HOMOLOGOUS CHROMOSOME INTERACTIONS IN MEIOSIS: DIVERSITY AMIDST CONSERVATION

Jennifer L. Gerton‡ and R. Scott Hawley*§*

Abstract | Proper chromosome segregation is crucial for preventing fertility problems, birth defects and cancer. During mitotic cell divisions, sister chromatids separate from each other to opposite poles, resulting in two daughter cells that each have a complete copy of the genome. Meiosis poses a special problem in which homologous chromosomes must first pair and then separate at the first meiotic division before sister chromatids separate at the second meiotic division. So, chromosome interactions between homologues are a unique feature of meiosis and are essential for proper chromosome segregation. Pairing and locking together of homologous chromosomes involves recombination interactions in some cases, but not in others. Although all organisms must match and lock homologous chromosomes to maintain genome integrity throughout meiosis, recent results indicate that the underlying mechanisms vary in different organisms.

**The Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, Missouri 64110, USA.*
‡University of Kansas Medical Center, Department of Biochemistry and Molecular Biology, 4011 Wahl Hall East, 3901 Rainbow Boulevard, Kansas City, Kansas 66160.
§University of Kansas, Department of Molecular Biosciences, 2034 Haworth Hall, 1200 Sunnyside Avenue, Lawrence, Kansas 66045.
Correspondence to J.L.G. e-mail: JEG@Stowers-Institute.org
doi:10.1038/nrg1614

From the point of view of the genome, meiosis is a matter of matching, locking and moving chromosomes. These processes are crucial for creating gametes that have the correct number of chromosomes. In humans, up to 30% of spontaneous miscarriages are estimated to be the result of chromosome missegregation events[1]. The results of only a few missegregation events are compatible with human life, and these include Down (trisomy 21), Turner (monosomic for X) and Klinefelter (XXY male) syndromes. In most organisms, chromosome missegregation events that occur during meiosis result in inviability. Although many of the mechanisms and proteins that participate in meiotic pairing and segregation are evolutionarily conserved, there are definite species-to-species differences in the main mechanisms used to achieve homologue segregation.

One of the main mechanisms by which homologous chromosomes are locked together involves crossing over, which is the result of recombination events that are initiated by double-strand breaks (DSBs). It is vitally important that in the face of the high frequency of recombination that occurs during meiosis, the genome is faithfully maintained. This is accomplished by adding specialized layers of regulation on top of the normal homologous recombination machinery. However, there is also evidence to indicate that at least some organisms, such as yeast and flies, are not entirely dependent on recombination for locking their homologous chromosomes together and instead can use other mechanisms to distribute their chromosomes[2].

In this review, we focus on meiosis-specific mechanisms that drive interaction between homologous chromosomes and how these vary between organisms. We begin with an overview of meiotic events, followed by a description and comparison of DSB-independent and dependent matching and locking mechanisms. In particular, we focus on one example of diversity amidst the conserved need to match and lock homologous chromosomes: the function of the Mnd1–Hop2 protein complex. We conclude by highlighting some of the future challenges in the field.

nrg0605-hawle.indd 477 © 2005 Nature Publishing Group 17/5/05 5:20:45 pm

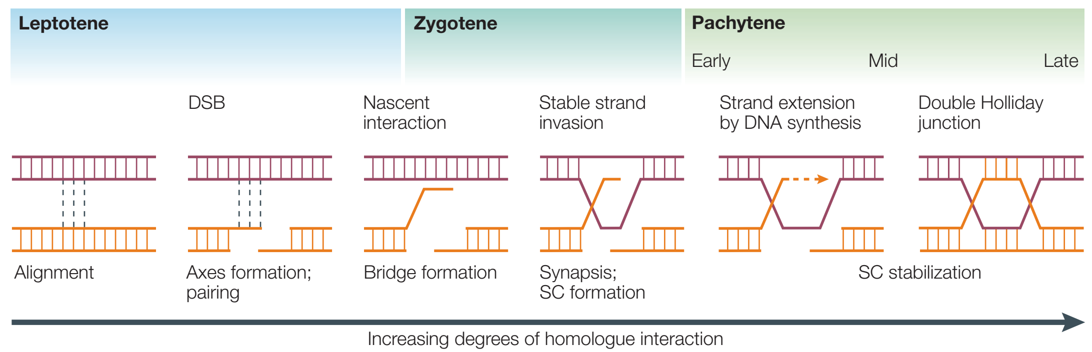

Figure 1 | **Homologue interactions during meiosis.** During chromosome pairing that is independent of double-strand break (DSB) formation (alignment), regions of local distortion might allow homology to be sensed. During DSB-dependent homologue interactions (pairing and nascent interactions), 3′ single-stranded regions engage in interactions with the homologous chromosome. During synapsis and synaptonemal complex (SC) formation, 3′ ssDNA ends stably invade the homologue. The synaptonemal complex, a proteinaceous structure, forms between homologous chromosomes. During this phase, the invading strand is extended by DNA synthesis. Once the strand is recaptured, a double HOLLIDAY JUNCTION forms. Adapted, with permission, from REF. 9 © (2001) Elsevier Science.

Overview of meiotic events

Meiosis is a special set of cell divisions that produces gametes in organisms that reproduce sexually. Cells enter meiosis in much the same way as they enter mitosis — having replicated their chromosomes. However, in cells that undergo meiosis, this replication might involve further specialized features that facilitate later phases of meiosis. For example, replicated sister chromatids in both mitosis and meiosis are held together by COHESIN; all eukaryotes studied so far have meiosis-specific versions of this complex. One of the unique aspects of meiosis is that homologous chromosomes must be identified and locked together in a way that allows them to segregate from each other at the first meiotic division (meiosis I). Homologous chromosomes in most organisms interact through recombination to produce at least one cross-over per chromosome so that they can segregate properly at the first nuclear division. The segregation of homologues at the first meiotic division is followed by the dissolution of chromosome cohesion and the segregation of sister chromatids at the second meiotic division (meiosis II). As there is not an intervening S phase, the result is a gamete that contains half the number of chromosomes of the starting cell.

What are the mechanisms used to identify homologues and lock them together? Homologue identification is perhaps the most mysterious aspect of meiosis. It might involve any or all of the following, depending on the species: interactions between DNA duplexes, interactions between DNA and proteins at specialized pairing centres, interactions between centromeres and centromeric heterochromatin, interactions between telomeres, and CHROMOSOME TERRITORIES. The evidence for each of these is discussed below.

Homologues are held together during much of the meiotic prophase by a proteinaceous structure known as the synaptonemal complex[3]. A more permanent linkage occurs as a result of recombination and the associated crossing over of DNA that generates CHIASMATA, the cytological manifestations of cross-overs. The frequency of such reciprocal recombination events is not uniform; rather, in some regions of the euchromatin, levels of recombination are much higher than in others (heterochromatic regions do not undergo crossing over). Moreover, when analysed at the DNA-sequence level, even those exchanges that occur in recombination-rich regions of euchromatin tend to occur at certain places in the genome, which are termed recombination hotspots, where double-strand breaks occur at a high frequency[4]. In most organisms, homologues are held together by both cross-overs and the synaptonemal complex, although a few organisms have only the synaptonemal complex, such as *Bombyx mori* females, or only cross-overs, such as *Schizosaccharomyces pombe*.

Besides homologue matching and locking mechanisms, there are other mechanisms that are required for correct homologue segregation at the first meiotic division, which are the processes that facilitate the correct orientation of homologous centromeres on the meiotic spindle. However, for the purpose of this review we concentrate on mechanisms that promote interactions between homologous chromosomes.

