When specialized sites are important for synapsis and the distribution of crossovers

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Summary

In *C. elegans* and *D. melanogaster*, specialized sites have an important role in meiotic recombination. Recent evidence has shown that these sites in *C. elegans* have a role in synapsis. Here we compare the initiation of synapsis in organisms with specialized sites and those without. We propose that, early in prophase, synapsis requires an initiator to overcome inhibitory factors that function to prevent synaptonemal complex (SC) formation between nonhomologous sequences. These initiators of SC formation can be stimulated by crossover sites, possibly other types of recombination sites and also specialized sites where recombination does not occur. *BioEssays* 29:217–226, 2007.
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Meiosis: juggling synapsis and recombination

Meiotic prophase involves two striking interactions between the homologous chromosomes. The first is the tight alignment, or synapsis, of homologs along their entire length and the second is the recombination of genetic material. Instead of thinking about these two as parallel processes, it has become clear that the interaction between genetic recombination and synapsis is complex. Meiotic recombination initiates with a double-strand break (DSB) in probably all organisms. Furthermore, it is so far without exception that Spo11, a TopoVI-like protein, is required for meiotic DSB formation.[1] The pathway of meiotic DSB repair has been reviewed extensively[2,3] but two important features are (1) single-stranded DNA from the broken chromatid undergoes a homology search for a repair

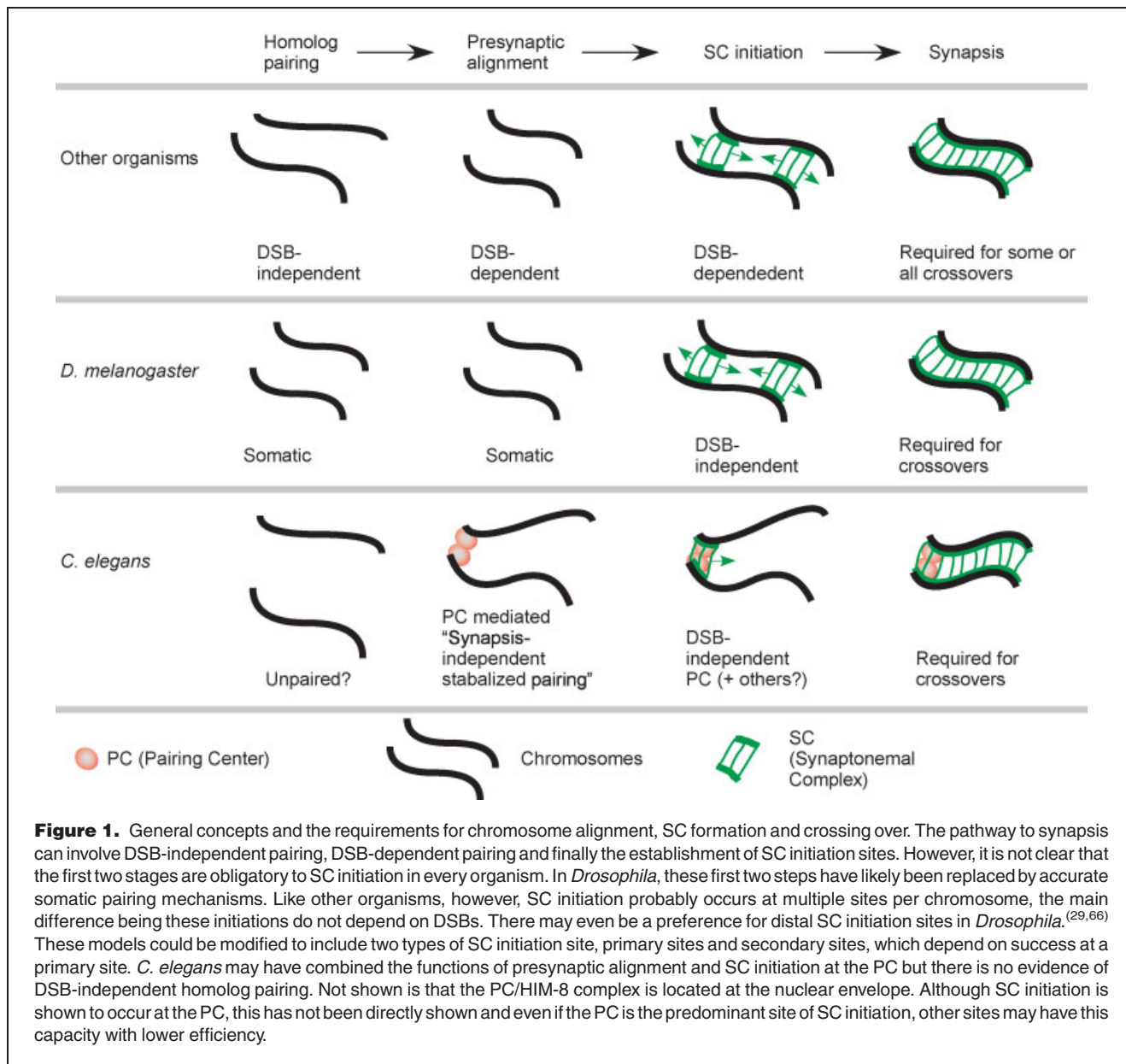Waksman Institute and Department of Genetics, Rutgers, the State University of New Jersey, Piscataway NJ.

