

# Early Decision: Meiotic Crossover Interference prior to Stable Strand Exchange and Synapsis

## Review

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**During meiosis, DNA double-strand breaks ultimately yield two types of recombinants: crossovers (CO) and noncrossovers (NCO). Recent studies in budding yeast show the CO/NCO designation occurs before stable strand exchange and thus well before Holliday junction resolution. Chromosome synapsis occurs after CO/NCO designation and is not required for the regulated distribution of COs along chromosomes manifested as CO interference.**

A cell faces the same challenge during both mitosis and meiosis: convey the intact genome to the next generation. Mitosis keeps the chromosome complement unchanged. The meiotic process, in contrast, reduces the diploid complement by half as required for sexual reproduction. This reduction in chromosome number is achieved by one round of DNA replication followed by two rounds of division, with no intervening replication. The need for each gamete to inherit a complete copy of the genome is satisfied by the segregation pattern of centromeres—the centromeres of paternal and maternal homologous chromosomes (each comprised of two sister chromatids) segregate to opposite poles at division I, whereas sister centromeres segregate at division II. Successful execution of the two meiotic divisions has three prerequisites: First, homologous chromosomes (homologs) must be connected to one another in order for the spindle apparatus to mediate regular segregation (“disjunction”) to opposite poles. This connection is achieved by homologous recombination, more specifically by reciprocal COs between one sister of each homolog plus links between sister chromatids all along their lengths (Figures 1A–1C). These connections are seen cytologically as chiasmata, whose correspondence to COs at the DNA level has been shown by differential BrdU labeling of sister chromatids (Figure 1D; reviewed in Jones, 1987). Connected homologs thus form a unit called a “bivalent” in which maternal and paternal centromeres/kinetochores can be attached to microtubules from opposite poles at metaphase I (reviewed in Zickler and Kleckner, 1999). Second, sister kinetochores must be modified such that they orient toward the same spindle pole at division I (e.g., Rabitsch et al., 2003). Third, at division I, links between sister chromatids (formed by cohesins) must be degraded along arms but not centromeres, thus permitting release

of chiasmata without loss of sister centromere association. Following cosegregation of sister centromeres, the division II spindle forms with bipolar attachment of sister kinetochores followed by centromeric cohesin release as in mitosis (reviewed in Lee and Orr-Weaver, 2001).

Given that interhomolog connections are required for proper division I segregation, it is not surprising that many of the most unique aspects of meiosis are devoted to providing these connections. Thus, while meiotic recombination is often thought of in terms of its role in promoting genetic diversity, it is also mechanically indispensable for the basic meiotic process itself. This conclusion is supported by the extreme phenotypes of meiotic recombination-deficient mutants.

### Nonrandom Distribution of Crossovers/Chiasmata

The distribution of crossovers/chiasmata among and along chromosomes is strikingly nonrandom (e.g., Carpenter, 1988; Jones, 1987; Lynn et al., 2002). This nonrandomness can be observed cytologically by determination of the positions of chiasma, recombination nodules (RNs), or immunostaining foci for certain recombination proteins (see below). Sites of COs/chiasmata occur at different positions along each pair of homologs in different meiotic nuclei, indicating that locus-specific effects do not account for the distribution of CO/chiasmata in a given nucleus. Despite this, the distribution of COs exhibits two specific features. First, there is always at least one CO/chiasma per pair of homologs, the so-called “obligatory chiasma,” reflecting the strong requirement for chiasma for division I segregation. The obligatory chiasma forms irrespective of chromosome length and despite the usually low average number of COs per bivalent (often one). Thus, formation of the obligatory chiasma is not ensured by induction of a large number of randomly distributed events. Second, if two or more COs are present along a bivalent, they tend not to occur near one another, i.e., they exhibit “interference” and, as a result, tend to be evenly spaced. The strength of this interference is inversely correlated with distance. Interference was first detected genetically in *Drosophila melanogaster* almost a century ago by the demonstration that double exchanges involving linked markers were less frequent than expected from the frequency of single exchanges (references in Shinohara et al., 2003).