Matching of homologues

During the early stages of meiotic prophase, pairs of homologous chromosomes are matched by mechanisms that are incompletely understood. The process of homologue interaction can be conceptualized as increasing degrees of physical association between homologues over time, culminating in close, stable homologue juxtaposition (FIG. 1). The term alignment usually refers to bringing homologous chromosomes into rough apposition along their entire length. Pairing refers to the intimate association of homologues. Synapsis is often used to refer to chromosomes that are connected by the synaptonemal complex that comes to lie between homologues and connects them along their entire length. Homologue matching in some species depends on the formation of a set of meiosis-specific DSBs, which are induced by proteins that are

HOLLIDAY JUNCTION
A point at which the strands of two dsDNA molecules exchange partners, which occurs as an intermediate during genetic recombination.

COHESIN
A multi-protein complex that maintains tight association of sister chromatids.

CHROMOSOME TERRITORY
A domain of the nucleus occupied by a pair of homologous chromosomes.

CHIASMA
(Pl. chiasmata.) A cytologically visible physical connection between homologous chromosomes that corresponds to the position of a meiotic cross-over.

nrg0605-hawle.indd 478 © 2005 Nature Publishing Group 17/5/05 5:20:49 pm

homologous to the *Saccharomyces cerevisiae* meiosis-specific sporulation protein (Spo11) and that initiate the recombination process; in other species, matching can occur by DSB-independent means. Although DSB-independent processes seem to be sufficient for homologue matching in flies and worms, DSB-dependent processes seem to be essential for accurate homologue interactions in yeast, *Arabidopsis thaliana*, mice and humans.

DSB-independent matching of homologues

DSB-independent pairing processes seem to be widespread, and at least in some cases, these interactions precede DSB-dependent pairing. In *S. cerevisiae*, for example, DSB-independent pairing is detected at the beginning of meiosis and then becomes undetectable during the meiotic S phase, but pairing is quickly re-established in a DSB-independent manner after S phase[5]. The molecular nature of these DSB-independent pairing interactions is unclear, but might simply involve interactions that are based on homology between two DNA duplexes. It has been argued that these types of 'kissing' interaction would be transient and unstable, and would not require an active process to dissociate them[6]. Their stabilization could occur through several sites of interaction along the length of each chromosome or through stabilizing proteins. Live imaging of chromosome movements in *S. pombe* revealed an initial steep increase in homologous pairing at the beginning of meiosis in wild-type cells and in a mutant lacking DSBs (a mutant for *rec12*; the *S. pombe* homologue of *S. cerevisiae SPO11*). This pairing was not detected in a *meu13* (the *S. pombe* homologue of *S. cerevisiae HOP2*) mutant, arguing that *meu13* might have a role in early DSB-independent pairing interactions[7]. DSB formation soon follows, however, and at least in *S. cerevisiae*, mice and *A. thaliana*, formation of the synaptonemal complex between homologues seems to depend on DSBs and is concomitant with stable strand invasion — when a single-stranded 3′ DNA end from one homologue stably invades the homologous sequence on the other homologue[8–12].

In some organisms, DSB-independent pairing processes seem to be sufficient for synapsis and synaptonemal complex formation. *Caenorhabditis elegans* chromosomes enter meiosis unpaired and then undergo a rapid alignment. This alignment requires neither the initiation of recombination nor the function of proteins that will later facilitate synapsis[13]. Homologue alignment and pairing also occur normally in the complete absence of DSBs in both sexes of *Drosophila melanogaster*[14,15]. In *D. melanogaster* mutants in which recombination levels are at least 100-fold below normal, the synaptonemal complex assembles perfectly normally between homologues[15]. Similarly, in the fungus *Coprinus cinereus*, a significant amount of homologue pairing occurs even when meiotic DSBs are absent[16]. The lack of a requirement for DSBs to initiate synapsis in these organisms probably reflects the ability of flies and worms to use other DSB-independent means to mediate homologue recognition, such as somatic pairing and the use of pairing centres.

Somatic pairing as a basis for homologue pairing. One reason that DSBs might not be necessary for homologue pairing in *D. melanogaster* is that the chromosomes are already aligned when meiosis begins. Mitotic chromosomes in somatic and germline cells in *D. melanogaster* and other Dipterans have been known for almost a century to have high levels of homologue pairing[17–19]. Hexaploid wheat also has somatically paired homologues[20]. Using chromosomes that were tagged

Table 1 | **Methods to monitor homologue interactions**

Method	Use	Advantage	Disadvantage
One-dimensional gel followed by Southern blot	To monitor DSBs, heteroduplex DNA and recombination products	Easy to quantify	Only monitors one genomic locus
Two-dimensional gel followed by Southern blot	To monitor the formation of single-end invasions and joint molecules	Easy to quantify	Only monitors one genomic locus
Tetrad dissection	To monitor recombination outcomes and spore viability	Can monitor multiple chromosome intervals	Monitoring recombination requires live meiotic products
FISH; GFP–lac repressor	To monitor pairing of particular sites on homologues (for example, centromeres, telomeres or individual sites)	An established technique	Might require spreading of chromosomes; timing can be crucial; might only be able to monitor one locus at a time
Immunostaining	To monitor foci formation of specific proteins (for example, antibodies against Dmc1 or Rad51 identify recombination sites)	An established technique	Might require spreading of chromosomes; timing can be crucial
Live chromosome imaging	Homologue pairing; chromosome movements	Can monitor all chromosomes at all times	Technically challenging

FISH, fluorescence *in situ* hybridization.

nrg0605-hawle.indd 479 © 2005 Nature Publishing Group 17/5/05 5:20:51 pm

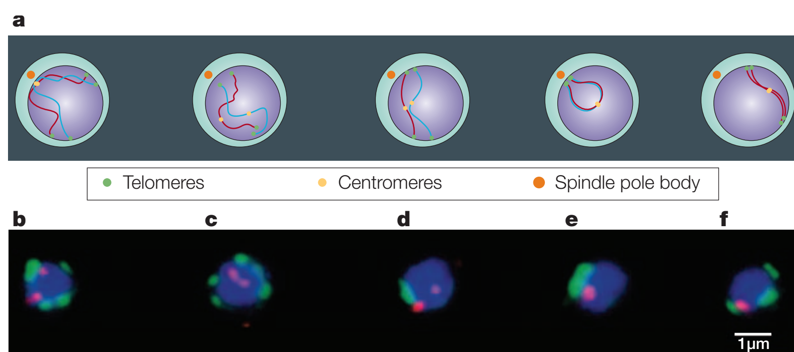

Figure 2 | **Telomere clusters during meiosis in budding yeast. a** | A schematic representation of telomere configuration during meiosis in budding yeast. Telomeres are released from the nuclear envelope at the time of DNA replication. Through recombination initiation in the late leptotene, telomeres reattach to the nuclear envelope. At the leptotene–zygotene transition, the telomeres cluster tightly near the spindle pole body in a 'bouquet'. At pachytene, telomeres again disperse around the nuclear envelope. Adapted, with permission, from *Nature* REF. 44 © (2005) Macmillan Magazines Ltd. **b–f** | A confocal image of intact *Saccharomyces cerevisiae* nuclei that are stained with DAPI (blue; indicates DNA), FITC (green; indicates telomeres) and rhodamine (red; indicates a cosmid of chromosome 11). **b** | The pre-meiotic nucleus with several peripheral telomere clusters and separate cosmid signals. **c** | The pre-meiotic nucleus with a similar telomere distribution, but with associated cosmid signals. **d** | The meiotic nucleus with telomere clusters and separate cosmid signals. **e** | The meiotic nucleus with clustered telomeres and paired cosmids. **f** | The late meiotic nucleus, with peripheral clustered telomeres and paired cosmids. Reproduced, with permission, from REF. 38 © (1999) Company of Biologists Ltd.