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Abbreviations: DSB, double-strand break; SC, synaptonemal complex; PC, pairing center; IH, intercalary heterochromatin; FISH, fluorescent in situ hybridization; ZMM, Zip Mer2 Msh complex; RN, recombination nodule; EM, electron microscopy.

template and (2) progression into the repair process leads to recombination intermediates that can be resolved into either simple gene conversions or a crossovers. Crossovers are important due to their role in chromosome segregation. At diplotene, crossovers appear as chiasmata and provide a link between the homologs, facilitating homolog orientation and segregation on the meiosis I spindle.

The whole process may begin with a type of DSB-independent homolog interaction,[4–6] which could include events near the telomeres[7,8] and whose relationship to the later DSB-dependent events is poorly defined. Subsequently, but prior to synapsis, it is often possible to identify a stage of presynaptic alignment that may depend on DSBs and serves to align the axis of homologous chromosomes at a distance of 300–400 nm.[6,9] Finally, synapsis stabilizes the homologs at a distance of approximately 100nm as they are held together by the SC, a meiosis specific structure conserved in most organisms.[10,11] Organisms with SC depend on its components for many or all of their crossovers. For example, all crossing over is eliminated in mutants lacking transverse element components of the SC in *C. elegans*[12,13] and *D. melanogaster*.[14,15]

It might have been expected that the close pairing of the homologs would precede DSBs in order to promote recombinational repair between homologs. In fact, the opposite approach appears to be the favored mechanism as a recombination-based process stimulates synapsis in a variety of organisms, such as budding yeast and other fungi, mice and plants such as *Arabidopsis*.[1,16] While DSBs can generate a substrate for homology searching and a mechanism for aligning chromosomes,[17] synapsis initiation appears to be more complicated. Studies in fungi have suggested that there are two stages of DSB-dependent pairing, presynaptic alignment and synapsis, and that each require a different number of DSBs[18–20] (Fig. 1). Presynaptic alignment requires fewer DSBs than synapsis. To explain the requirement for additional DSBs to promote synapsis, is has been suggested that SC only initiates at a subset of DSB sites (see below).[6]

Some organisms, however, do not require DSBs for synapsis. Studying meiosis in these systems has the advantage that the mechanism of SC formation has been

Figure 1. General concepts and the requirements for chromosome alignment, SC formation and crossing over. The pathway to synapsis can involve DSB-independent pairing, DSB-dependent pairing and finally the establishment of SC initiation sites. However, it is not clear that the first two stages are obligatory to SC initiation in every organism. In *Drosophila*, these first two steps have likely been replaced by accurate somatic pairing mechanisms. Like other organisms, however, SC initiation probably occurs at multiple sites per chromosome, the main difference being these initiations do not depend on DSBs. There may even be a preference for distal SC initiation sites in *Drosophila*.[29,66] These models could be modified to include two types of SC initiation site, primary sites and secondary sites, which depend on success at a primary site. *C. elegans* may have combined the functions of presynaptic alignment and SC initiation at the PC but there is no evidence of DSB-independent homolog pairing. Not shown is that the PC/HIM-8 complex is located at the nuclear envelope. Although SC initiation is shown to occur at the PC, this has not been directly shown and even if the PC is the predominant site of SC initiation, other sites may have this capacity with lower efficiency.

uncoupled from DSB formation. The objective of this review is to examine two well-studied cases, in *C. elegans* and *D. melanogaster*, where it has been shown that synapsis occurs without delay in the absence of DSBs.[21,22] Two important questions to address are: how is synapsis initiated when it does not require DSBs, and are there common features in the mechanism for SC initiation among those organisms that require DSBs for synapsis and those that do not? If there are similarities, studying the arguably simpler synapsis initiation mechanism in *C. elegans* and *D. melanogaster* may provide insights into the core of the synapsis initiation mechanism. These two organisms have another feature that has not been described in other organisms. In both organisms, special sites

are required for normal levels of crossing over. Here we will review the evidence for specialized meiotic sites and ask why they exist? Is their presence in these organisms related to the fact that they do not require DSBs for synapsis?