### The Orientation of Holliday Junction Resolution Does Not Account for Crossover versus Noncrossover Recombination

Meiotic recombination is initiated by programmed formation of DNA double-strand breaks (DSBs) followed by the sequential appearance of two stable species in which sequences from the broken chromatid are joined to corresponding sequences on a homologous chromatid (see below for details). Two types of recombination products are then formed: reciprocal exchanges, also called crossovers (COs or CRs), and noncrossovers

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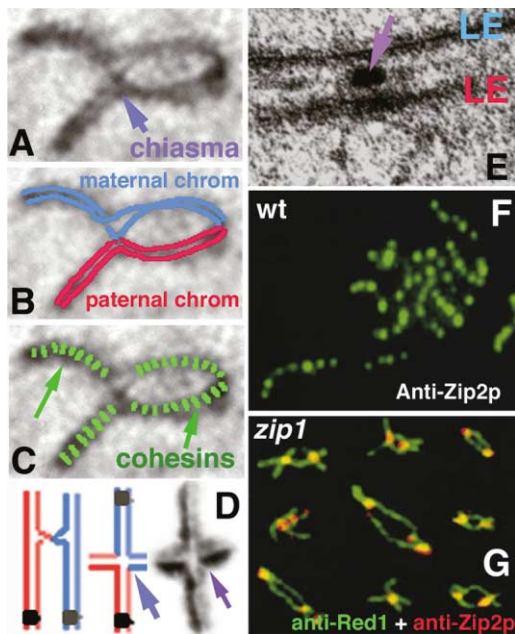


Figure 1. Meiotic Chromosomes

(A) Bivalent of *Locusta* with one chiasma (purple arrow).  
 (B) Same picture with homologous chromosomes drawn in blue and red.  
 (C) Cohesins maintain connections between sister chromatids on both sides of the chiasma, as manifested by the fact that axes are perfectly parallel (inferred position indicated by green lines).  
 (D) Monochiasmate bivalent of *Locusta* after BrdU incorporation to differentially “stain” the sister chromatids and corresponding drawings (from Jones, 1987). The exchange site between white and black chromatids corresponds exactly with the chiasma (purple arrows); black and gray rectangles mark the centromere positions.  
 (E) Recombination nodule (purple arrow) of *Sordaria macrospora* located on the central element of the SC. LE (in blue and red) indicates maternal and paternal lateral elements of the SC.  
 (F) Localization of Zip2p by antibodies to Zip2p on a spread wild-type budding yeast nucleus.  
 (G) Localization of Zip2p and axial/lateral element component Red1p in a *zip1* mutant. Red1p (green) localizes on chromosome axis and Zip2p as foci (red/yellow) on SICs.  
 note: (F) and (G) are from Chua and Roeder, 1998. (A) and (E) were previously unpublished (D. Zickler).

(NCOs or NCRs). NCOs are often referred to as “gene conversions.” However, gene conversions are more properly defined as sites where recombination events result in non-Mendelian segregation of one or more genetic markers among the products of a single meiotic cell. Such events reflect the local degradation and re-synthesis of DNA sequences that occurs at the center of both CO and NCO events. Only CO recombination products are ultimately manifested as chiasmata. What determines if a DSB will mature into a CO as opposed to an NCO? The view canonized in textbooks is that COs and NCOs form via alternative modes of resolution of a common precursor, the Holliday Junction (Holliday, 1964). This explanation for the CO/NCO decision remained when physical and EM studies (Bell and Byers, 1983) as well as genetic modeling (Szostak et al., 1983) introduced the idea that each recombination event creates a branched intermediate that involves two Holliday

Junctions (Figure 2A). The Double-Strand-Break Repair (DSBR) model accounts for the CO versus NCO outcome by the relative orientation of the two Holliday junction resolution events that occur at each branched intermediate.