with the GFP–lac repressor protein at different positions (TABLE 1), Vazquez *et al.* followed the behaviour of meiotic chromosomes during male *D. melanogaster* meiosis[21]. Because meiosis in *D. melanogaster* males occurs in the absence of recombination or a recognizable synaptonemal complex, this is a good system in which to study homologue matching and segregation in the complete absence of recombination. The results indicated that the pairing events observed in male meiosis I were simply the result of the continuation of previous somatic pairings, and not the result of a meiosis-specific mechanism[21]. The authors proposed that heterochromatic associations, including centromere associations (see below) or chromatid entanglement, might be responsible for the maintenance of homologue association. They also demonstrated that the sequestering of paired homologues into chromosome territories might have an active role in ensuring the specificity of meiotic segregation (see below).

Pairing centres. There is mounting evidence for the existence of specific *cis*-acting sequences that aid homologue identification in worms and flies. In *C. elegans*, specific *cis*-acting regions, or homologue-recognition regions (HRRs), have been identified at the ends of each of the 6 chromosomes. Only a piece of a chromosome that retains the HRR is capable of recombining with its intact homologue[22,23]. These sites might be required for the initiation or maintenance of pairing or synapsis[24]. How they function is still rather mysterious.

A different type of pairing centre is exemplified by homologous regions that are used for pairing of otherwise non-homologous chromosomes. Perhaps the best example of this type of pairing site is the rDNA region of the X and Y chromosomes that mediates sex-chromosome pairing in *D. melanogaster* males[25–27]. A pseudoautosomal region on the human X and Y chromosomes, PAR1, could also be considered an example of a pairing site or region. Most X and Y chromosomes are non-homologous. The PAR1 region is the single largest region of homology between the X and Y chromosomes, and it is therefore the site of obligatory pairing and exchange between the HETEROSOMES. The W and Z chromosomes in birds are similarly non-homologous and their pairing and exchange is regulated by a pseudoautosomal region. The sequencing of the platypus genome has recently revealed that this organism has 10 sex chromosomes, which indicates that these 5 pairs of heterosomes must each have a unique pseudoautosomal region, or pairing and recombination centre, to generate XXXXX gametes for females and YYYYY gametes for males[28].

HETEROSOMES
Homologous chromosomes that are not identical in appearance; for example, the sex chromosomes.

Pairing that involves centromeres and heterochromatin. Centromeres and/or pericentric heterochromatin might function as a type of pairing centre in the sense that these *cis*-acting regions on each chromosome might help homologues to find one another. In an interesting exception to the locking of homologous chromosomes through recombination, homologues that lack cross-overs (non-exchange homologues) in *D. melanogaster* oocytes are matched and segregated in meiosis in a way that depends on the pairing of the pericentric heterochromatin[29,30].

Other organisms also seem to have mechanisms for distributing chromosomes in the absence of reciprocal exchange, often referred to as distributive mechanisms. In *S. cerevisiae*, a single chromosome pair that does not undergo exchange segregates correctly in 90% of meioses[31–34]; this phenomenon also seems to be mediated by centromere association. Centromeres of non-exchange chromosomes seem to pair in meiosis and mediate bipolar attachment to the spindle at the first meiotic division[35]. So, centromeres and pericentric heterochromatin might be special *cis*-acting regions that can facilitate homologue segregation in the absence of recombination.

Pairing that involves telomeres. Cytogenetic studies (TABLE 1) of the first meiotic prophase of many organisms have revealed the clustering of telomeres at the nuclear periphery during the leptotene–zygotene transition[36–40] (FIG. 2). Because this configuration, known as the chromosomal bouquet, precedes the initiation of synapsis, the possibility has been raised that this early localization of the telomeres to a small region of the nuclear envelope facilitates the alignment of homologous chromosomes. *Saccharomyces cerevisiae* strains that contain a mutation in *spo11* (which lack DSBs) or that carry a specific mutation in the DNA repair gene *rad50* (the *rad50S* mutants make DSBs but cannot further process them) form bouquets[41], demonstrating that in *S. cerevisiae*, bouquet formation is independent of recombination and synapsis[38]. In a *spo11* mutant in *Sordaria macrospora*,

nrg0605-hawle.indd 480 © 2005 Nature Publishing Group 17/5/05 5:20:53 pm

ASTRAL MICROTUBULES
Microtubules that extend from each pole of the mitotic spindle without attaching to any other visible structure.

CHROMOSOME PAINTING
Fluorescence *in situ* hybridization (FISH) to chromosomes using a probe that represents a whole chromosome or part of a chromosome.

the bouquet also forms normally, arguing that DSBs are not needed for this type of chromosome organization[42]. However, *spo11* mutants in both *S. macrospora* and budding yeast cannot exit from this phase of meiosis[38,42]. Exogenous DSBs rescue this delay in *S. macrospora*, which indicates that exit is mediated by regulatory processes that sense the progression of recombination beyond the DSB stage[42].

Ndj1, a meiosis-specific telomere protein required for bouquet formation, is required for the localization of telomeres to the nuclear periphery during meiotic prophase in budding yeast. In an *ndj1* mutant, linear chromosomes (but not ring chromosomes, which lack telomeres) missegregate at a high rate[36,43]. Meiotic divisions and synaptonemal complex formation are delayed[36,43], as is the processing of DSBs into recombination products[44]. Together, these results have been taken to indicate that Ndj1 might facilitate recombination through its effect on meiotic telomere organization. It might do this by kinetically facilitating the homologue search through contributions to coalignment, or by reducing the effective volume between homologous sites in the nucleus[45].

During homologue pairing in *S. pombe*, the nucleus oscillates between the cell poles, a process that is driven by ASTRAL MICROTUBULES. During these oscillations, the telomeres are clustered at the spindle pole body, located at the leading edge of the moving nucleus, and the rest of each chromosome follows behind. This oscillatory nuclear movement during meiotic prophase depends on cytoplasmic dynein[46]. A mutation in the dynein heavy chain gene (*dhc1*) reduces nuclear movement; consequently, homologous centromeric and arm regions fail to associate[47]. This gene is required for chromosome segregation in the presence and absence of recombination[118]. Another mutation, in a gene that encodes a telomere-binding protein and length regulator, *taz1*, allows association of homologous centromeres, but disrupts the association of arm and telomere-proximal loci[47]. In addition, *S. pombe* mutants that have disrupted telomere clustering show reduced recombination[48,49], which indicates that telomere clustering facilitates the homologue alignment that is crucial for recombination. In mice and humans, centromere and telomere movements during the early meiotic prophase are associated with the onset of chromosome pairing[50].