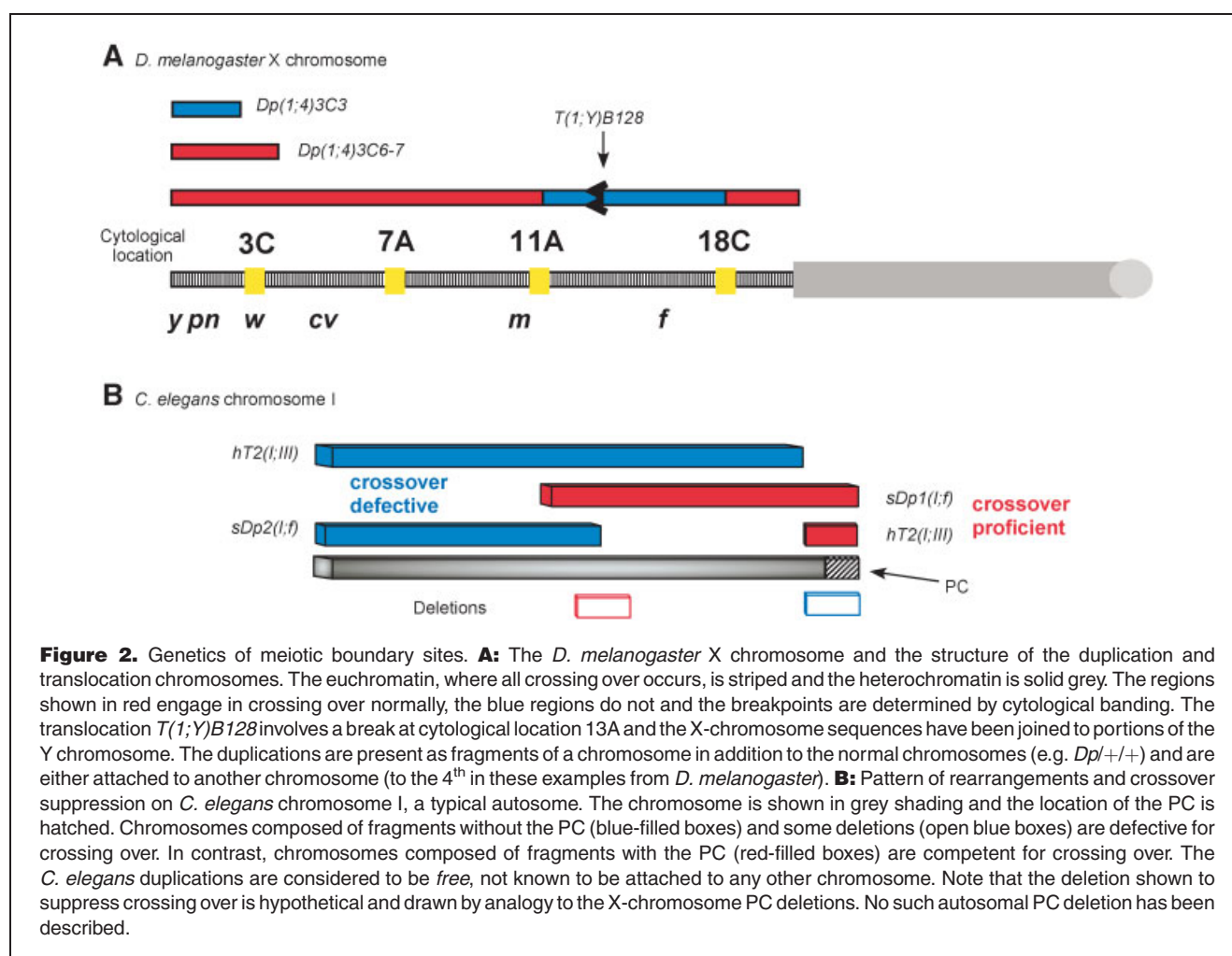**Translocations and inversions provide evidence for specialized meiotic sites in *D. melanogaster***
Translocations are region-specific crossover suppressors (as assessed by progeny counts) in *D. melanogaster*. A long-held belief has been that crossover suppression in translocation heterozygotes was due to defects in homolog pairing or synapsis.[23,24] Since heterozygosity for a single breakpoint reduces crossing over between two discrete boundaries, it has

been proposed that there are pairing sites that mediate homolog interactions. This idea was also inspired by the characterization of collochores in *Drosophila* males, which are sites where the X and Y chromosomes are attached in the absence of chiasmata,[25,26] and of heterochromatic sequences in *Drosophila* females, where achiasmate chromosomes pair.[27] As described below, however, the sites important for crossing over do not appear to be required for homolog pairing.

Whether involved in pairing or not, comparing the pattern of crossover suppression to the location of rearrangement breakpoints has been a successful method for identifying sites important for meiotic recombination. Based on the pattern of crossover suppression in translocation heterozygotes, Hawley[28] mapped four "pairing sites" on the X-chromosome (Fig. 2A). For example, the translocation *T(1;Y)B128*, with a break in cytological band 13A, exhibited 8.8% of wild-type crossing over in the *m–f* interval while having 82.0% of wild-type crossing over in the *w–m* interval (Fig. 2A).