Key intermediates and features of the DSBR model, including the existence of double Holliday junctions (DHJs), have been confirmed by studies in the budding yeast *Saccharomyces cerevisiae* (reviewed in Allers and Lichten, 2001). However, several observations challenge the idea that the orientation of Holliday junction resolution accounts for the relative frequencies of COs and NCOs. First, several mutants have been identified that retain high levels of DSBs and NCOs, but reduced levels of COs (Engebrecht et al., 1990; and references for the genes/proteins discussed below in Börner et al., 2004 [this issue of *Cell*]; Fung et al., 2004). Proteins implicated specifically in promoting meiotic CO recombination include (1) a DNA helicase Mer3; (2) Msh4 and Msh5, relatives of *E. coli* MutS not involved in mismatch repair; (3) Zip1, a structural component of the central region of the synaptonemal complex; and (4) Zip2 and Zip3, two proteins required for initiating Zip1 polymerization along homologs. Mutation of any member of this group reduces CO (but not NCO) frequency. These findings suggested that COs form via a more elaborate mechanism than NCOs. Second, the configuration of heteroduplex DNA on NCO recombinants did not conform to expectations of the DSBR model (Figure 2A; Gilbertson and Stahl, 1996; Porter et al., 1993). Third, Allers and Lichten (2001) used physical detection methods to provide evidence that most or all of the DHJ intermediates are pre-CO intermediates rather than intermediates on the road to forming either COs or NCOs. Fourth, two new studies in budding yeast (discussed below) show that the positions of COs are determined much earlier in prophase than the stage at which Holliday junction resolution occurs. These new studies support and extend an earlier proposition based on timing of DHJ resolution relative to that of recombination nodule appearance (Storzazzi et al., 1996). These studies, particularly the work from the Kleckner and Lichten labs, support an “Early CO decision” (ECD) model of meiotic recombination (Figure 2B).

Allers and Lichten (2001) suggested that NCO recombinants form by a pathway that does not involve a Holliday junction intermediate (see Figure 2B). They proposed that NCOs form by transient invasion of one or both of the two DNA ends formed by a DSB. Such transient invasion could allow an invading 3' end to be extended via limited DNA synthesis using the invaded duplex as template. Following ejection of the extended ends, annealing of partner ends could then lead to repair of the DSB. Evidence that such a recombination pathway can occur has been found in several mitotic systems and is referred to as “synthesis-dependent strand annealing” (SDSA; reviewed in Nassif et al., 1994; Paques and Haber, 1999). While it remains to be determined if meiotic NCOs form via SDSA, this hypothesis is attractive and accounts for available data. The mechanism through which recombination intermediates can be assigned a CO fate prior to Holliday junction resolution is, at present, mysterious.

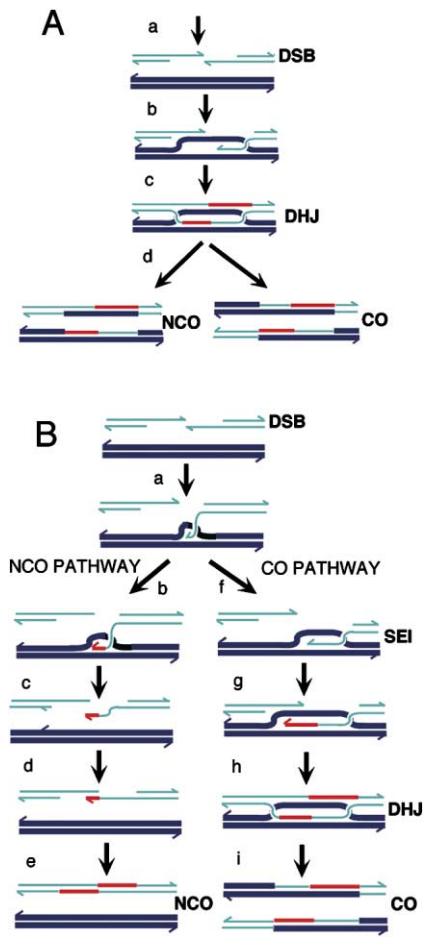


Figure 2. The DSBR and ECD Models of Meiotic Recombination

(A) The DSBR model.