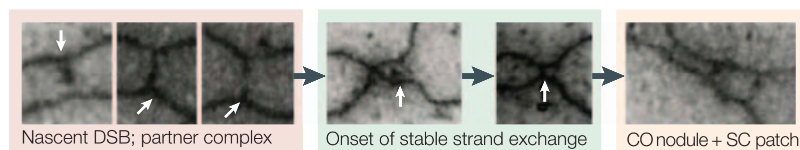

Figure 3 | **Visualization of chromosomal bridges in *Allium fistulosum* and *Allium cepa* (plant) meiocytes.** The sites of double-strand break (DSB)-dependent homologue interaction can be seen as ~400-nm bridges between chromosome axes[55,56]. These bridges, which probably contain a DSB that is already engaged in a nascent interaction with its partner DNA, occur in large numbers[9,57,58]. Their formation depends on the RecA (recombination protein) homologues that are expressed in this species. In the next phase of homologue interaction, these nascent interactions are converted to stable strand-invasion events. This nucleates the formation of the synaptonemal complex (SC). Reproduced, with permission, from REF. 119 © (1987) Springer.

Chromosome territories. Cytogenetic studies in several organisms show that different chromosomes occupy particular domains or territories in the nucleus. The separation of chromosomes into domains might facilitate their pairing by effectively reducing the volume of the nucleus. A deletion of the homologous pairing suppressor locus (*ph1b*) in rye results in a low rate of chromosome synapsis[51]. Comparison of isogenic lines with and without the *ph1* mutant locus demonstrates similar pre-meiotic chromosome arrangement and telomere configurations. However, although homologous chromosomes in the wild-type line maintain separate territories in the nucleus, they become intermingled in the *ph1b* mutant line[51]. Furthermore, although centromere associations occur in the mutant, unlike in the wild type, these associations are non-homologous[52].

In the nucleus of *D. melanogaster*, spermatocyte chromosome territories might also promote the maintenance of meiotic pairing[21]. CHROMOSOME PAINTING in human spermatogonia reveals compacted, largely mutually exclusive chromosome territories[50], again indicating that localization of homologues to a domain might aid in maintaining alignment and pairing.

DSB-dependent matching of homologues

In most organisms, the interaction of meiotically induced DNA DSBs with matching sequences on the homologous chromosome brings homologous chromosomes into alignment during the early- to mid-leptotene stage. DSBs are made by the topoisomerase type II-like protein, Spo11 (REFS 53,54). The sites of DSB-dependent homologue interactions can be seen as ~400-nm bridges between chromosome axes[55,56] (FIG. 3). These bridges, which probably contain a DSB that is already engaged in a nascent interaction with its partner DNA, occur in large numbers[9,57,58]. As leptotene proceeds, a small fraction of these bridges seem to mature into structures known as axial associations that connect the paired lateral elements[59]. These axial associations will eventually nucleate the formation of the synaptonemal complex (that is, as synapsis-initiation sites) between paired chromosomes. The topic of synaptonemal complex formation is beyond the scope of this review, but has been the subject of other recent reviews (see for example REFS 2,4).

The formation of DSBs might be linked to chromatin structure. It has been shown that DSBs tend to occur in regions of open chromatin, or regions that are hypersensitive to nucleases[60–62]. They are also positively correlated with double promoter regions or head-to-head promoters in *S. cerevisiae*[63]. Binding of transcription factors, but not transcription itself, is crucial for hotspot activity at certain locations in the genomes of yeast and mice[64–69].

Certain chromatin states might be specific to meiosis or facilitate recombination. Some might promote DSB formation, whereas others might follow DSB formation to promote resolution of breaks. Recently, it has been shown that ubiquitylation of histone H2B is necessary

nrg0605-hawle.indd 481 © 2005 Nature Publishing Group 17/5/05 5:20:55 pm

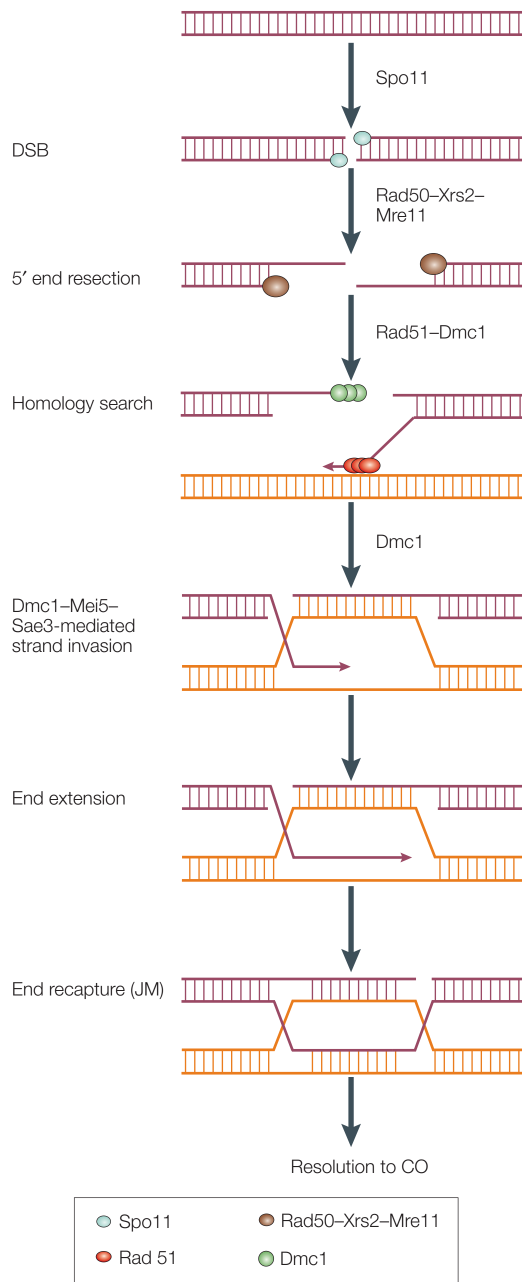

Figure 4 | **The molecular mechanism of meiotic crossover recombination.** Meiotic recombination begins with a double-strand break (DSB) made by Spo11 (a meiosis-specific sporulation protein) through a transesterification reaction (see main text for details). The 5′ ends of this break are resected, leaving stretches of 3′ ssDNA. This DNA is probably used in a homology search that involves the RecA (recombination protein) homologues Dmc1 and Rad51. Once the appropriate homologue is identified, Dmc1 begins a crossing-over recombination event by mediating stable invasion of the homologous chromosome using one of the 3′ ends. This is followed by DNA synthesis that extends the end of the invading strand, and then by recapture of this strand, which generates a joint molecule (JM) that contains a double Holliday junction. This can then be resolved into a cross-over. The connection between two homologous chromosomes through a double Holliday junction is crucial for accurate homologue segregation at the first meiotic division.

for recruiting and/or stabilizing the DSB-forming machinery that contains Spo11 in budding yeast[70]. HIM-17 (high incidence of males due to increased X-chromosome loss 17), which is required for the correct accumulation of histone H3 methylation at lysine 9 on meiotic prophase chromosomes, is essential for DSB formation but dispensable for homologous synapsis in *C. elegans*[71]. In mammalian cells, regions that contain DSBs become phosphorylated on the histone variant H2AX (γ-H2AX)[72]. Although there is certainly a link between chromatin structure and homologue pairing and recombination, the nature and the hierarchy of these events are just beginning to be explored.

