

Clarifying the mechanics of DNA strand exchange in meiotic recombination

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During meiosis, accurate separation of maternal and paternal chromosomes requires that they first be connected to one another through homologous recombination. Meiotic recombination has many intriguing but poorly understood features that distinguish it from recombination in mitotically dividing cells, and several of these features depend on the meiosis-specific DNA strand exchange protein Dmc1 (disrupted meiotic cDNA1). Many questions about this protein have arisen since its discovery more than a decade ago, but recent genetic and biochemical breakthroughs promise to shed light on the unique behaviours and functions of this central player in the remarkable chromosome dynamics of meiosis.

Sexual organisms must halve the chromosome number in gametes to maintain genome size with each generation. This goal is achieved through meiosis, in which two rounds of chromosome segregation follow a single round of DNA replication. The first meiotic division separates maternal and paternal copies of each chromosome, but in order for this segregation to occur properly, the chromosomes must first pair with their correct partner and then become physically connected so that they orient together on the meiotic spindle. Connection is established by the exchange of homologous chromosome arms (the point of crossover being called a chiasma) plus cohesion between sister chromatids^{1,2} (Fig. 1). Exchange uses a specialized pathway of homologous recombination that repairs DNA double-strand breaks (DSBs)³—basically, the cell deliberately damages its own DNA and then uses the repair process to lock homologous chromosomes together. A central step in recombination involves proteins related to bacterial RecA that catalyse the pairing and exchange of DNA strands between single-stranded DNA (ssDNA) formed at the break and intact, homologous double-stranded DNA (dsDNA)⁴. Most eukaryotes have two RecA homologues, the ubiquitous Rad51 and its meiosis-specific counterpart Dmc1 (ref. 5).

Meiotic recombination is subject to many layers of control that dictate, for example, the choice of DNA substrate for DSB repair, the distribution and timing of recombination events, and the integration of recombination with higher order chromosome structures^{2,6}. Executing these controls requires meiosis-specific factors (such as Dmc1) in addition to proteins (such as Rad51) that function during normal repair of DSBs in other cell types. The widely conserved Dmc1 has a critical role in meiosis in most sexual organisms, but what its molecular functions are and how and why it differs from its cousin Rad51 are not well understood⁵. Recent genetic studies have uncovered new information about the roles of Dmc1 and accessory proteins that modulate Dmc1 function *in vivo*. These advances, coupled with breakthroughs in biochemical analysis of Dmc1 activities, promise to answer long-standing questions about the mechanism of meiotic recombination.

The challenges of meiotic recombination

The molecular events of meiotic recombination have been most thoroughly described in the budding yeast *Saccharomyces cerevisiae* (Fig. 2). DSBs are formed by the meiosis-specific Spo11 protein,

which cuts DNA via a topoisomerase-like reaction to generate covalent protein–DNA linkages to the 5′ DNA ends on either side of the break³. After Spo11 is removed from the DNA ends, one or more exonucleases process the DSB to generate 3′ ssDNA tails. DNA strand exchange proteins bind these tails, forming helical nucleoprotein filaments, which are the active structures that carry out the search for a homologous target and catalyse strand exchange. The detailed biochemical steps in this process are described below. Further processing of the strand exchange intermediates yields intact recombinant products that either have exchanged the flanking DNA arms (crossovers) or have not undergone exchange (non-crossovers) (Fig. 2).

The task of DNA strand exchange proteins in meiosis is remarkable. Each DSB generates about 1 kilobase of ssDNA, for which the homologous target duplex must be located and engaged. In yeast, this is the equivalent of searching through a 10-km-long string to find a particular 20-cm-long region. In human cells, that 20-cm stretch would need to be picked out of some 2,500 km, enough to reach from

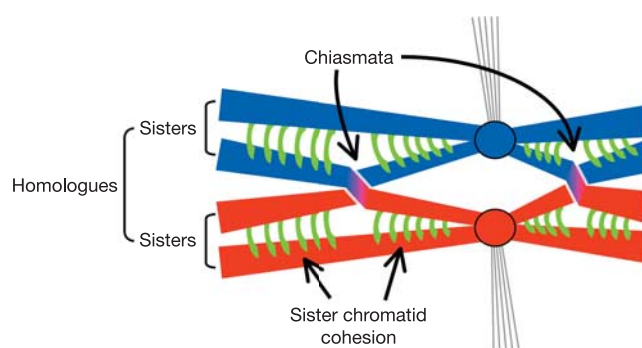


Figure 1 | Connections formed between homologous chromosomes during meiosis. Meiotic cells of most sexual organisms contain two copies of most chromosomes, one from each of the parents (red and blue). After DNA replication, each chromosome comprises a pair of sister chromatids held together by cohesion complexes (green). Sister centromeres (circles) attach as a single unit to microtubules (thin lines) from a spindle pole (not shown). Exchange of chromosome arms between non-sister chromatids yields a chiasma. Dissolution of sister chromatid cohesion along the arms allows the homologues to separate at the first meiotic division (not shown).

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London to Moscow. The problem is more complex, however, because within that 2,500-km length would be three exact matches to the target (one on the sister chromatid, and one on each of the two chromatids of the homologous chromosome), but only the two targets on the homologue can be used. This strong preference for interhomologue recombination in meiosis is important because intersister recombination would not join homologues. This bias contrasts with mitotic cells, in which the sister chromatid is greatly preferred as the target for Rad51-mediated recombination⁷. The problem gets even worse because every meiotic cell makes many DSBs (hundreds per cell in yeast and mouse)^{8,9}. Thus, repair has to occur simultaneously at numerous places in each meiosis. Finally, the search for homology and the catalysis of strand exchange must be spatially and temporally coordinated with the development of higher order chromosome structures (for example, synaptonemal complex, chromosome axes and chromatin loops) that are required for the recombination event to successfully connect the homologues^{2,6}. All of this is accomplished in just a few hours in yeast and a few days in mammals. With all these issues to consider, it is clear why meiotic recombination is so tightly controlled, and why this process has fascinated chromosome biologists for decades.