Indeed, X-chromosome translocations with breaks anywhere between cytological divisions 11A and 18C suppressed crossing over between *m* and *f* but not between others markers. Experiments with free duplications showed that these sites did not act only in *cis*, but could compete with each other as well. A duplication [*Dp(1;4)/X/X*], which included the site at 3C, suppressed crossing over between *w* and *m* on two full-length X-chromosomes, even though the duplication included almost no material in this interval (Fig. 2A). A slightly shorter duplication, differing only by the lack of the 3C sequences, did not affect *w–m* crossing over. This result suggested that, by interacting with a normal X-chromosome in the 3C region, the longer duplication could prevent the other X-chromosome from engaging in crossovers between *w* and *m*.

Sherizen et al.[29] extended this type of study, using the extent of crossover suppression in translocation heterozygotes to map four sites on chromosome 3R. The most-surprising result came from fluorescent in situ hybridization

Figure 2. Genetics of meiotic boundary sites. **A:** The *D. melanogaster* X chromosome and the structure of the duplication and translocation chromosomes. The euchromatin, where all crossing over occurs, is striped and the heterochromatin is solid grey. The regions shown in red engage in crossing over normally, the blue regions do not and the breakpoints are determined by cytological banding. The translocation *T(1;Y)B128* involves a break at cytological location 13A and the X-chromosome sequences have been joined to portions of the Y chromosome. The duplications are present as fragments of a chromosome in addition to the normal chromosomes (e.g. *Dp/+/+*) and are either attached to another chromosome (to the 4th in these examples from *D. melanogaster*). **B:** Pattern of rearrangements and crossover suppression on *C. elegans* chromosome I, a typical autosome. The chromosome is shown in grey shading and the location of the PC is hatched. Chromosomes composed of fragments without the PC (blue-filled boxes) and some deletions (open blue boxes) are defective for crossing over. In contrast, chromosomes composed of fragments with the PC (red-filled boxes) are competent for crossing over. The *C. elegans* duplications are considered to be *free*, not known to be attached to any other chromosome. Note that the deletion shown to suppress crossing over is hypothetical and drawn by analogy to the X-chromosome PC deletions. No such autosomal PC deletion has been described.

(FISH) experiments, which showed that homolog pairing in translocation heterozygotes was normal throughout the crossover-suppressed region. This result was confirmed with the analysis of inversions on the X chromosomes by Gong et al.[30] These results led to the conclusion that homolog pairing defects are not the cause of the crossover suppression in translocation or inversion heterozygotes. For this reason, we now refer to these chromosome locations as boundary sites to represent the observation that a rearrangement breakpoint causes crossover suppression only between two sites (with one exception, see below). Indeed, both Sherizen et al.[29] and Gong et al.[30] found that homologs most likely enter meiotic prophase paired at multiple sites along their lengths. This highly accurate homolog pairing may be mechanistically related to similar events in somatic cells[31,32] and the mitotically dividing oogonial cells of the female germline.[33] It has not been determined if pairing is established rapidly after pre-meiotic DNA replication since pairing may be lost at S-phase,[34] or if pairing is maintained from an event earlier in development. Interestingly, the somatic pairing mechanism(s) is not sufficient to hold the homologs together throughout meiotic prophase. In c(3)G mutants, which lack SC, the homologs begin to separate as prophase progresses.[29,30] In short, D. melanogaster females initiate SC between homologs that are already precisely aligned and require synapsis to stabilize homolog pairing later in meiotic prophase.

To date, no DNA sequences have been associated with boundary sites. Hawley[28] suggested that the locations of the X-chromosome D. melanogaster sites corresponded to the known sites of intercalary heterochromatin (IH). The sites mapped by Sherizen et al.[29] may also correspond to these locations. IH contains repetitive DNA, is late replicating, enriched for certain proteins like HP1 and associates with the nuclear envelope.[35] Indeed, upwards of 15 sites per chromosome arm are predicted to interact with the nuclear envelope in somatic cells.[36] Although these sites have not been shown to correspond to known locations of IH and IH has only been described in somatic cells, all IH sites have probably not yet been identified. The suggestion by Hawley is intriguing, however, because it raises the possibility that the D. melanogaster boundary sites have some common characteristics to the C. elegans sites described below, such as interactions with the nuclear envelope.

The C. elegans pairing center and its role in synapsis and crossing over

As in D. melanogaster, crossover suppression is observed for a long distance from a C. elegans translocation breakpoint.[37,38] Unlike D. melanogaster, however, each C. elegans chromosome has only a single specialized site located near one end of each chromosome, referred to as a Pairing Center (PC). Chromosome fragments require only the PC end of a chromosome to be able to crossover with the homolog, while the complementary fragment lacking the PC, no matter how large, rarely experiences a crossover (Fig. 2B). The degree of crossover suppression is quite impressive. In the study of eT1(III;V), an interval of 8.6 cM was reduced to less than 0.3 cM.[37] Similar observations were made with duplications of the X chromosome[39] and the autosomes.[40,41] For example, crossing over between the free duplication sDp2(1:f) and the regular chromosome I was rare, occurring at a frequency of $<10^4$ even though the expected frequency based on the genetic length of the same interval on the intact chromosome I was ~25%.[40] Conversely, a duplication carrying the PC end of chromosome I engaged in crossing over frequently. These results show that long regions of homology are not sufficient to promote crossing over and the PC is required crossing over between homologs.