The nature of the nascent interactions between a DSB and its partner DNA is unclear. Spo11 makes a DSB using its catalytic tyrosine to attack the phosphodiester backbone of the DNA, creating a covalent bond between Spo11 and the 5′ end of the break. The resection of the 5′ end of the break requires a protein complex that contains the DNA repair proteins, Xrs2, Mre11 and Rad50, exposing a 3′ single-stranded tail (FIG. 4). This 3′ tail might be involved in paranemic interactions (in which one strand is in close proximity to the dsDNA with which it shares homology) and then increasingly stable plectonemic interactions (in which one strand is wound around the dsDNA with which it shares homology) with homologous sequences before any type of stable strand invasion. The interactions between the 3′ single-stranded tail and duplex DNA might constitute a DSB-dependent homology search that allows DSB-dependent homologue pairing to occur. As these types of interactions occur in several locations along the length of homologous chromosomes, they would probably increase the extent of homologue pairing. Once several of these interactions are established, the chromosome axes might become stably aligned[56]. A homology search is probably mediated at least in part by the *Escherichia coli* recombination protein RecA homologues Rad51 and Dmc1, which are thought to bind to the 3′ single-stranded DNA tail. RecA has been shown to mediate this type of homology recognition[73].

Studies in many organisms have shown that synapsis depends on DSBs. In yeast, mouse and *A. thaliana* individuals that carry mutations in the *SPO11* homologues, the synapsis of homologous chromosomes is much reduced or undetectable[8,12,74]. In the absence of Spo11-induced DSBs, synapsis can be restored if DSBs are induced by other means, as has been shown experimentally for mutations in *SPO11* or its homologues in *S. cerevisiae*, *C. cinereus* and the mouse[12,16,75]. It is most likely that further processing of the DSBs into recombination intermediates is also required for correct and efficient synapsis. Analysis of yeast mutants in which the production or processing of DSBs is impaired reveals various synaptonemal complex formation defects[11,76].

Recombination creates a physical association

Stable strand invasion, which can be detected by two-dimensional gel electrophoresis[9] (TABLE 1) or can

nrg0605-hawle.indd 482 © 2005 Nature Publishing Group 17/5/05 5:20:58 pm

be inferred by the presence of HETERODUPLEX DNA[77,78], occurs at the beginning of synapsis and synaptonemal complex formation in *S. cerevisiae*[9]. The appearance of stable strand-invasion events depends on the *E. coli* RecA homologue Dmc1 (REFS 9,79). Both Rad51 and Dmc1 have been shown to mediate strand invasion *in vitro*[80,81]. Other proteins are required for strand exchange, including Tid1–Rdh52, Rad52, and Rad54 (for a detailed review of the role of Rad52 epistasis group proteins in recombination and DSB repair see REF. 82). The protein complexes Mei5–Sae3 and Mnd1–Hop2 have recently been suggested to assist in meiotic strand invasion; we will concentrate on these newcomers in this review. Stable strand-invasion events can mature into the cross-overs and chiasmata that are needed to lock homologues together for correct segregation.

Recombination is biased towards interhomologue recombination by Red1–Hop1. Once DSBs are made, ostensibly they can be resealed, undergo recombination with the sister chromatid, undergo recombination with the homologue or undergo recombination with an ectopic location that probably shares some homology. Each DSB hotspot is likely to have different frequencies of each fate, but the most likely fate of a DSB is recombination with a homologue. Budding yeast contains genes that seem to specifically channel breaks into interhomologue recombination. Red1 and Hop1 are part of the chromosome axis and Mek1 is a meiosis-specific serine–threonine kinase[83,84]. Together, these proteins are thought to channel DSBs into a Dmc1-dependent interhomologue-recombination pathway[85]. In their absence, the number of DSBs is reduced, and the remaining DSBs are mainly resolved by intersister recombination[86]. This does not prevent sporulation, but spores have poor viability as they lack the cross-overs necessary to facilitate appropriate homologue segregation. At present, it is not clear whether other organisms have similar proteins that direct DSBs into interhomologue recombination.

The meiotic recombinases Rad51 and Dmc1 and Mnd1–Hop2. At least two RecA homologues function during meiosis in *S. cereviseae*. One is Rad51, which also participates in homologous recombination in mitosis, and some organisms contain multiple paralogues of this protein. The second protein, Dmc1, is a meiosis-specific RecA homologue which is present in some organisms[87] (see below). Both Rad51 and Dmc1 have been shown to mediate strand invasion *in vitro*[80,81]. Yeast that lack Rad51 can still carry out some recombination and form spores, although with low efficiency and viability[88]. However, without Dmc1, *S. cerevisiae* carry out almost no recombination in meiosis and form extremely small numbers of spores[79], leading to the proposal that Dmc1 is primarily responsible for interhomologue strand invasion during meiosis in *S. cerevisiae*.

In *A. thaliana*, a mutation in *RAD51* has been isolated that does not affect any mitotic functions, but has defective chromosome pairing and synapsis during meiosis[89]. A mutation in *DMC1* results in random chromosome segregation and a residual fertility of 1.5% of the wild type[90]. In mice, homozygous *Rad51* loss-of-function mutation results in early embryonic lethality[91,92], whereas *Dmc1*$^{-/-}$ mice are infertile and their chromosomes do not synapse[93]. So, in many organisms, it seems that although RAD51 has a role in both mitotic and meiotic homologous recombination, DMC1 is essential and specific for homologous recombination in meiosis.

The Mnd1–Hop2 complex has recently been characterized as comprising meiotic genes encoding proteins that form a heterodimer, probably through predicted coiled-coil domains[94,95]. The proteins localize to meiotic chromosomes[95,96]. *mnd1* and *hop2* mutants in *S. cerevisiae* have a similar phenotype to *dmc1* mutants, in that very little interhomologue strand invasion is detected[97]. Like *Dmc1*$^{-/-}$ mice, *Hop2*$^{-/-}$ mice are completely infertile, but do not have any overt mitotic defects[98]. Spermatogenesis in *Hop2*$^{-/-}$ mice reaches only the primary spermatocyte stage, which is consistent with a defect before the first meiotic nuclear division; this is consistent with what has been observed in *S. cerevisiae*. *hop2*-mutant *S. cerevisiae* show extensive synapsis of chromosomes, as measured by staining for the synaptonemal complex component Zip1 (molecular ZIPper), but this synapsis is non-homologous[96]. Although synapsis (presumably non-homologous) is extensive in an *mnd1* mutant, 16 fully synapsed bivalents were never observed[99,100]. In contrast to the extensive synapsis in a *hop2* mutant in *S. cerevisiae*, limited synapsis is seen in chromosome spreads of *Hop2*$^{-/-}$ mouse spermatocytes. Only 25% of cells had undergone more than 10% synapsis, most of which was non-homologous[98]. Therefore, mutations that affect Mnd1–Hop2 cause a synapsis defect in both yeast and mice.

Interestingly, synapsis in a mouse *Hop2*$^{-/-}$ null mutant is more severely affected than it is in a *Dmc1*$^{-/-}$ mutant, in which 61% of cells have undergone at least 10% synapsis[98]. The *Hop2*$^{-/-}$ *Dmc1*$^{-/-}$ double mutant resembles the *Hop2*$^{-/-}$ mutant, with 26% of these cells having undergone 10% or more synapsis, which implies that HOP2 functions upstream of DMC1. However, in *S. cerevisiae*, *hop2 dmc1* or *hop2 rad51* double mutants have 40–50% pairing for a given chromosome, similar to the homologue pairing observed in a *dmc1* or *rad51* single mutant, and better than the 20% that is observed in a *hop2* single mutant. This result indicates that in terms of homologue pairing, Hop2 might function downstream of Dmc1. Although these results might indicate true species differences in the order of action of Hop2 and Dmc1, another interpretation is that Mnd1–Hop2 might have roles both upstream and downstream of Dmc1. This issue will need to be resolved in future experiments. A common feature of the *mnd1–hop2* mutant phenotype in yeast and mice is a defect in homologue pairing and synaptonemal complex formation between the wrong chromosomes.