The bacterial DNA strand exchange protein RecA had already been extensively characterized⁴ when the first eukaryotic homologues were identified in *S. cerevisiae*^{10–12}. The widely conserved Rad51 protein gets its name from the radiation-sensitive phenotype caused by mutations in the gene. Not surprisingly then, Rad51 is required for

most homologous recombination reactions in both mitotic and meiotic cells¹³. In contrast, Dmc1 is meiosis-specific and, in many organisms, *dmc1* mutants display reduced or absent meiotic recombination⁵. It was appreciated early on that Dmc1 activities must be central to the unique aspects of meiotic (as opposed to mitotic) recombination^{10,14}. As such, several fundamental questions have stood since its discovery: which aspects of meiotic recombination reflect specialized roles for Dmc1? Is it a DNA strand exchange protein, and what are the intrinsic biochemical properties that differentiate it from Rad51? What are the extrinsic factors that control and direct Dmc1, and to what extent do these dictate the unique properties of Dmc1?

Genetics and cytology of Dmc1

The physiological function and specialized roles of Dmc1 have been best characterized in *S. cerevisiae*⁵. In one commonly used laboratory strain (the SK1 background), *dmc1* mutants show a near-complete block to recombination¹⁰. When the DNA intermediates of recombination were assayed, no stable strand invasion or double-Holliday-junction formation was detected^{14,15} (Fig. 2). Based in part on these observations, it was suggested that Dmc1 promotes an interhomologue-only recombination pathway that is unique to meiosis¹⁴; this role was also suggested by the observation that *dmc1* mutants have increased recombination between ectopic sequence repeats (that is, homologous sequences located at positions other than the equivalent position on the homologous chromosome)¹⁶. *rad51* mutants are similarly profoundly defective for meiotic recombination, revealing that both DNA strand exchange proteins contribute critical, non-overlapping functions⁵. In every organism examined, Rad51 and Dmc1 are components of multiprotein complexes, or nodules, that form on meiotic chromosomes at the sites where DSBs are being repaired^{8,9}.

A striking hallmark of meiotic recombination in most organisms is that crossovers are not randomly distributed along chromosomes. Instead, the presence of a crossover makes it less likely that another will form nearby¹⁷. This phenomenon, known as crossover interference, is poorly understood, but Dmc1 may be involved because interference is lost or diminished when Dmc1 is absent^{18,19}.

Interestingly, the 5' strands of DSBs are more extensively processed by exonuclease(s) when Dmc1 and/or Rad51 is missing^{10,11,14}. This finding indicates that the nuclease(s) and DNA strand exchange proteins are coordinated in some fashion, for example through competition for the same substrate or by direct protein–protein interaction. This coordination probably serves to strike a balance between too little processing (and thus, not enough ssDNA to carry out the homology search and strand exchange) and too much processing (which may be deleterious for integrity of the genome). The idea of a hand over from the DSB processing activity to the strand exchange proteins is attractive because functional coupling of sequential steps in the pathway might help meiotic DSB repair to operate so efficiently that it can handle larger numbers of DSBs than would be tolerated by a mitotic cell.

Dmc1 is important for meiotic recombination in many organisms; for example, mice with targeted mutation of the *Dmc1* gene are sterile and show hallmarks of poorly repaired DSBs²⁰. However, Dmc1 is not absolutely essential in all circumstances. Some organisms, such as *Drosophila melanogaster*, *Caenorhabditis elegans* and *Neurospora crassa*, have Rad51 but lack a Dmc1 orthologue¹⁷. In another frequently used *S. cerevisiae* laboratory strain (the BR background), and in *Schizosaccharomyces pombe*, *dmc1* mutants still exhibit significant levels of meiotic recombination^{21,22}. Moreover, the recombination defect in *S. cerevisiae dmc1* mutants can be largely suppressed by overexpression of Rad51 or Rad54 (a protein that stimulates Rad51; see below)^{19,23}. These findings may indicate that Rad51 and Dmc1 operate in multiple meiotic recombination pathways that only partially overlap in budding yeast¹⁹. However, it is also possible that under normal circumstances the two proteins function only together, and that only aberrant recombination occurs when one is missing.

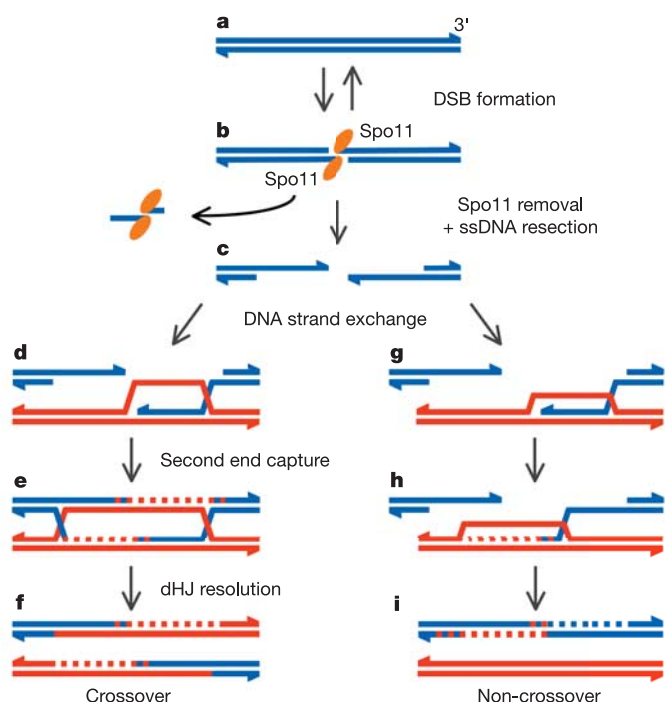


Figure 2 | DNA events in meiotic recombination. **a–c**, Presynapsis. Spo11 (elipses) cleaves dsDNA, yielding a covalent Spo11–DNA complex. Endonuclease releases Spo11 bound to a short oligonucleotide, and 5' DNA strands are degraded to yield 3' ssDNA tails, which are bound by Rad51 and Dmc1 (not shown). **d–f**, Crossover formation. **d**, Invasion of ssDNA from one end of the break forms an asymmetric strand exchange intermediate. **e**, DNA synthesis (dashed line) is primed from the invading 3' end; the second DSB end is captured and primes DNA synthesis. Ligation yields a pair of Holliday junctions (dHJ). **f**, Resolution yields a mature product with exchanged flanking DNA. **g–i**, A non-crossover pathway. Strand invasion (**g**) and DNA synthesis (**h**) are inferred but have not been directly detected. A transient strand invasion complex may be dissociated, perhaps by DNA helicases, allowing newly synthesized DNA to anneal to complementary ssDNA on the other side of the break (**i**). Further DNA synthesis and ligation yield a mature non-crossover product.