The most-detailed studies on the C. elegans PC function have been conducted on the X-chromosome. These studies revealed a dual role for the X-chromosome PC. The first is in homolog pairing as revealed by the observation of "synapsis-independent stabilization of pairing" which refers to the ability of the PC region of the chromosome to maintain pairing in the absence of synapsis.[12,13] Since synapsis-independent stabilization of pairing involves the interaction of two homologously paired copies of the PC in the absence of SC proteins, it may have similarities to presynaptic alignment observed in other organisms (Fig. 1). The second function is the promotion of synapsis. A deletion of the X-chromosome PC results in a disruption in synapsis, as shown by lack of staining with SC proteins like SYP-1.[42] Unlike the pairing function, it appears that one copy of the PC can initiate a low level of synapsis (see below). One consequence of these defects is that the PC deletions cause a decrease in crossing over and an increase in nondisjunction that is specific to the X-chromosome.[43]

The genetic evidence for the PC has been confirmed with the molecular analysis of him-8.[44] HIM-8 is a C2H2 zinc-finger protein required for synapsis and crossing over on the X-chromosome but not the autosomes. Since both him-8 mutants and PC deficiencies have similar effects on pairing, synapsis and crossing over, HIM-8 may be a protein required for PC activity. Indeed, HIM-8 localizes to the end of the X-chromosome containing the PC and this complex is closely associated with the nuclear envelope. These results raise the interesting possibility that pairing and synapsis in C. elegans involves the PC interacting with several proteins at the nuclear envelope. Interactions between telomeres and the nuclear envelope are thought to be important for homolog pairing in other organisms as well, but the link to synapsis is poorly understood.[7,8] These results in C. elegans may be the best current example linking chromosome contacts at the nuclear envelope to synapsis. There are, however, several important questions to be answered. For example, is the nuclear envelope association of the PC is important for synapsis and does it depend on HIM-8? Although HIM-8 may primarily

function at the PC, there is a small but significant increase in the severity of the synaptic defects in *him-8* mutants compared to a PC deficiency and synapsis is occasionally observed in PC deficiency heterozygotes. This could be explained by HIM-8 interacting with X-chromosome sequences other than those in the PC to promote synapsis and crossing over.

Do the specialized sites in *C. elegans* and *D. melanogaster* function in SC initiation?

An attractive model for both *C. elegans* and *D. melanogaster* is that these specialized pairing sites are locations for SC initiation. Since PC deletions severely disrupt SC formation in *C. elegans*, the primary location of synapsis initiation may be at or near the PC.[42] While synapsis may initiate at additional locations on a *C. elegans* chromosome, these events are relatively infrequent. Furthermore, *C. elegans* may have combined the steps of pairing (presynaptic alignment) and synapsis initiation at the PC. Unlike *Drosophila*, there is little evidence for premeiotic or DSB-independent homolog pairing mechanisms as a force for the meiotic alignment of homologs (Fig. 1). In *him-8* mutants, there is no difference in pairing of X-chromosome sites in premeiotic and meiotic cells.[44] Interestingly, when SC forms between homologs in the absence of the PC, synapsis is complete, suggesting that, once initiated, SC formation is highly processive. This is supported by the synapsis behavior of translocation heterozygotes. The results of FISH studies indicate that the translocations do not form a classical quadrivalent structure. Instead, six bivalents are formed because the crossover-suppressed regions (those sequences ''distal'' to the breakpoint relative to the PC) nonhomologously synapse.[42]

The idea that homologous synapsis proceeds from the PC up to the breakpoint nicely explains the close correspondence between crossover-suppression boundaries and the translocation breakpoint.[38,41] The continuation of synapsis into nonhomologous regions is both a striking example of processive synapsis and a failure to respect any constraints that prevent nonhomologous synapsis. These mechanisms depend on the *hop2* and *mnd1* gene products in yeast and mammals and prevent SC formation between nonhomologous sequences.[11,45,46] While these genes are not present in *C. elegans*, a similar mechanism to block nonhomologous synapsis probably exists, as shown by the recent findings that *htp-1* mutants exhibit nonhomologous synapsis.[47,48] In contrast, the PC appears to give sequences a license to synapse. Since synapsis occurs rapidly between homologous and nonhomologous sequences in translocation heterozygotes, homology may not be checked once initiated by the PC. The consequence of PC function, therefore, appears to promote synapsis regardless of proteins, like HTP-1, that function to prevent nonhomologous synapsis.

The relationship between the boundary sites in *D. melanogaster* and synapsis is less clear than in *C. elegans*.