3' ends of DNA are indicated with a half arrowhead. Newly synthesized regions of DNA are indicated in red. The version of the model shown is modified from that originally proposed. The original model, that 5' and 3' ends were nucleolytically processed prior to invasion. Subsequent studies indicated that 3' ends undergo little or no degradation.

- (a) A DNA double-strand break occurs and the 5' strands at DNA ends are resected to yield 3' ssDNA overhangs.
- (b) Strand invasion of both ends forms a D loop.
- (c) DNA synthesis from the invading ends fills in regions lost by end resection, ligation of extended strands forms two Holliday junctions.
- (d) Resolution of Holliday junctions forms CO and NCO recombination products. Alternative modes of resolution give rise to NCO (right) and CO (left) recombinants.

(B) The Early Crossover Decision (ECD) Model.

- (a) DSB end forms an unstable ("nascent") interaction with a homologous chromatid.
- (b) En route to forming an NCO, the end involved in the nascent interaction is extended by DNA synthesis. This intermediate is either unstable or too transient to detect by current 2D gel methods.
- (c) The extended end is ejected from the nascent joint.
- (d) The extended end anneals with its partner.
- (e) DNA synthesis and ligation completes formation of the NCO.
- (f) En route to forming a CO, the nascent end is stabilized to form an SEI intermediate that can be detected by 2D gels.
- (g) The second end engages the SEI via strand invasion or annealing.
- (h) DNA synthesis from the invading ends fills in regions lost by end resection, ligation of extended strands forms two Holliday junctions.
- (i) Resolution of Holliday junctions occurs such that only a CO is formed.

**The Crossover/Noncrossover Decision Is Made Very Early, prior to or during Formation of Stable Strand Exchange**

Comparison between the onset of strand exchange and timing of the synaptonemal complex formation in budding yeast suggested that differentiation of CO versus NCO occurs at a very early stage (Hunter and Kleckner, 2001). Börner et al. (2004) examined a set of five mutants *mer3*, *msh5*, *zip1*, *zip2*, and *zip3*, collectively referred to as *zmm* mutants. As discussed above, each of these mutants had been shown previously to reduce CO efficiency (for a more complete discussion, see Fung et al., 2004). Two of these mutants (*mer3* and *zip1*) also display defects in CO interference when assayed genetically; *msh5* is inferred to have a similar phenotype based on the interference defect of *msh4*. At 33°C, the phenotype of all five *zmm* null single mutants was stronger and more uniform than at 23°C (budding yeast strains are conventionally examined at 30°C). Four different double mutant combinations were also examined; all showed phenotypes similar to the corresponding single mutants (consistent with previous analysis of two other double mutant combinations, see Fung et al., 2004). These results suggest that the five ZMM proteins function in concert on the same recombination pathway.

At 33°C, each of the *zmm* mutants arrests in meiotic prophase and produces only about 15% of the normal level of CO products. Importantly, DSBs and NCO products appeared to form with normal timing and efficiency in the three *zmm* mutants examined in this regard (*mer3*, *zip1*, and *zip2*). Two-dimensional gel analysis showed that the deficit in COs is a result of a block (or a delay) in the progress of recombination reactions after formation of DSBs, but before formation of the first detectable joint molecule intermediate. This early joint intermediate, the "single-end invasion intermediate" (SEI), is formed when one of the two ends created by a DSB invades a homologous chromatid (Hunter and Kleckner, 2001). An important implication of the defect seen in *zmm* mutants at 33°C is that the CO versus NCO decision is made prior to or during formation of SEIs (Figure 2B, f) as originally suggested by Hunter and Kleckner (2001). Because NCO recombinants form normally in *zmm* mutants, while CO formation is strongly blocked, the ZMM proteins are *not* required for the CO/NCO decision. This conclusion has implications for the role of ZMM proteins in CO interference (as discussed further below). It should be noted that all analysis of phenotypic effects of the *zmm* mutants on recombination intermediates has been done at the *HIS4* locus. It will be important to determine if ZMMs play an identical role at other loci.