Dmc1-mediated DNA strand exchange

Whereas *in vivo* studies identify aspects of meiotic recombination that are influenced by Dmc1, they do not reveal the biochemical mechanism of Dmc1 action. Given its homology to RecA, it was long suspected that Dmc1 would have DNA strand exchange activity^{9,24}, but only relatively recently has this been demonstrated. Strand exchange by RecA-related proteins can be divided into three phases⁴ (Fig. 3a). During the presynaptic phase, the strand exchange protein assembles on ssDNA, creating a helical nucleoprotein filament in which the DNA is stretched and underwound relative to B-form DNA. Formation of a ternary complex of the ssDNA–protein filament with dsDNA initiates the synaptic phase. Juxtaposition of three DNA strands within the synaptic filament permits rapid homology sampling through transient Watson–Crick base pairing between the ssDNA and the complementary strand of the duplex partner. Sequential cycles of binding, sampling and release of dsDNA are performed until homology is detected. A stable DNA joint is then formed by intertwining of the ssDNA with its complement from the homologous target. During the postsynaptic phase, the exchange of DNA strands is completed and the filament is dismantled. The generation of mature recombinant products (that is, intact DNA duplexes with or without reciprocal exchange of flanking sequences) requires many other postsynaptic processes such as more extensive strand exchange via migration of branched DNA structures (D loops or Holliday junctions); resolution of branched DNA intermediates by helicases, nucleases and/or topoisomerases; DNA synthesis; and resealing of DNA strand interruptions with DNA ligase^{13,25}.

Two types of *in vitro* DNA strand exchange assay are often used. The first detects transfer of one strand of a linear dsDNA onto a circular single-stranded nucleoprotein complex, creating a nicked duplex circle and a linear ssDNA (Fig. 3b). (Note that this situation contrasts with recombination *in vivo*, where a 3' DNA end is provided by the initiating ssDNA.) The second assay detects invasion of a short ssDNA nucleoprotein complex into a negatively supercoiled duplex circle, displacing the non-complementary strand into a D-loop structure (Fig. 3c). Because RecA has high specificity for

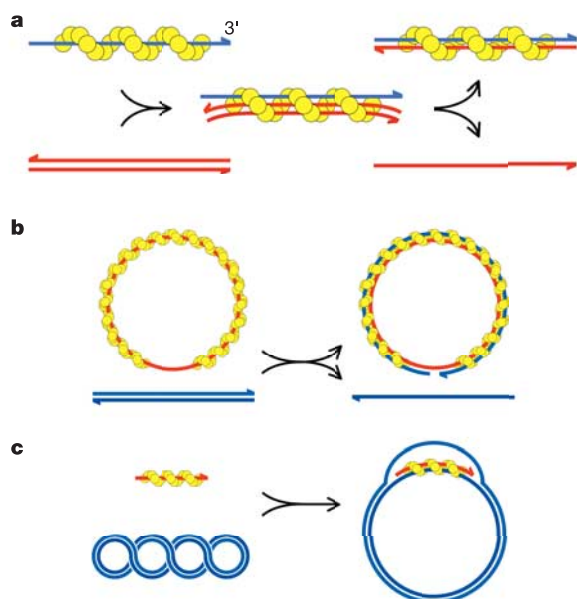


Figure 3 | The DNA strand exchange reaction. **a**, Three stages of strand exchange: presynaptic helical filament on ssDNA (left); synaptic complex with homologous dsDNA (middle); postsynaptic stage with the original ssDNA fully base-paired with the complementary strand from the dsDNA donor (right). **b**, **c**, Typical *in vitro* DNA strand exchange assay systems. **b**, Strand transfer from a linear duplex to a circular nucleoprotein filament. **c**, D-loop assay. Invasion of a short nucleoprotein filament into a negatively supercoiled circular duplex, creating a bubble, or D-loop.

binding ssDNA over dsDNA, strand exchange is efficient even when all reaction components are mixed simultaneously⁴. In contrast, Dmc1 is more sensitive to the order of addition because its ability to bind dsDNA can lead to non-productive complexes being formed²⁶.

In early studies, Dmc1 displayed poor strand exchange activity relative to RecA or Rad51 (refs 27–30). Although RecA and its other homologues (eukaryotic Rad51, bacteriophage UvsX and archaeal RadA) form hexameric to octameric rings in solution, the active oligomeric form for strand exchange is a helical nucleoprotein filament⁴. Electron microscopy of Dmc1, however, revealed nucleoprotein complexes that were exclusively rings, never helices^{29,31} (Fig. 4a). The lack of helical filaments was surprising because Dmc1 has high sequence conservation with RecA-related proteins and because biophysical studies revealed that Dmc1 and Rad51 recognize homology in the same way³². It was suggested that rings were an artefact caused by lack of essential cofactors³², but models for ring-based DNA strand exchange were also proposed³³.

More recently, a robust Dmc1-mediated strand exchange reaction has been described, which differs from previous studies in using physiological pH and higher salt concentrations^{26,34–36}. Under these conditions, helical Dmc1–ssDNA filaments were formed^{26,35,37} (Fig. 4b), strongly indicating that the filament is the active oligomeric form for DNA strand exchange. Inefficient filament formation probably accounts for the weak strand exchange activity of earlier studies.

Yet, is there a function for the Dmc1 ring? It has been proposed that stacks of Dmc1 rings on DNA might be converted into an active helix, perhaps upon binding ATP³⁸. As Dmc1 rings are oriented with alternating head-to-tail polarity^{31,33}, however, it is impossible to convert a stack of rings into a continuous helical filament without dismantling and reorienting at least half of the DNA-bound protein. A more likely possibility is that free, but not DNA-bound, rings are the form of Dmc1 (and of other RecA relatives) that assembles on DNA (Fig. 4c).

