oocytes arrest in meiosis I or exhibit aberrant spindle and chromosome configurations, and cortical microtubules fail to rearrange in response to activation. Cortex is a Cdc20-related protein that, with the mitotic Cdc20 homolog Fizzy (Fzy), works in the anaphase-promoting complex (APC) to destroy Cyclins A, B, and B3 in female meiosis to allow for proper cell cycle progression (Swan and Schupbach 2005, 2007; Pesin and Orr-Weaver 2007). Cks30A promotes the activation of the APC that utilizes Cortex (Swan and Schupbach 2007), and Cortex is also required for the destruction of the Polo inhibitor Mtrm during meiosis II to allow proper entry into the embryonic mitoses (Whitfield *et al.* 2013). These studies demonstrate the importance of the degradation of meiotic proteins before the onset of embryonic mitoses.

Because meiosis II is completed so rapidly, much of our knowledge is based on a limited set of mutants that cause arrest at various stages of meiosis II. There is still much to learn about the regulation of meiosis II.

Conclusions

A century of genetic studies in *Drosophila* are now embedded in superb cytological techniques. More than ever, we can imagine taking the genetic formalisms that resulted from the first 100 years of meiotic mutant analysis and inserting them in the realities of meiotic cell biology. Indeed, studies examining the genetics and biology of female meiosis have revealed:

1. The SC is assembled and disassembled in a highly organized fashion.
2. Components of the SC play important roles in regulating CO formation, centromere clustering, and pairing.

3. There are two classes of COs and multiple proteins play roles promoting the formation of class I COs (interference-sensitive) over class II COs (interference-insensitive).
4. Formation of a tapered, bipolar meiosis I spindle is important for proper segregation of homologous chromosomes.
5. The distributive system helps ensure the segregation of achiasmate chromosomes.
6. Meiosis II occurs rapidly with sister chromatids segregating away from each other on twin spindles.

However, many important questions remain to be fully answered. For example, how are the number and placement of DSBs and COs chosen? What regulates the assembly and disassembly of the SC? What proteins play a role in the RN? How are meiosis I and II entry and exit controlled? As new advances in imaging, sequencing, and biochemistry are developed, our understanding of the processes regulating meiosis will only deepen.

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