The results of a recent biochemical study of one of the ZMM proteins, the helicase Mer3, is in good agreement with the phenotypic analysis implicating Mer3 formation of SEIs (Mazina et al., 2004 [this issue of *Cell*]). Mer3 can stimulate heteroduplex extension during Rad51-mediated strand exchange reaction. The directionality of this extension activity is that expected to stabilize joints formed by invasion of 3' ssDNA ends.

**The SC Initiates at Sites of COs in Budding Yeast**

The synaptonemal complex (SC) consists of three elongated proteinaceous components that lie in parallel, two

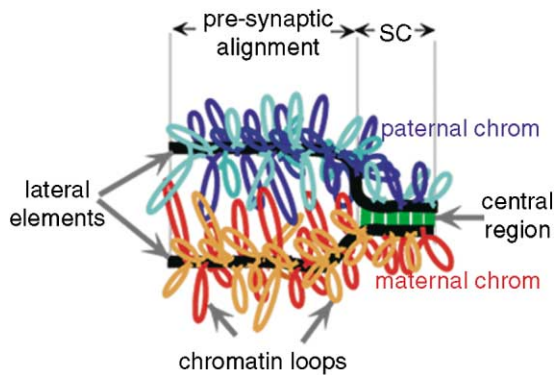


Figure 3. Schematic of a Zygotene Chromosome

The maternal sister chromatids are represented in red and orange, the paternal sisters in blue and purple. Chromatids are arranged as a set of loops along the lateral element (black). In the Presynaptically aligned region, loops emanate from the axes and can contact one another. The schematic is not meant to imply that loop contacts are responsible for establishing or maintaining the parallel configuration of lateral elements at the presynaptic stage; structures not depicted, such as proteinaceous bridges, may serve this function (reviewed in Zickler and Kleckner, 1999). In the SC portion, lateral elements are held in parallel alignment by the central region (green bars).

lateral elements flanking a central element (Figure 3; reviewed in Zickler and Kleckner, 1999). Each lateral element is built along a sister chromatid pair and, together with cohesins, forms the chromosome axes along which sister chromatin loops are arrayed. Close “synapsis” of lateral elements then occurs by assembly of the central region of the SC. Several studies provide strong evidence for functional interplay between SC formation and recombination events. In most analyzed species, DSBs are generated before, and are required for, SC formation. In addition, mutations in structural components of the SC cause recombination defects even in the two organisms known to form SC in absence of DSBs (e.g., Börner et al., 2004; Colaiacovo et al., 2003; Hunter and Kleckner, 2001; Page and Hawley, 2001; Smith and Roeder, 1997; Sym et al., 1993). Electron microscopy reveals two types of nodules along the SC as it is forming and after its formation is complete (Figure 1E). At zygotene, nodules correspond to the immunostaining foci formed by Rad51 and Dmc1, two homologs of the bacterial recombinase RecA (e.g., Bishop, 1994; Tarsounas and Moens, 2001). Thus, these early nodules appear to reflect the sum of all homologous recombination events (CO plus NCO). In contrast, the late nodules seen at pachytene correspond to the subset of recombinational interactions that become COs (Carpenter, 1988; reviewed in Zickler and Kleckner, 1999). When monitored in synchronous budding yeast cells, SEI formation occurs as the central region of the SC assembles (zygotene) and DHJs formation occurs shortly after the SC is fully assembled (early pachytene; Hunter and Kleckner, 2001).

Zip2 and Zip3 promote initiation of assembly of the SC central region (reviewed in Fung et al., 2004). Zip1 assembly requires both Zip2 and Zip3, which colocalize with Zip1 as soon as Zip1 is visible. Zip2/3 foci (Figure 1F) form prior to and independently of Zip1. In a *zip1*

mutant, Zip2/3 foci are found at sites of homologous axial element associations as manifestations of their roles at these sites (Figure 1G). Roeder and colleagues propose that Zip2 and Zip3 form a “synaptic initiation complex” or “SIC.” Previous work (reviewed in Fung et al., 2004) and the new results from Börner et al. indicate that the other ZMM proteins function with Zip2 and Zip3 at this initiation step.