Ca^{2+} stimulates both filament formation on ssDNA and DNA strand exchange by bacterially expressed human and yeast Dmc1 proteins^{35,36}. Ca^{2+} also stimulates Rad51 (ref. 39), so it has been suggested that Dmc1 and Rad51 activity might be modulated *in vivo* by variation in intracellular calcium levels^{35,36}. However, the free Ca^{2+} concentration required for optimal Dmc1 stimulation ($\sim 100 \mu\text{M}$) is much higher than the free concentration in the cytosol ($0.1\text{--}1 \mu\text{M}$ in yeast), and Ca^{2+} was not necessary for efficient DNA strand exchange by human DMC1 expressed in insect cells and assayed in higher salt concentrations²⁶. Therefore it is possible that Ca^{2+} acts as a non-physiological stimulant, perhaps substituting for more

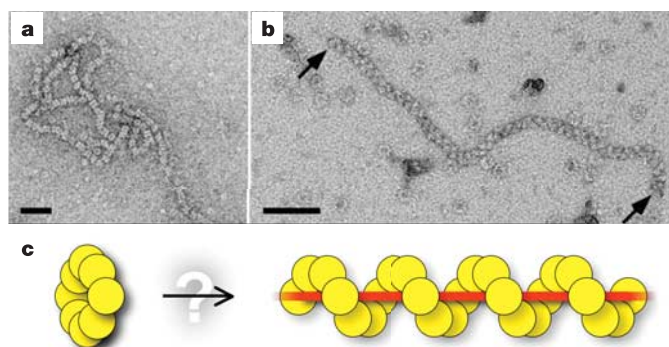


Figure 4 | Oligomeric structure of Dmc1 nucleoprotein complexes. **a**, **b**, Electron micrographs of Dmc1–DNA complexes (from refs 26, 31, with permission). Scale bars, 50 nm. **a**, Stacks of octameric Dmc1 rings on DNA. **b**, A helical Dmc1–ssDNA filament, similar to structures created by other RecA family members. **c**, A Dmc1 ring and a right-handed helical nucleoprotein filament. The dynamic relationship between rings and helical filaments is not known, but it is possible that filaments are formed by the opening and deposition of Dmc1 rings.

physiological monovalent cations and/or mimicking the effects of accessory proteins.

DNA strand exchange proteins RecA, Rad51 and Dmc1 require nucleotide cofactor, but whether Dmc1 requires ATP hydrolysis remains controversial. Some studies indicate a strong requirement for ATP hydrolysis^{26,28,34}, whereas others show strand exchange even when hydrolysis is hindered^{27,35,36,40–42}. Notably, neither RecA nor Rad51 requires ATP hydrolysis for strand invasion, but both require energy to turn over and release product DNA⁴.

The flurry of recent papers describing robust Dmc1-catalysed DNA strand exchange represent an important landmark because they open the door to a more detailed mechanistic understanding of Dmc1. The key question is, what are the intrinsic biochemical differences that functionally distinguish Dmc1 from Rad51? One apparent difference is the greater sensitivity of Dmc1 to reaction conditions *in vitro*, which seems to be related, at least in part, to the dynamics of ring versus filament formation. At the moment, it is not obvious if or how these properties relate to the unique requirements of meiotic recombination. The challenge will be to sort out whether these are meaningful differences (as opposed to by-products of suboptimal *in vitro* conditions), and if so, to translate the differences into functional consequences *in vivo*.

Modulation of Dmc1 activity by extrinsic factors

RecA family members are remarkable enzymes, but do not function alone—RecA and Rad51 are profoundly affected by accessory proteins *in vivo* and *in vitro*^{13,43}. These accessory factors can function at one or more steps (presynaptic, synaptic, postsynaptic), and they serve to enhance (or sometimes restrain) catalytic efficiency and/or appropriately target strand exchange activities. Similarly, Dmc1 is also highly modulated⁵. Understanding how these factors affect Dmc1 is critical to understanding the mechanisms of the extremely regulated pathways of meiotic recombination.

RPA and recombination mediators. The ssDNA-binding protein RPA is required for efficient strand exchange by Rad51 but its effect is complex⁴³. RPA minimizes secondary structure in ssDNA, promoting Rad51 filament formation; it also sequesters both free ssDNA substrate and the displaced single strand after strand exchange, preventing either from competing with dsDNA. However, RPA can also directly compete with Rad51 for binding to ssDNA, which is inhibitory. RPA is required for extensive Dmc1-catalysed strand exchange, presumably for the same reasons as for Rad51 (ref. 26).

In vivo, RPA probably competes with Dmc1 for binding to ssDNA at meiotic DSBs. For Rad51-dependent reactions *in vitro*, inclusion of Rad52 or a complex of Rad55 and Rad57 overcomes this inhibitory effect and promotes assembly of Rad51 filaments on RPA-coated ssDNA^{13,43}. Proteins such as these are generally referred to as recombination 'mediators'⁴³. It is not yet known if they directly stimulate Dmc1 as well, but all are involved in meiotic recombination (at least in some organisms)¹³. The tumour suppressor protein Brca2 has also been implicated as a Rad51 mediator⁴⁴. In many organisms, Brca2-deficient mutants have meiotic recombination defects^{45–48} and, in plants at least, Brca2 and Dmc1 interact physically^{46,49}.

Rdh54. Genetic studies in yeast indicate an important role in meiotic recombination for Rdh54 (also known as Tid1), a homologue of the DNA repair protein Rad54. Rdh54 is required for the timely conversion of DSBs into mature recombinant products⁵⁰. In its absence, there is decreased co-localization of Dmc1 and Rad51 in cytological nodules on chromosomes⁵¹, presumably reflecting a role for Rdh54 in dissociating Dmc1 from duplex DNA not associated with DSBs (D. K. Bishop, personal communication). Rad54 is less important in the meiotic context in yeast, so the role of Rdh54 is distinct^{50,51}. Rdh54 promotes D-loop formation by Rad51 *in vitro*^{52,53}, so it may function similarly with Dmc1. Fission and budding yeast Rdh54 and Dmc1 interact^{54,55}. Mammals have two known Rad54 family members, Rad54 and Rad54B. Human RAD54B interacts with and stimulates DMC1 *in vitro*²⁶, but the significance of this interaction is

not yet clear because there is little or no meiotic recombination defect in mice lacking Rad54B, Rad54, or both⁵⁶.