HETERODUPLEX DNA
DNA that contains a strand from each homologue.

nrg0605-hawle.indd 483 © 2005 Nature Publishing Group 17/5/05 5:20:59 pm

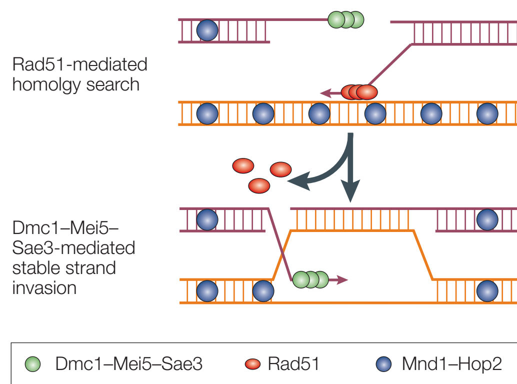

Figure 5 | **A model for Rad51, Dmc1–Mei5–Sae3 and Mnd1–Hop2 function in meiosis.** Dmc1–Mei5–Sae3 (a recombination protein complex) assembles on 3′ ssDNA on one side of a double-strand break (DSB) and the recombination protein Rad51 assembles on the other side. Dmc1–Mei5–Sae3 and Rad51 might be present in multiple copies, in multiprotein complexes or in different multimeric forms. In this model, the Rad51-bound end is primarily involved in the homology search that identifies the homologous chromosome. The interaction between homologous chromosomes is stabilized through interactions between Mnd1–Hop2 complexes that are likely to be present in multiple copies. Once the homologues are engaged in a stable interaction, Dmc1–Mei5–Sae3 can initiate a stable strand invasion. At this point, the Rad51-containing complex might dissociate from the DNA.

It has been proposed that Mnd1–Hop2 might cooperate with Dmc1 to promote stable strand invasion[97]. Dmc1 and Rad51 form foci in *S. cerevisiae mnd1* and *hop2* mutants[95,97,100]. Foci of recombination proteins (RAD51, DMC1 and RPA) were also observed on chromosomes of the mouse *Hop2*$^{-/-}$ mutant[98]. So recombination proteins can still assemble onto DNA, but they seem to be unable to mediate a successful extensive homology search or to catalyse stable strand invasion. Overexpression of Rad51 (but not Dmc1) suppresses defects in meiotic recombination in *S. cerevisiae hop2* mutants[101]. Taken together, these results indicate that there might be two pathways for recombination-mediated pairing of homologues — one that depends on Rad51 to sense homology and one in which Dmc1 depends on Mnd1–Hop2 to ensure correct pairing and recombination[101] (FIG. 5). Consistent with this proposal, a recent study showed that a purified Mnd1–Hop2 heterodimer stimulates the *in vitro* strand-invasion activity of Dmc1 by threefold or more[94,120]. However, in cytological studies, Mnd1–Hop2 does not significantly co-localize with Rad51 (which does co-localize with Dmc1), arguing against a direct interaction of Mnd1–Hop2 with recombination proteins *in vivo*[100]. The issue of direct versus indirect interactions between recombinases and Mnd1–Hop2 will have to be determined in future experiments.

Not all organisms have *DMC1*, *HOP2* and *MND1* orthologues. Although mammals, plants, protists and yeasts possess these genes, worms, flies and *Neurospora crassa* do not. This phyletic pattern correlates with the requirement for DSBs for synapsis and synaptonemal complex formation. Synaptonemal complex formation seems to be dependent on DSBs in yeast and mice[5,10,12,102]. By contrast, in *C. elegans* and *D. melanogaster*, synaptonemal complexes form efficiently in the absence of the respective Spo11 homologues of these species (REFS 14,15). As discussed earlier, fly and worm chromosomes have specific DNA sequences that can facilitate pairing in *cis*[24,103,104]. Furthermore, somatic pairing of fly chromosomes might alleviate the need for other pairing mechanisms during meiosis. Although it is possible that flies and worms have genes that serve a similar function to Dmc1–Mnd1–Hop2 that cannot be identified on the basis of sequence similarity, it seems more likely that pairing centres and/or somatic pairing can provide effective substitutes for Dmc1–Mnd1–Hop2-mediated pairing and recombination. In these organisms, meiotic recombination depends on Rad51 homologues.

Mei5–Sae3 assists Dmc1. Mei5 and Sae3 form a heterodimer during meiosis that has been proposed to interact with Dmc1 (REFS 105,106). In *mei5*, *sae3* or *dmc1* mutants, Rad51 associates with chromosomes, but does not seem to subsequently disassociate from them. Sporulation, spore viability and crossing over are reduced to similar levels in all three mutants. All three proteins co-localize on meiotic chromosomes and their localization is mutually dependent. Taken together, these results indicate that Mei5–Sae3 complexes are Dmc1-specific accessory factors required for catalytic and structural roles in interhomologue recombination during meiosis.

Genome integrity in the face of high levels of recombination. In *S. cerevisiae*, recombination rates are elevated about 1,000-fold in meiosis as compared with mitosis[107]. Surprisingly, recombination between homologous sequences located on homologous chromosomes is not particularly favoured over recombination between homologous sequences on non-homologous chromosomes[107–109], making recombination between dispersed homologous elements a rather frequent event in *S. cerevisiae*. These types of event could potentially be lethal if a translocation, or a dicentric or acentric chromosome is created. However, ectopic recombination during meiosis in flies is infrequent[110–113]. The rapid and DSB-independent synapsis and formation of synaptonemal complexes in flies and worms might help prevent the recombination of homologous sequences on non-homologous chromosomes in these organisms.

Mode 1 (e.g. flies, worms)	**Mode 2** (e.g. yeast, mice, humans)
• DSB-independent homologue matching	• DSB-dependent homologue matching
• Low ectopic recombination	• High ectopic recombination (yeast)
• No Mnd1–Hop2	• Mnd1–Hop2
• Recombination dependent on Rad51	• Recombination dependent on Dmc1

Figure 6 | **A comparison of the main features of recombination-dependent (mode 2) and independent (mode 1) matching and locking of homologous chromosomes.**

nrg0605-hawle.indd 484 © 2005 Nature Publishing Group 17/5/05 5:21:02 pm

FLUORESCENCE *IN SITU* HYBRIDIZATION
(FISH). A technique in which a fluorescently labelled DNA probe is used to hybridize with and therefore detect a particular chromosome or gene with the help of fluorescence microscopy.

The synapsis of non-homologous chromosomes in a *hop2* mutant in *S. cerevisiae* indicates that Mnd1–Hop2 might normally promote interactions that prevent recombination between non-homologous chromosomes. It has recently been shown that although ectopic recombination between homologous sequences can be detected in *dmc1*, *mnd1* and *rad51* single mutants, *mnd1 rad51* and *hop2 rad51* double mutants have much higher levels of ectopic recombination between repetitive sequences, indicating that the proteins that these genes encode might normally have a synergistic role in preventing these types of potentially deleterious events (Henry, Rice and J.L.G., unpublished observations). Therefore, Mnd1–Hop2, in conjunction with recombination proteins, might provide an alternative mechanism for helping to prevent ectopic events in the absence of rapid and DSB-independent synapsis.