In *D. melanogaster*, SC is usually present in the crossover-suppressed regions of translocation[29] and inversion[30] heterozygotes. Part of the reason why crossover suppression is usually more severe in *C. elegans* could be the more-extensive SC formation in *D. melanogaster* translocation heterozygotes. However, the resolution of these immunofluorescence studies could not rule out that SC structure is affected in *D. melanogaster*. The failure to observe frequent disruptions in synapsis could be explained if SC assembly progresses bidirectional from the initiation sites in *D. melanogaster*. Thus, even if SC assembly initiates at boundary sites, most regions in a translocation heterozygote would be associated with SC because either side of the breakpoint is still be linked to an SC initiation. The breakpoint will, however, prevent the SC from becoming continuous between two boundary sites. That breakpoints cause defects in synapsis is also supported the observation that, in a low frequency of oocytes, the staining of the SC protein C(3)G was missing or reduced in the crossover-suppressed regions of translocation[29] or inversion[30] heterozygotes. As described below, crossover suppression may occur due to the break in the chromosome axis, rather than simply the absence of SC.

Missing from the analysis of synapsis initiation in *C. elegans* and *D. melanogaster* is cytological observations of homolog pairing during zygotene. Part of the problem is that zygotene, when one would expect to observe evidence of SC initiation sites, is rapid in both organisms. In *D. melanogaster*, the axial elements do not form prior to assembly of transverse filaments.[49] Therefore, while zygotene has been described, the location of synaptic initiation sites cannot be determined by EM. Similarly, immunofluorescence studies have shown that two SC proteins, C(2)M and C(3)G, appear simultaneously during zygotene.[50] Since C(2)M is a Rec-8 family member and C(3)G is a transverse filament protein, these results are consistent with the EM data that lateral and transverse elements assemble at the same time in *D. melanogaster*. Based on immunofluorescence studies of zygotene in wild-type or early prophase in *c(2)M* mutants,[15,50] there is definitely more than one SC initiation site per arm in *D. melanogaster*, consistent with the mapping of approximately four sites per arm from the genetic studies. In *C. elegans*, there appears to be a brief time when the axial elements (detected using antibodies to Hop1 homologs HIM-3 and HTP-3 or REC-8)[12,13] form prior to the transverse filaments, making it formally possible to map SC initiation sites.

Chromosomal rearrangements create crossover suppression without affecting the initiation of recombination

In neither *C. elegans* nor *D. melanogaster* is the crossover suppression in rearrangement heterozygotes due to reductions in the initiation of recombination. Using γ-His2Av staining as a marker for DSBs in *D. melanogaster*, Gong et al.[30]

demonstrated that DSBs are induced in the crossover-suppressed regions. Similarly, Rad51 staining has been used to show that DSBs are induced when synapsis fails due to a PC deletion in *C. elegans*.[42] Therefore, in both organisms, the crossover defects may be a secondary consequence of a synapsis defect. Consistent with this conclusion is the observation that mutants lacking SC in *C. elegans*[12,13] and *D. melanogaster*[14,15] lack crossovers. Nonetheless, it is unclear how the DSBs induced in crossover-suppressed regions are repaired. Sherizen et al.[29] reported that gene conversion was also reduced in the crossover-suppressed regions. To reconcile this with the observation that DSBs are induced, it is possible that DSBs are repaired using the sister chromatids. This could explain what happens to the *C. elegans* DSBs in the crossover-suppressed regions, since they are present on unsynapsed chromosomes. Normally there are barriers to sister chromatid exchanges, such as axial element proteins HIM-3[51] and HTP-1.[47,48] But since at least some of these axial element proteins localize in synapsis-defective mutants and PC deletions,[42,44] DSB repair involving the sister chromatids would be occurring despite the presence of proteins that are supposed to prevent it.

The absence of crossing over can be attributed to the lack of SC in *C. elegans*. In *D. melanogaster*, however, the effect on crossing over in translocation heterozygotes is more severe than the synapsis defect. As described above, extensive SC may form in the crossover-suppressed regions, but it is not continuous between two boundary sites.[29] This could be critical. There is circumstantial evidence that crossover suppression can be caused by breaks in the structure of the SC. For example, in either a *c(3)G* mutant with a internal deletion of its coiled-coil[15] or *c(2)M* mutants,[50] many small segments of SC are assembled but never joined into long continuous threads and crossing over is severely reduced. One interpretation of these results is that repair of a DSB into a crossover requires long continuous segments of SC. A similar idea has been proposed by Zickler and Kleckner[10] based on the transmission of physical stresses, such as tension, along the chromosome cores. A role for properly assembled chromosomes axis has also been suggested in studies of *C. elegans*.[52] Since crossover suppression occurs despite the presence of SC proteins, continuity of SC structure, and not simply having SC proteins assembled, may be critical to stimulate crossing over in *D. melanogaster*.[29]

SC initiation sites in other organisms

A link between synapsis initiation and crossover sites has been proposed in several organisms. The evidence for this is strongest in budding yeast[20,53,54] and has been extensively reviewed.[2,6,11,16] The ZMM complex of proteins (including budding yeast Zip1, Zip2, Zip3, Mer3, Msh4, Msh5) is thought to be involved in both crossover-specific processing of DSBs and the nucleation of SC.[54] Consistent with this idea,

crossover specification in *S. cerevisiae* occurs very early in the recombination pathway, prior to DSB formation or during the initial stages of strand exchange.[2]