Insight into possible biochemical functions of Rdh54 with Dmc1 comes from consideration of its relative, Rad54, a member of the SWI2/SNF2 family of ATPases, which appears to stimulate Rad51 activity through multiple mechanisms^{13,43}. Rad54 couples ATP hydrolysis with translocation along the DNA, thereby generating superhelical torsion in the DNA^{57,58}. Rad51 stimulates this activity, and the topological changes in the DNA in turn promote the ability of Rad51 to catalyse strand invasion and D-loop formation^{57,58}. Rad54 also possesses chromatin remodelling activity, stimulated by Rad51, indicating that another Rad54 function is to enhance the ability of Rad51 to target chromatinized dsDNA for strand exchange^{59,60}. Finally, Rad54 dismantles Rad51 complexes bound to dsDNA⁶¹; this activity may be useful postsynaptically to recycle limiting amounts of Rad51.

Hop2 and Mnd1. Genetic studies in yeast and mouse have identified a chromosome-associated, heterodimeric complex of Hop2 and Mnd1 proteins. Loss of these proteins causes chromosomes to synapse non-homologously and meiotic DSBs to persist^{40,62–67}. Purified human, mouse and budding yeast Hop2–Mnd1 complexes all stimulate D-loop formation and, where tested, DNA strand exchange by the Dmc1 proteins from the same organism^{40,42,68}. The mammalian complexes also stimulate Rad51 (refs 42, 68). Whether yeast Hop2–Mnd1 affects Rad51 activities has not been reported, but *hop2* and *mnd1* mutant phenotypes are much closer to those of *dmc1* mutants than *rad51*, leading to the hypothesis that Hop2–Mnd1 functions primarily with Dmc1 (refs 40, 65, 66). Indeed, organisms that do not contain a Dmc1 orthologue also lack Hop2 and Mnd1 (ref. 17). However, because Rad51 and Dmc1 are so closely tied together (see below), it is plausible that Hop2–Mnd1 could be specific for a Dmc1-dependent pathway and yet function biochemically to stimulate both Rad51 and Dmc1.

The molecular function of Hop2–Mnd1 remains unclear. The biochemical studies are consistent with direct effects on the catalytic activities of Dmc1 and/or Rad51. A presynaptic role in aiding the binding of Dmc1 to ssDNA has been suggested⁴², but the genetics and biochemistry are currently most consistent with a synaptic and/or postsynaptic role^{40,42,64,66,67}. Klein and colleagues have suggested a different model, in which Hop2–Mnd1 promotes Dmc1 activity indirectly by acting on the chromatin and/or higher order chromosome structures of the homologous target⁶⁶. This model is motivated in part by the observation that Hop2–Mnd1 associates with chromatin independent of DSB formation, and does not co-localize with DSB sites or with Rad51 and Dmc1 complexes⁶⁶.

Mei5 and Sae3. The evolutionarily conserved Mei5 and Sae3 proteins of budding yeast form DSB-dependent chromosomal complexes that co-localize with each other and with Dmc1 in a mutually dependent manner^{69,70}. Dmc1, Mei5 and Sae3 physically interact, and Mei5 and Sae3 co-purify as a complex⁶⁹. Unrepaired meiotic DSBs accumulate in *mei5* and *sae3* mutants, indicating a defect in strand exchange^{69,70}. Rad51 foci form in the absence of Mei5, Sae3 or Dmc1, suggesting that the role of Mei5–Sae3 is Dmc1-specific^{69,70}. *S. pombe* contains two Mei5 homologues, Swi2 and Sfr1, and one Sae3 homologue, Swi5. Unlike in budding yeast, these proteins interact with Rhp51 (the *S. pombe* Rad51 orthologue) and function in recombination in both vegetative and meiotic cells^{71,72}.

The biochemical function(s) of Mei5–Sae3 is not yet known. The fact that Dmc1 is not loaded onto chromosomes in *mei5* and *sae3* mutants (unlike in *hop2* and *mnd1* mutants) suggests a direct presynaptic role in promoting Dmc1 filament formation^{69,70}. Perhaps Mei5–Sae3 converts inactive Dmc1 rings into active helical filaments, or helps to load Dmc1 onto RPA-coated ssDNA⁶⁹ (as Rad52, Rad55 and Rad57 perform for Rad51). Indeed, genetic experiments in *S. pombe* support a mediator role for Swi5–Sfr1 in Rhp51-mediated recombination⁷¹. Now that Mei5 and Sae3 can be purified⁶⁹, these ideas can be tested.

Rad51. The function of Dmc1 in meiotic recombination is closely

coordinated with Rad51 (ref. 5). As noted above, both proteins localize together to the sites where DSBs are being repaired^{8,9}. In the absence of Rad51, localization of yeast Dmc1 is impaired, although Rad51 localization seems normal in the absence of Dmc1 (refs 9, 24). Precisely how the proteins are coordinated is not yet clear. Based in part on co-localization experiments, it has been proposed that they work together by forming mixed nucleoprotein filaments⁸. Homopolymeric Rad51 and Dmc1 filaments arranged consecutively on the same ssDNA is another possibility. On the other hand, side-by-side immunostaining patterns have been observed for the two proteins under at least some circumstances on spread meiotic chromosomes^{8,51}, leading to the hypothesis that Rad51 and Dmc1 bind to opposite ends of each DSB^{15,51}. The idea of asymmetrical protein binding is attractive because the two ends of a DSB themselves behave differently—one end carries out the initial strand invasion, while the other end is captured in a separate reaction¹⁵ (see Fig. 2). If this idea is correct, it raises a couple of interesting questions: which protein is responsible for initial strand invasion, and how is the asymmetric distribution of the proteins accomplished? It has been proposed that the Dmc1 nucleoprotein filament is the end that invades first¹⁵, although this remains to be verified. Recent studies of the early steps in DSB processing, when Spo11 is removed from its covalent attachment to DNA ends, have led to the hypothesis that asymmetry is set up very early, at or before DSB formation⁷³. This also awaits confirmation.