Challenges for the future

Although all sexually reproducing organisms must match, lock and segregate their homologues during meiosis, it is clear that some organisms rely on certain mechanisms more than others. One of the biggest differences is that some organisms rely on recombination for synapsis (yeast and mice) whereas others apparently have other mechanisms that allow for synapsis in the absence of recombination (worms and flies, see FIG. 6). Still others, such as *S. pombe* and male *D. melanogaster*, do not form synaptonemal complexes at all. Why and how these differences have evolved is an intriguing question, and one for which there is no clear explanation. Even within a species there can be extreme differences in meiosis between the sexes, such as the difference between male *D. melanogaster*, which does not have recombination or synaptonemal complexes on any chromosome, and female *D. melanogaster*, which has recombination on at least two and usually three of the four chromosomes. However these differences arose, the choice of model system helps to focus studies on specific mechanisms[114]. As meiosis is studied in more model systems, we will come to have a better understanding and appreciation of each mechanism.

In terms of physical assays for recombination intermediates that are currently available for *in vivo* studies (TABLE 1), it is possible to detect DSBs, at least in yeast. The next detectable intermediate is stable strand invasion. Currently, there is no simple physical assay to monitor the progress of the homology search. It is possible to look at pairing of specific loci by FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) in chromosome spreads[115] (TABLE 1). Unfortunately, this method can give different results depending on the spreading technique used, the specific loci monitored, and the time at which the assay is carried out. It is also possible to look at pairing by Cre-mediated recombination of *loxP* sites placed throughout the genome[116,117]. This method can also yield different results depending on the sites that are being monitored. One drawback to most of the cytological methods used until now to monitor homologue pairing in meiosis is that they monitor a single locus at a time in fixed cells. To fully appreciate homologue matching will probably require dynamic methods for monitoring homologue interactions in real time *in vivo*, as has been done for one study in *D. melanogaster*[21] and two studies in *S. pombe*[7,47].

1. Hassold, T. & Hunt, P. To err (meiotically) is human: the genesis of human aneuploidy. *Nature Rev. Genet.* **2**, 280–291 (2001).
A useful and informative review of human meiosis, tightly linked to current questions in meiotic biology.
2. Page, S. L. & Hawley, R. S. Chromosome choreography: the meiotic ballet. *Science* **301**, 785–789 (2003).
3. Page, S. L. & Hawley, R. S. The genetics and molecular biology of the synaptonemal complex. *Annu. Rev. Cell Dev. Biol.* **20**, 525–558 (2004).
4. Petes, T. D. Meiotic recombination hot spots and cold spots. *Nature Rev. Genet.* **2**, 360–369 (2001).
5. Cha, R. S., Weiner, B. M., Keeney, S., Dekker, J. & Kleckner, N. Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. *Genes Dev.* **14**, 493–503 (2000).
6. Kleckner, N. & Weiner, B. M. Potential advantages of unstable interactions for pairing of chromosomes in meiotic, somatic, and premeiotic cells. *Cold Spring Harb. Symp. Quant. Biol.* **58**, 553–565 (1993).
7. Nabeshima, K., Kakihara, Y., Hiraoka, Y. & Nojima, H. A novel meiosis-specific protein of fission yeast, Meu13p, promotes homologous pairing independently of homologous recombination. *EMBO J.* **20**, 3871–3881 (2001).
8. Grelon, M., Vezon, D., Gendrot, G. & Pelletier, G. AtSPO11-1 is necessary for efficient meiotic recombination in plants. *EMBO J.* **20**, 589–600 (2001).
9. 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).
10. Baudat, F., Manova, K., Yuen, J. P., Jasin, M. & Keeney, S. Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol. Cell* **6**, 989–998 (2000).
11. Henderson, K. A. & Keeney, S. Tying synaptonemal complex initiation to the formation and programmed repair of DNA double-strand breaks. *Proc. Natl Acad. Sci. USA* **101**, 4519–4524 (2004).
12. Romanienko, P. J. & Camerini-Otero, R. D. The mouse *Spo11* gene is required for meiotic chromosome synapsis. *Mol. Cell* **6**, 975–987 (2000).
13. MacQueen, A. J., Colaiacovo, M. P., McDonald, K. & Villeneuve, A. M. Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. *Genes Dev.* **16**, 2428–2442 (2002).
14. Dernburg, A. F. *et al.* Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94**, 387–398 (1998).
A study that demonstrates that homologue pairing is independent of Spo11 homologues in *C. elegans*.
15. McKim, K. S. *et al.* Meiotic synapsis in the absence of recombination. *Science* **279**, 876–878 (1998).
The authors show that homologue synapsis is independent of recombination in *D. melanogaster*.
16. Celerin, M., Merino, S. T., Stone, J. E., Menzie, A. M. & Zolan, M. E. Multiple roles of Spo11 in meiotic chromosome behavior. *EMBO J.* **19**, 2739–2750 (2000).
17. Metzker, C. W. Chromosome studies on the Diptera: II. The paired association of chromosomes in the Diptera and its significance. *J. Exp. Zool.* **21**, 213–279 (1916).
18. Metz, C. W. Observations on spermatogenesis in *Drosophila*. *Z. Zellforsch. Mikrosk. Anat.* **4**, 1–28 (1926).
19. Stevens, N. M. A study of germ cells of certain Diptera, with reference to the heterochromosomes and the phenomena of synapsis. *J. Exp. Zool.* **5**, 359–374 (1908).
20. Prieto, P., Santos, A. P., Moore, G. & Shaw, P. Chromosomes associate premeiotically and in xylem vessel cells via their telomeres and centromeres in diploid rice (*Oryza sativa*). *Chromosoma* **112**, 300–307 (2004).
21. Vazquez, J., Belmont, A. S. & Sedat, J. W. The dynamics of homologous chromosome pairing during male *Drosophila* meiosis. *Curr. Biol.* **12**, 1473–1483 (2002).
This paper provides strong evidence that the meiotic pairing in *D. melanogaster* males is a continuation of previous somatic pairings.
22. McKim, K. S., Howell, A. M. & Rose, A. M. The effects of translocations on recombination frequency in *Caenorhabditis elegans*. *Genetics* **120**, 987–1001 (1988).
One of the first papers to provide clear evidence for a role for specific chromosomal sites in mediating pairing and synapsis.
23. McKim, K. S., Peters, K. & Rose, A. M. Two types of sites required for meiotic chromosome pairing in *Caenorhabditis elegans*. *Genetics* **134**, 749–768 (1993).
24. Villeneuve, A. M. A *cis*-acting locus that promotes crossing over between X chromosomes in *Caenorhabditis elegans*. *Genetics* **136**, 887–902 (1994).
25. McKee, B. D., Habera, L. & Vrana, J. A. Evidence that intergenic spacer repeats of *Drosophila melanogaster* rRNA genes function as X–Y pairing sites in male meiosis, and a general model for achiasmatic pairing. *Genetics* **132**, 529–544 (1992).
26. McKee, B. D. & Karpen, G. H. *Drosophila* ribosomal RNA genes function as an X–Y pairing site during male meiosis. *Cell* **61**, 61–72 (1990).
A study that demonstrates that the rDNA functions as a pairing site in *D. melanogaster* males.
27. Merrill, C. J. *et al.* Promoter-containing ribosomal DNA fragments function as X–Y meiotic pairing sites in *D. melanogaster* males. *Dev. Genet.* **13**, 468–484 (1992).
28. Grutzner, F. *et al.* In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. *Nature* **432**, 913–917 (2004).
29. Karpen, G. H., Le, M. H. & Le, H. Centric heterochromatin and the efficiency of achiasmate disjunction in *Drosophila* female meiosis. *Science* **273**, 118–122 (1996).

nrg0605-hawle.indd 485 © 2005 Nature Publishing Group 17/5/05 5:21:04 pm

A study that demonstrates that heterochromatic homology is sufficient for proper meiotic chromosome segregation in *D. melanogaster* females.