Zip2/3 focus formation is DSB dependent. Furthermore, immunolocalization, coimmunoprecipitation, and two-hybrid experiments indicate that these proteins are associated with recombination proteins that act at sites of DSBs (e.g., Mre11 and the recombinase Rad51, reviewed in Rockmill et al., 2003). These results suggest that Zip2/3 promotes initiation of SC at recombination sites. This conclusion is confirmed and extended by new evidence that Zip2 foci assemble specifically at sites of COs. Measurement of the number and position of Zip2 foci along four different yeast chromosomes indicates that Zip2 specifically localizes at sites that will form COs (Fung et al., 2004). Most importantly, Zip2 foci are spaced more evenly than expected if their placement were random; they display interference just as COs do. Consistent with the properties of CO interference when measured genetically, interference of Zip2 foci is stronger on long chromosomes than short ones (Fung et al., 2004; Kaback et al., 1999). Based on prior colocalization experiments, foci formed by three of the remaining ZMM proteins, Zip3, Msh4/5, and Zip1 (in its early focal pattern) can be inferred to display interference as well. In addition to these experiments, quantitative analyses in a series of mutants that generate different levels of DSBs revealed a close correspondence between Zip3 foci, SC initiation, and CO frequency, providing further evidence for the role of CO sites in SC initiation (Henderson and Keeney, 2004). Also, an *sgs1* null mutant increases the number of COs and Zip3/Zip2 foci to a similar degree (Sgs1 is a member of the RecQ family of DNA helicases; Rockmill et al., 2003). Thus, there is evidence for a synaptic initiation complex (which we will call ZMM-SIC) that initiates SC assembly at sites of COs in budding yeast. Given that the CO/NCO decision occurs prior to the step at which the ZMM-SIC promotes progression of the CO pathway, the results indicate that the CO/NCO decision precedes synapsis (Börner et al., 2004).

Correspondence between SC assembly sites and COs sites is also found in other organisms as illustrated by the following examples (additional examples are cited within the references given). In a short heterozygous inversion of maize, there is a one-to-one correspondence between the frequencies of COs (estimated by recombination nodules and translocation bridge frequencies at anaphases I and II) and the frequency of SC initiations within the inverted region (Maguire and Riess, 1994). In the fungus *Sordaria macrospora*, mutants with decreased CO frequencies show a parallel decrease in the number of late recombination nodules, chiasmata, and SC initiation sites. Furthermore, a quantitative correlation between DSBs (indicated by Rad51 foci), Cos, and SC initiation sites is also observed in different *ski8* mutants of *Sordaria* (Ski8 is an antiviral protein required for meiotic DSB formation); SC formation requires more DSBs than does presynaptic homolog

alignment and an even higher level of DSBs is required for full-length SC formation (Tesse et al., 2003; Zickler et al., 1992).