Structural components of meiotic chromosomes. Meiotic recombination and large-scale chromosome structures are highly integrated, in part because a functional chiasma itself is a multicomponent structure comprising local exchange of homologous DNA duplexes (that is, recombination) plus exchange of the proteinaceous axes of the homologues and local separation of sister chromatids^{2,6} (Fig. 1). Achieving this integration requires cross-talk spanning size scales that differ by orders of magnitude; that is, between the enzymes executing the DNA events of recombination and the structural proteins that make up micrometre-scale chromosome structures. The detailed molecular mechanisms involved are poorly understood. An extensive discussion is beyond the scope of this review (for further information see refs 1, 2, 6, 74), but a few points pertaining specifically to Dmc1 will be mentioned here.

The Red1 protein in *S. cerevisiae* provides one type of functional connection between Dmc1 and chromosome organization. Red1 is a major structural component of meiotic chromosomes⁷⁵. *In vivo*, one function of Red1 is to promote, directly or indirectly, the loading of Dmc1 onto ssDNA at meiotic DSBs⁷⁴. Another role of Red1 is to prevent stable DNA strand exchange between homologues when Dmc1 is absent^{14,15}. Whereas DSBs persist in *dmc1* mutant cells, cells lacking both Dmc1 and Red1 are able to repair meiotic DSBs by Rad51-mediated recombination between sister chromatids instead of between homologues. Thus, it seems that interplay between Dmc1 and Red1 is integral to the interhomologue bias of meiotic recombination.

Another connection between Dmc1 and chromosome structure is through Rec8, an evolutionarily conserved, meiosis-specific subunit of cohesin, the multiprotein complex that holds sister chromatids together in both mitosis and meiosis^{1,76}. In addition to defects in sister chromatid cohesion, *rec8* mutants in *S. cerevisiae* have recombination phenotypes similar in some respects to *dmc1* mutants, including inefficient repair and over digestion by exonucleases⁷⁶. Recombination defects are also apparent in a *Rec8*-defective mouse, suggesting that at least some of the roles of this protein in recombination are conserved⁷⁷.

Outlook

This is an exciting time for students of meiotic chromosome dynamics in general, and of meiotic recombination in particular. The field faces several challenges. On the biological front, there is a need for more information about the specific processes influenced by Dmc1 (for example, interhomologue bias, crossover interference), particularly in organisms other than budding yeast. Which aspects of

Dmc1 function are conserved, and which are not? How does recombination differ in organisms that have Dmc1 versus those that do not? On the biochemical front, there is a need for deeper mechanistic understanding of the mediators and other accessory proteins that influence Dmc1 activities. How do they modify Dmc1 activity? What is the interplay between Rad51 and Dmc1, and how do other factors impinge?

The biggest challenge, however, will be to connect the dots between the biochemical properties of Dmc1 and the *in vivo* functions revealed by genetics. One route to this goal will be to develop *in vitro* reactions that recapitulate increasingly complex aspects of chromosome behaviour, such as the connection between Dmc1 and chromosome structure proteins. Another route will be to apply the powerful genetic and cytological methods available in many organisms to test specific predictions from biochemical experiments, for example by examining phenotypes of mutants with specific biochemical defects in Dmc1 or other proteins. At such a point, we will be able to say that these too often disparate fields—biochemistry and genetics—have finally (re)combined.

- Petronczki, M., Siomos, M. F. & Nasmyth, K. Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112**, 423–440 (2003).
- Kleckner, N. Chiasma formation: chromatin/axis interplay and the role(s) of the synaptonemal complex. *Chromosoma* **115**, 175–194 (2006).
- Keeney, S. Mechanism and control of meiotic recombination initiation. *Curr. Top. Dev. Biol.* **52**, 1–53 (2001).
- Bianco, P. R., Tracy, R. B. & Kowalczykowski, S. C. DNA strand exchange proteins: a biochemical and physical comparison. *Front. Biosci.* **3**, D570–D603 (1998).
- Shinohara, A. & Shinohara, M. Roles of RecA homologues Rad51 and Dmc1 during meiotic recombination. *Cytogenet. Genome Res.* **107**, 201–207 (2004).
- Zickler, D. & Kleckner, N. Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* **33**, 603–754 (1999).
- Johnson, R. D. & Jasin, M. Double-strand-break-induced homologous recombination in mammalian cells. *Biochem. Soc. Trans.* **29**, 196–201 (2001).
- Tarsounas, M., Morita, T., Pearlman, R. E. & Moens, P. B. RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. *J. Cell Biol.* **147**, 207–220 (1999).
- Bishop, D. K. RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. *Cell* **79**, 1081–1092 (1994).
- Bishop, D. K., Park, D., Xu, L. & Kleckner, N. DMC1: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell* **69**, 439–456 (1992).
- Shinohara, A., Ogawa, H. & Ogawa, T. Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69**, 457–470 (1992).
- Aboussekhra, A., Chanet, R., Adjiri, A. & Fabre, F. Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the RAD51 gene, whose sequence predicts a protein with similarities to prokaryotic RecA proteins. *Mol. Cell. Biol.* **12**, 3224–3234 (1992).
- Krogh, B. O. & Symington, L. S. Recombination proteins in yeast. *Annu. Rev. Genet.* **38**, 233–271 (2004).
- Schwacha, A. & Kleckner, N. Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. *Cell* **90**, 1123–1135 (1997).
- Hunter, N. & Kleckner, N. The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. *Cell* **106**, 59–70 (2001).
- Grushcow, J. M. et al. *Saccharomyces cerevisiae* checkpoint genes MEC1, RAD17 and RAD24 are required for normal meiotic recombination partner choice. *Genetics* **153**, 607–620 (1999).
- Stahl, F. W. et al. Does crossover interference count in *Saccharomyces cerevisiae*? *Genetics* **168**, 35–48 (2004).
- Shinohara, M., Sakai, K., Shinohara, A. & Bishop, D. K. Crossover interference in *Saccharomyces cerevisiae* requires a TID1/RDH54- and DMC1-dependent pathway. *Genetics* **163**, 1273–1286 (2003).
- Tsubouchi, H. & Roeder, G. S. The importance of genetic recombination for fidelity of chromosome pairing in meiosis. *Dev. Cell* **5**, 915–925 (2003).
- Di Giacomo, M. et al. Distinct DNA damage-dependent and independent responses drive the loss of oocytes in recombination-defective mouse mutants. *Proc. Natl Acad. Sci. USA* **102**, 737–742 (2005).
- Rockmill, B., Sym, M., Scherthan, H. & Roeder, G. S. Roles for two RecA homologs in promoting meiotic chromosome synapsis. *Genes Dev.* **9**, 2684–2695 (1995).
- Young, J. A., Hyppa, R. W. & Smith, G. R. Conserved and nonconserved proteins for meiotic DNA breakage and repair in yeasts. *Genetics* **167**, 593–605 (2004).
- Bishop, D. K. et al. High copy number suppression of the meiotic arrest caused by a *dmc1* mutation: REC114 imposes an early recombination block and RAD54 promotes a DMC1-independent DSB repair pathway. *Genes Cells* **4**, 425–444 (1999).