Some ZMM proteins are conserved in other organisms (such as Mer3, Msh4 and Msh5) but the link between crossovers and SC initiation has not been characterized to the same level of detail. Interestingly, mouse *msh4* and *msh5* mutants exhibit defects in synapsis.[55–57] Furthermore, some interesting correlations have been found between the cytologically observed distribution of recombination sites and patterns of synapsis. Recombination nodules (RNs) as seen through EM analysis are associated with meiotic chromosomes and SC during prophase. They are believed to be the sites of initiating and ongoing recombination and contain the appropriate proteins for the molecular events leading to crossover formation as well as other products of DSB repair. There are two types of RN based on morphology and timing: early RNs may be the sites of the earliest stages of DSB repair while late RNs are less frequent, have a distribution similar to crossovers and may indeed be those DSB sites that become crossovers.[58] Since RNs and the stages of SC assembly can be visualized simultaneously, the study of RN distribution has provided insights into the processes by which SC initiation and meiotic recombination are regulated. This type of analysis is not informative in *Drosophila*, however, since both types of RN do not appear until pachytene.[58]

SC often first initiates in distal regions[10] although, in many cases, particularly in plants, there are secondary interstitial initiations as well. In mammals, SC initiation may occur at fewer sites. A recent study of meiosis in human males concluded that SC initiation is reproducible, occurring at one site per chromosome arm at a subtelomeric location.[59] Similarly, a large fraction of the crossovers occur in these distal regions. Studies of early and late RNs in maize have shown a correlation between where synapsis initiates and crossovers form. This could also be related to the placement of early RNs, which show distal enrichment in some cases.[60] Interestingly, early RNs often appear at synaptic forks, providing evidence that sites engaged in the early stages of DSB repair can be synapsis initiation sites.[60,61] It is not known, however, if these are the subset that will become crossovers. Since it is only the earliest appearing RNs that are associated with synaptic forks and only if the first appearing early RNs are also the ones to become crossover sites, it is possible that the relationship between crossover distribution and synapsis initiation is indirect.

Synapsis initiation is not always associated with a crossover site. Particularly in plants, there are more initiation sites than crossover sites, and the distribution of chiasmata does not always reflect the pattern of SC initiation.[10,62,63] Thus, in these cases, there must be mechanisms for SC initiation that do not proceed through the crossover-specification mechanism. There are also situations in organisms with the

recombination-dependent pathway, such as yeast, where SC can occur in the absence of DSBs.[6,64] Together, these results show that SC can initiate independent of crossovers and possibly DSBs.

If SC initiates in distal regions, it might be predicted that chromosome rearrangements in these regions could have long-distance effects on synapsis and crossing over. An observation like this was made by Burnham et al.,[65] who cytologically characterized pairing in translocation heterozygotes of maize and concluded that the probability for an initial pairing between homologs was highest in the distal regions of each arm. Remarkably, almost identical conclusions were made by Roberts[66] in *D. melanogaster* based on the observation that translocations with distal break points suppressed crossing over throughout the arm of a chromosome (this phenomenon does not occur with X-chromosome translocations). These results were confirmed by Sherizen et al.[29] Why an organism that does not require DSBs for synapsis shows such a similarity with one that may require DSBs (although it has not been confirmed that maize requires DSBs for synapsis) is yet to be determined. One possibility is that there are dominant SC initiation sites even in organisms that have the potential to initiate SC at many (crossover or noncrossover) sites. Once SC is initiated at these presumably distal sites, subsequent events of SC initiation can occur at secondary sites.

In summary, there are several types of sites that may initiate SC in different organisms. Recombination sites destined to be crossovers, the earliest recombination sites to be initiated, recombination sites in a particular region of the chromosome or some other subset of recombination sites have been proposed to be sites where SC assembly can initiate. In addition, particularly in organisms where DSBs are not required, SC could be initiated by other types of sites, such as those at defined locations. In any of these cases, however, the connection between selecting a site to initiate SC formation and actually triggering the assembly of SC proteins has not yet been determined.

Specialized sites for checking homology

The studies in *C. elegans* and *D. melanogaster* summarized above emphasize the importance of chromosome structure on synapsis and meiotic crossing over. It has been demonstrated in these two organisms that the function of specialized sites can be disrupted by breaking the axial backbone of the meiotic chromosomes. We propose that specialized sites evolve for different reasons (see below) but their function is related to a requirement for all organisms to check for homology before the initiation of SC assembly (Fig. 1). Secondarily, this can have an effect on crossover control since the strength of interference or the distance over which SC can assemble from an initiation site may influence the distribution of crossovers.