#### The SC Is Not Required for Interference

Several related models maintain that interference is mediated by assembly of, or signal transduction through, the SC (reviewed by Shinohara et al., 2003). The first version of this class of model, proposed by Egel in 1978 (reviewed by Egel, 1995), was based on Maguire's 1977 finding in *maize* that the choice of CO sites appeared to be made in advance of synapsis (within the inverted segment of an inversion heterozygote, see Maguire and Riess, 1994). Further support was provided by the fact that two species of fungi, *Schizosaccharomyces pombe* and *Aspergillus nidulans*, lack both interference and the SC (Egel, 1995). Such models also gained support by the finding that the budding yeast *zip1* mutant lacks both SC and interference (when measured genetically; Sym and Roeder, 1994). It now appears that the elegant experiments demonstrating that Zip1 is an essential structural component of the SC central region (Sym et al., 1993; Sym and Roeder, 1995) were misleading with respect to the protein's involvement in interference (as anticipated by Storlazzi et al., 1996). As discussed above, Zip2 foci display interference. Surprisingly, the same nonrandom pattern of Zip2 foci observed in wild-type cells is also seen in *zip1* mutants, implying that interference can occur in the absence of Zip1 and hence in the absence of the SC (Fung et al., 2004). This finding is consistent with the finding that the CO/NCO decision appears to occur normally in *zmm* mutants including *zip1* (Börner et al., 2004). Both sets of results imply that the subset of sites that will engage ZMM-SIC is chosen prior to, and independently of, the SC (Figure 4). Further, normal designation of CO sites does not depend upon the ZMM-SIC. Thus, neither the SC nor a functional ZMM-SIC is required for establishing the nonrandom pattern of CO sites. Work in *Drosophila* also indicates that the SC is not required for interference; a *c(3)G* mutant, defective in a protein with structural similarity to Zip1, has a strong defect in SC assembly, but exhibits CO interference (Page and Hawley, 2001). If not via the SC, how might interference be mediated? Kleckner and colleagues propose a solution (Börner et al., 2004; Zickler and Kleckner, 1999). Prior to SC assembly, homologous chromosomes with well-formed lateral (axial) elements are aligned at a distance and linked by connections that include recombination complexes. Thus, a CO designation event could initiate an interference signal that could then be transmitted along the axes, blocking nearby CO designations as it travels (see Figure 4).

#### Are the *zmm* Mutants Really Interference Mutants?

Zip2 foci display an interference pattern in *zip1* and *msh4*, two mutants that lack interference when assayed genetically (Fung et al., 2004). If interference is normal when assayed cytologically, why isn't it detected genetically? One possibility is that the ZMM complex, the SC, or both are needed to maintain crossover designation until the stage at which DHJs are finally resolved at the end of pachytene (Börner et al., 2004). Without this

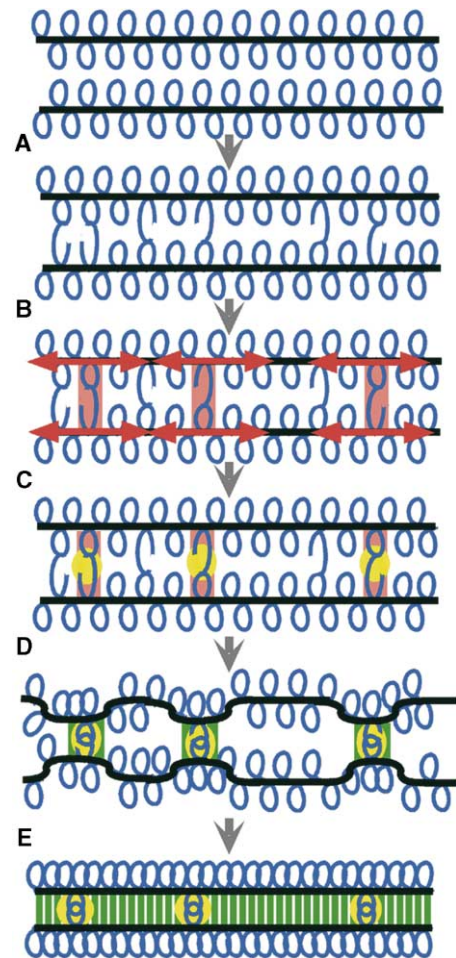


Figure 4. Pre-SC Model for CO Designation and Interference  
(A) DSBs are formed on chromatin loops and engage in unstable/nascent interactions with corresponding sequences on the loop of a non-sister chromatid.  
(B) A subset of nascent interactions is designated to become a CO, setting up interference along the chromosome axis. CO designation is indicated by a pink box. Interference signals, indicated as red lines with double arrowheads, spread from sites of CO designation preventing nearby nascent interactions from acquiring their own CO designation.  
(C) ZMM-SIC (yellow circles) loads only at designated pre-COs.  
(D) Polymerization of the SC central region is initiated coupled with conversion of the nascent interaction to an SEI. Green bars represent components of the SC central region.  
(E) SC elongation occurs and SEIs are converted to double Holliday junctions. Nascent interactions that failed to obtain CO designation go on to form NCOs.

function, intermediates designated to be COs may instead be resolved aberrantly into both COs and NCOs. The resulting reduction in the density of designated COs may make it difficult to detect interference because non-interfering, ZMM-independent COs (discussed below) make up a greater fraction of the total than in wild-type.