24. Shinohara, A., Gasior, S., Ogawa, T., Kleckner, N. & Bishop, D. K. *Saccharomyces cerevisiae* recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. *Genes Cells* **2**, 615–629 (1997).
25. Paques, F. & Haber, J. E. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **63**, 349–404 (1999).
26. Sehorn, M. G., Sigurdsson, S., Bussen, W., Unger, V. M. & Sung, P. Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. *Nature* **429**, 433–437 (2004).
27. Hong, E. L., Shinohara, A. & Bishop, D. K. *Saccharomyces cerevisiae* Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous super-coiled duplex DNA. *J. Biol. Chem.* **276**, 41906–41912 (2001).
28. Li, Z., Golub, E. I., Gupta, R. & Radding, C. M. Recombination activities of HsDmc1 protein, the meiotic human homolog of RecA protein. *Proc. Natl Acad. Sci. USA* **94**, 11221–11226 (1997).
29. Masson, J. Y. *et al.* The meiosis-specific recombinase hDmc1 forms ring structures and interacts with hRad51. *EMBO J.* **18**, 6552–6560 (1999).
30. Nara, T., Hamada, F., Namekawa, S. & Sakaguchi, K. Strand exchange reaction *in vitro* and DNA-dependent ATPase activity of recombinant LIM15/DMC1 and RAD51 proteins from *Coprinus cinereus*. *Biochem. Biophys. Res. Commun.* **285**, 92–97 (2001).
31. Passy, S. I. *et al.* Human Dmc1 protein binds DNA as an octameric ring. *Proc. Natl Acad. Sci. USA* **96**, 10684–10688 (1999).
32. Gupta, R. C., Golub, E., Bi, B. & Radding, C. M. The synaptic activity of HsDmc1, a human recombination protein specific to meiosis. *Proc. Natl Acad. Sci. USA* **98**, 8433–8439 (2001).
33. Kinebuchi, T. *et al.* Structural basis for octameric ring formation and DNA interaction of the human homologous-pairing protein Dmc1. *Mol. Cell* **14**, 363–374 (2004).
34. Sauvageau, S. *et al.* Fission yeast Rad51 and Dmc1, two efficient DNA recombinases forming helical nucleoprotein filaments. *Mol. Cell. Biol.* **25**, 4377–4387 (2005).
35. Lee, M. H. *et al.* Calcium ion promotes yeast Dmc1 activity via formation of long and fine helical filaments with single-stranded DNA. *J. Biol. Chem.* **280**, 40980–40984 (2005).
36. Bugreev, D. V., Golub, E. I., Stasiak, A. Z., Stasiak, A. & Mazin, A. V. Activation of human meiosis-specific recombinase Dmc1 by Ca^{2+} . *J. Biol. Chem.* **280**, 26886–26895 (2005).
37. Chang, Y. C. *et al.* Molecular visualization of the yeast Dmc1 protein ring and Dmc1–ssDNA nucleoprotein complex. *Biochemistry* **44**, 6052–6058 (2005).
38. Kinebuchi, T., Kagawa, W., Kurumizaka, H. & Yokoyama, S. Role of the N-terminal domain of the human DMC1 protein in octamer formation and DNA binding. *J. Biol. Chem.* **280**, 28382–28387 (2005).
39. Bugreev, D. V. & Mazin, A. V. Ca^{2+} activates human homologous recombination protein Rad51 by modulating its ATPase activity. *Proc. Natl Acad. Sci. USA* **101**, 9988–9993 (2004).
40. Chen, Y. K. *et al.* Heterodimeric complexes of Hop2 and Mnd1 function with Dmc1 to promote meiotic homolog juxtaposition and strand assimilation. *Proc. Natl Acad. Sci. USA* **101**, 10572–10577 (2004).
41. Enomoto, R. *et al.* Positive role of the mammalian TBPIP/HOP2 protein in DMC1-mediated homologous pairing. *J. Biol. Chem.* **279**, 35263–35272 (2004).
42. Petukhova, G. V. *et al.* The Hop2 and Mnd1 proteins act in concert with Rad51 and Dmc1 in meiotic recombination. *Nature Struct. Mol. Biol.* **12**, 449–453 (2005).
43. Sung, P., Krejci, L., Van Komen, S. & Sehorn, M. G. Rad51 recombinase and recombination mediators. *J. Biol. Chem.* **278**, 42729–42732 (2003).
44. Yang, H., Li, Q., Fan, J., Holloman, W. K. & Pavletich, N. P. The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA–ssDNA junction. *Nature* **433**, 653–657 (2005).
45. Sharan, S. K. *et al.* BRCA2 deficiency in mice leads to meiotic impairment and infertility. *Development* **131**, 131–142 (2004).
46. Siau, N. *et al.* Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. *EMBO J.* **23**, 1392–1401 (2004).
47. Kojic, M., Kostrub, C. F., Buchman, A. R. & Holloman, W. K. BRCA2 homolog required for proficiency in DNA repair, recombination, and genome stability in *Ustilago maydis*. *Mol. Cell* **10**, 683–691 (2002).
48. Martin, J. S., Winkelmann, N., Petalcorin, M. I., McIlwraith, M. J. & Boulton, S. J. RAD-51-dependent and -independent roles of a *Caenorhabditis elegans* BRCA2-related protein during DNA double-strand break repair. *Mol. Cell. Biol.* **25**, 3127–3139 (2005).
49. Dray, E., Siau, N., Dubois, E. & Doutriaux, M. P. Interaction between *Arabidopsis* Brca2 and its partners Rad51, Dmc1, and Dss1. *Plant Physiol.* **140**, 1059–1069 (2006).