In most organisms, SC may initiate at a select group of sites such as a subset of recombination sites and, in some cases, sites determined to become crossovers. These sites, however, are not at specific locations because DSBs occur at many sites. Linking synapsis to a select group of recombination sites, such as crossover sites, could be a method to regulate SC initiation and ensure its formation between aligned homologs. In contrast, specialized sites at defined locations could be the basis for initiating SC without DSBs. In either case, this process generates a small number of SC initiation sites at a stage in prophase when SC formation is strictly homologous. In contrast, nonhomologous synapsis occurs in some organisms at later stages of prophase and may reflect a relaxation of the constraints limiting SC formation to a homology check.[9,10] Therefore, in early prophase, there may be blocks to form SC that are alleviated at specific initiation sites providing a "license" to synapse.

As proposed by MacQueen et al.,[42] the PC could have a role in the check for homology between chromosomes. Transient stabilization at the PC end of the chromosome might allow homology to be checked, either in the region of the PC or chromosome wide. It remains to be determined whether homologous PCs preferentially interact or any two PCs can interact prior to a check for homology. The former would be consistent with the finding that other chromosomes probably use different HIM-8-like proteins. Although there is less supporting data in *D. melanogaster*, the current evidence does not rule out a role for the boundary sites at the interface between a homology check and SC initiation. Even in *D. melanogaster* when the chromosomes are prealigned, it may be necessary to check homology prior to SC formation.

The number of SC initiation sites may have an important impact on crossover frequency. This is based on the suggestion that interference is related to the capacity to assemble continuous SC for a long distance from the initiation point.[6] Below we invoke this to explain why *C. elegans* has only a single specialized site. For example, highly processive SC formation in *C. elegans* could lead to the high levels of interference observed in this organism.[67,68] This leads to a 1:1 relationship between SC initiation and crossover sites even though the two occur at distinctly separate locations.

Conclusion

We suggest that the specialized sites in *D. melanogaster* and *C. elegans* substitute for the function of recombination (or crossover) sites in providing a homology check prior to initiating SC formation (Fig. 1). This is a level of control that is probably present in all organisms, and involves restricting SC formation in early prophase to occur only between homologous regions of a chromosome. We also suggest that there are features of the mechanism of SC initiation in *D. melanogaster* and *C. elegans* that are conserved in many organisms. While in the minority when it comes to synapsis in

the absence of DSBs, *C. elegans* and *D. melanogaster* are unlikely to be alone. There are several examples of organisms that form SC in the absence of crossovers, such as *B. mori* females[69] and several others, mostly insects.[6,10] Therefore, mechanisms for SC formation in the absence of recombination must be present in these organisms. Finally, the experiments to determine if there are specialized sites that mediate synapsis or crossing over have not often been performed. Therefore, it is not known how widespread are these specialized sites for synapsis and recombination.

In *C. elegans*, the presence of a single PC could be related to the lack of defined and localized centromeres. MacQueen et al.[42] suggested that a single pairing center on each chromosome could ensure that chromosome fragments are not efficiently segregated. One problem with this model is that many large free duplications are almost as stable as full chromosomes (being transmitted at close to 50% of gametes).[70] Alternatively, the presence of a single PC may be related to the problems associated with segregating chromosomes that are holokinetic. There must be a mechanism to restrict centromere activity to one side of a crossover site at meiosis I or a bivalent could be pulled in two directions at anaphase.[37,71] Restriction of centromere activity has clearly been shown to occur in other mitotically holokinetic organisms.[72] In addition, recent studies have shown that SC disassembly and AIR-2 (an Aurora B homolog) localization is asymmetric relative to crossover position.[73] Perhaps a single pairing site is part of the mechanism that ensures a single crossover occurs (through high interference—see above), or to regulate the restriction of microtubule attachment sites, or both.

D. melanogaster has no need for a stage equivalent to presynaptic alignment and its recombination-dependent mechanisms. Therefore, a major role for DSBs has been negated. In the future, it will be important to determine if the boundary sites are the locations where SC initiates or define domains where the SC must be uninterrupted for normal levels of crossing over. While these are not mutually exclusive possibilities, there are several implications if the former is correct. SC does not initiate simply because the homologs are in close proximity. Instead, SC formation is regulated by initiating only at specialized sites.

It is simplest to presume that the mechanisms regulating synapsis in *D. melanogaster* and *C. elegans* evolved independently and for different reasons. Interestingly, both of these organisms have apparently lost three proteins during their evolution—Hop2, Mnd1 and Dmc1, which have roles in promoting strand exchange and DSB-dependent pairing.[74] This loss may not be a difficult transition, however, since it is possible to compensate for the loss of these proteins by overexpressing Rad51.[46] Conversely, it is not known why other organisms need to use a DSB-dependent mechanism to align chromosomes prior to synapsis. Although highly spec-

ulative, it may be necessary to override forces that normally prevent pairing of homologs in somatic cells. Despite the initial differences in how SC formation is initiated, our ignorance of the mechanism that starts the polymerization of SC subunits leaves the possibility open that, once a site for SC initiation has been established, the mechanism to carry out synapsis could be conserved amongst organisms that depend on DSBs and those that do not.

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