#### A Second CO Pathway

Not all COs depend on ZMM-SIC in budding yeast. COs form at 50% of normal levels at 30°C or below and at 15% of normal levels at 33°C. Börner et al. (2004) argue

that the low level of ZMM-SIC-independent COs seen at 33°C is likely to reflect the level at which this type of CO forms in wild-type at all temperatures; i.e., that the ZMM-SIC pathway predominates in normal budding yeast. Recent studies indicate that the formation of at least a fraction of ZMM-SIC-independent COs depends on two proteins, Mms4 and Mus81 (reviewed by Hollingsworth and Brill, 2004). Mms4/Mus81, and the orthologous Eme1/Mus81 in *S. pombe*, are heterodimeric complexes with structure-specific endonuclease activity. Mus81/Mms4 can cleave Holliday junctions, but the complex has more robust activity on other types of DNA branches, raising the possibility of a pathway for CO formation that does not involve Holliday junction formation (Osman et al., 2003). Thus, it appears that there are at least two mechanisms for forming COs in budding yeast that can function independently of one another. The first mechanism depends on the ZMM group and the second on Mms4/Mus81. The COs formed by the Mms4/Mus81 pathway may not obey the rules of interference, whereas those formed by the ZMM pathway clearly do.

#### Questions Remaining

In addition to the *zmm* mutations, other budding yeast mutants are interference defective when measured genetically. These mutants include *dmc1* and *tid1*. Tid1 is one of two paralogous accessory factors that stimulate Rad51- and Dmc1-mediated recombination in vitro, the other being Rad54. The *tid1* mutant shows a delay in DSB conversion and the *dmc1* mutant a stronger block that can be suppressed by overexpression of either Rad54 or Rad51. *tid1* strains and suppressed *dmc1* strains have a partial interference defect (Shinohara et al., 2003; Tsubouchi and Roeder, 2003). These results are fully consistent with the results discussed above, indicating that the CO/NCO decision occurs at the onset of stable strand exchange. Unlike the *zmm* mutants, *tid1* and suppressed *dmc1* mutants display near normal CO frequency. It remains to be seen if *TID1* and/or *DMC1* promote proper designation of CO sites.

Importantly, differences in *mus81* and *msh4* mutant phenotypes in different organisms suggest that the degree to which specific recombination proteins contribute to the formation of meiotic COs may differ substantially across species (reviewed by Hollingsworth and Brill, 2004). Specifically, most or all COs form by a *MSH4*-dependent mechanism in *Caenorhabditis elegans*. In contrast, most or all COs form by a *MUS81*-dependent mechanism in *S. pombe* (which lacks *Msh4/5*). Budding yeast appear to lie somewhere between the two extremes, with a significant number of COs contributed by both *Msh4/5*- and *Mus81*-dependent pathways. As mentioned above, there are also strong species-specific differences with respect to the relationship of DSBs to initiation of the SC (reviewed by Henderson and Keeney, 2004). A better understanding of the mechanisms underlying these and other species-specific differences is likely to elucidate the nature of selective forces driving meiotic evolution.

In summary, the two new studies by the Kleckner and Roeder labs shed new light on the regulation of meiotic recombination and its relationship to chromosome syn-

apsis. We are left with many unanswered questions, two of which are primordial. What is the function of the SC, and what is the specific mechanism governing CO distribution?

#### Acknowledgments

We thank the reviewers of this manuscript for their insightful suggestions. We regret that because of length restriction we were unable to discuss or cite many relevant and important studies.

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