50. Shinohara, M. *et al.* Characterization of the roles of the *Saccharomyces cerevisiae* RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. *Genetics* **147**, 1545–1556 (1997).
51. Shinohara, M., Gasior, S. L., Bishop, D. K. & Shinohara, A. Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination. *Proc. Natl Acad. Sci. USA* **97**, 10814–10819 (2000).
52. Petukhova, G., Stratton, S. & Sung, P. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature* **393**, 91–94 (1998).
53. Petukhova, G., Sung, P. & Klein, H. Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. *Genes Dev.* **14**, 2206–2215 (2000).
54. Dresser, M. E. *et al.* DMC1 functions in a *Saccharomyces cerevisiae* meiotic pathway that is largely independent of the RAD51 pathway. *Genetics* **147**, 533–544 (1997).
55. Catlett, M. G. & Forsburg, S. L. *Schizosaccharomyces pombe* Rdh54 (TID1) acts with Rhp54 (RAD54) to repair meiotic double-strand breaks. *Mol. Biol. Cell* **14**, 4707–4720 (2003).
56. Wesoly, J. *et al.* Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. *Mol. Cell. Biol.* **26**, 976–989 (2006).
57. Van Komen, S., Petukhova, G., Sigurdsson, S., Stratton, S. & Sung, P. Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54. *Mol. Cell* **6**, 563–572 (2000).
58. Ristic, D., Wyman, C., Paulusma, C. & Kanaar, R. The architecture of the human Rad54–DNA complex provides evidence for protein translocation along DNA. *Proc. Natl Acad. Sci. USA* **98**, 8454–8460 (2001).
59. Jaskelioff, M., Van Komen, S., Krebs, J. E., Sung, P. & Peterson, C. L. Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. *J. Biol. Chem.* **278**, 9212–9218 (2003).
60. Alexeev, A., Mazin, A. & Kowalczykowski, S. C. Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51–ssDNA nucleoprotein filament. *Nature Struct. Biol.* **10**, 182–186 (2003).
61. Solinger, J. A., Kiianitsa, K. & Heyer, W. D. Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. *Mol. Cell* **10**, 1175–1188 (2002).
62. Leu, J. Y., Chua, P. R. & Roeder, G. S. The meiosis-specific Hop2 protein of *S. cerevisiae* ensures synapsis between homologous chromosomes. *Cell* **94**, 375–386 (1998).
63. Gerton, J. L. & DeRisi, J. L. Mnd1p: an evolutionarily conserved protein required for meiotic recombination. *Proc. Natl Acad. Sci. USA* **99**, 6895–6900 (2002).
64. Petukhova, G. V., Romanienko, P. J. & Camerini-Otero, R. D. The Hop2 protein has a direct role in promoting interhomolog interactions during mouse meiosis. *Dev. Cell* **5**, 927–936 (2003).
65. Tsubouchi, H. & Roeder, G. S. The Mnd1 protein forms a complex with Hop2 to promote homologous chromosome pairing and meiotic double-strand break repair. *Mol. Cell. Biol.* **22**, 3078–3088 (2002).
66. Zierhut, C., Berlinger, M., Rupp, C., Shinohara, A. & Klein, F. Mnd1 is required for meiotic interhomolog repair. *Curr. Biol.* **14**, 752–762 (2004).
67. Henry, J. M. *et al.* Mnd1/Hop2 facilitates Dmc1-dependent interhomolog crossover formation in meiosis of budding yeast. *Mol. Cell. Biol.* **26**, 2913–2923 (2006).
68. Enomoto, R. *et al.* Stimulation of DNA strand exchange by the human TBPIP/Hop2–Mnd1 complex. *J. Biol. Chem.* **281**, 5575–5581 (2006).
69. Hayase, A. *et al.* A protein complex containing Mei5 and Sae3 promotes the assembly of the meiosis-specific RecA homolog Dmc1. *Cell* **119**, 927–940 (2004).
70. Tsubouchi, H. & Roeder, G. S. The budding yeast Mei5 and Sae3 proteins act together with Dmc1 during meiotic recombination. *Genetics* **168**, 1219–1230 (2004).
71. Akamatsu, Y., Dziadkowiec, D., Ikeguchi, M., Shinagawa, H. & Iwasaki, H. Two different Swi5-containing protein complexes are involved in mating-type switching and recombination repair in fission yeast. *Proc. Natl Acad. Sci. USA* **100**, 15770–15775 (2003).
72. Ellermeier, C., Schmidt, H. & Smith, G. R. Swi5 acts in meiotic DNA joint molecule formation in *Schizosaccharomyces pombe*. *Genetics* **168**, 1891–1898 (2004).
73. Neale, M. J., Pan, J. & Keeney, S. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. *Nature* **436**, 1053–1057 (2005).
74. Blat, Y., Protacio, R. U., Hunter, N. & Kleckner, N. Physical and functional interactions among basic chromosome organizational features govern early steps of meiotic chiasma formation. *Cell* **111**, 791–802 (2002).
75. Smith, A. V. & Roeder, G. S. The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J. Cell Biol.* **136**, 957–967 (1997).
76. Klein, F. *et al.* A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell* **98**, 91–103 (1999).
77. Xu, H., Beasley, M. D., Warren, W. D., van der Horst, G. T. & McKay, M. J. Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. *Dev. Cell* **8**, 949–961 (2005).

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