30. Dernburg, A. F., Sedat, J. W. & Hawley, R. S. Direct evidence of a role for heterochromatin in meiotic chromosome segregation. *Cell* **86**, 135–146 (1996).
The authors present evidence that heterochromatin is necessary for the proper segregation of non-exchange chromosomes in *D. melanogaster*.
31. Guacci, V. & Kaback, D. B. Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. *Genetics* **127**, 475–488 (1991).
32. Dawson, D. S., Murray, A. W. & Szostak, J. W. An alternative pathway for meiotic chromosome segregation in yeast. *Science* **234**, 713–717 (1986).
33. Ross, L. O., Rankin, S., Shuster, M. F. & Dawson, D. S. Effects of homology, size and exchange of the meiotic segregation of model chromosomes in *Saccharomyces cerevisiae*. *Genetics* **142**, 79–89 (1996).
34. Mann, C. & Davis, R. W. Meiotic disjunction of circular minichromosomes in yeast does not require DNA homology. *Proc. Natl Acad. Sci. USA* **83**, 6017–6019 (1986).
35. Kemp, B., Boumil, R. M., Stewart, M. N. & Dawson, D. S. A role for centromere pairing in meiotic chromosome segregation. *Genes Dev.* **18**, 1946–1951 (2004).
36. Rockmill, B. & Roeder, G. S. Telomere-mediated chromosome pairing during meiosis in budding yeast. *Genes Dev.* **12**, 2574–2586 (1998).
37. Zickler, D. & Kleckner, N. The leptotene–zygotene transition of meiosis. *Annu. Rev. Genet.* **32**, 619–697 (1998).
38. Trelles-Sticken, E., Loidl, J. & Scherthan, H. Bouquet formation in budding yeast: initiation of recombination is not required for meiotic telomere clustering. *J. Cell Sci.* **112**, 651–658 (1999).
39. Chikashige, Y. *et al.* Meiotic nuclear reorganization: switching the position of centromeres and telomeres in the fission yeast *Schizosaccharomyces pombe*. *EMBO J.* **16**, 193–202 (1997).
40. Chikashige, Y. *et al.* Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270–273 (1994).
41. Cao, L., Alani, E. & Kleckner, N. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell* **61**, 1089–1101 (1990).
42. Storlazzi, A. *et al.* Meiotic double-strand breaks at the interface of chromosome movement, chromosome remodeling, and reductional division. *Genes Dev.* **17**, 2675–2687 (2003).
43. Conrad, M. N., Dominguez, A. M. & Dresser, M. E. Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. *Science* **276**, 1252–1255 (1997).
44. Wu, H.-Y. & Burgess, S. The meiotic recombination defects and checkpoint response in *Ndj1*. *Nature* (in the press).
45. Peoples-Holst, T. & Burgess, S. Multiple branches of the meiotic recombination pathway contribute independently to homolog pairing and stable juxtaposition during meiosis in budding yeast. *Genes Dev.* (in the press).
46. Yamamoto, A., West, R. R., McIntosh, J. R. & Hiraoka, Y. A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. *J. Cell Biol.* **145**, 1233–1249 (1999).
47. Ding, D. Q., Yamamoto, A., Haraguchi, T. & Hiraoka, Y. Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. *Dev. Cell.* **6**, 329–341 (2004).
48. Cooper, J. P., Watanabe, Y. & Nurse, P. Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. *Nature* **392**, 828–831 (1998).
49. Nimmo, E. R., Pidoux, A. L., Perry, P. E. & Allshire, R. C. Defective meiosis in telomere-silencing mutants of *Schizosaccharomyces pombe*. *Nature* **392**, 825–828 (1998).
50. Scherthan, H. *et al.* Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing. *J. Cell Biol.* **134**, 1109–1125 (1996).
51. Maestra, B., Hans de Jong, J., Shepherd, K. & Naranjo, T. Chromosome arrangement and behaviour of two rye homologous telosomes at the onset of meiosis in disomic wheat-5RL addition lines with and without the *Ph1* locus. *Chromosome Res.* **10**, 655–667 (2002).
52. Martinez-Perez, E., Shaw, P. & Moore, G. The *Ph1* locus is needed to ensure specific somatic and meiotic centromere association. *Nature* **411**, 204–207 (2001).
This study presents evidence for genetic control of chromosome territories and centromere associations in plants.
53. Keeney, S., Giroux, C. N. & Kleckner, N. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**, 375–384 (1997).
54. Bergerat, A. *et al.* An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* **386**, 414–417 (1997).
55. Albini, S. M. & Jones, G. H. Synaptonemal complex-associated centromeres and recombination nodules in plant meiocytes prepared by an improved surface-spreading technique. *Exp. Cell Res.* **155**, 588–592 (1984).
56. Tesse, S., Storlazzi, A., Kleckner, N., Gargano, S. & Zickler, D. Localization and roles of Ski8p protein in *Sordaria* meiosis and delineation of three mechanistically distinct steps of meiotic homolog juxtaposition. *Proc. Natl Acad. Sci. USA* **100**, 12865–12870 (2003).
The authors present evidence for three stages in the recognition, alignment and synapsis of homologous chromosomes in *Sordaria macrospora*.
57. Franklin, A. E. *et al.* Three-dimensional microscopy of the Rad51 recombination protein during meiotic prophase. *Plant Cell* **11**, 809–824 (1999).
58. 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).
59. 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).
60. Ohta, K., Shibata, T. & Nicolas, A. Changes in chromatin structure at recombination initiation sites during yeast meiosis. *EMBO J.* **13**, 5754–5763 (1994).
61. Fan, Q. Q. & Petes, T. D. Relationship between nuclease-hypersensitive sites and meiotic recombination hot spot activity at the *HIS4* locus of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**, 2037–2043 (1996).
62. Keeney, S. & Kleckner, N. Communication between homologous chromosomes: genetic alterations at a nuclease-hypersensitive site can alter mitotic chromatin structure at that site both in *cis* and in *trans*. *Genes Cells* **1**, 475–489 (1996).
63. Gerton, J. L. *et al.* Inaugural article: global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **97**, 11383–11390 (2000).
64. White, M. A., Wierdl, M., Detloff, P. & Petes, T. D. DNA-binding protein RAP1 stimulates meiotic recombination at the *HIS4* locus in yeast. *Proc. Natl Acad. Sci. USA* **88**, 9755–9759 (1991).
65. Kirkpatrick, D. T., Fan, Q. & Petes, T. D. Maximal stimulation of meiotic recombination by a yeast transcription factor requires the transcription activation domain and a DNA-binding domain. *Genetics* **152**, 101–115 (1999).
66. White, M. A., Dominska, M. & Petes, T. D. Transcription factors are required for the meiotic recombination hotspot at the *HIS4* locus in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **90**, 6621–6625 (1993).
67. Kon, N., Krawchuk, M. D., Warren, B. G., Smith, G. R. & Wahls, W. P. Transcription factor Mts1–Mts2 (Atf1–Pcr1, Gad7–Pcr1) activates the M26 meiotic recombination hotspot in *Schizosaccharomyces pombe*. *Proc. Natl Acad. Sci. USA* **94**, 13765–13770 (1997).
68. Shenkar, R., Shen, M. H. & Arnheim, N. DNaseI-hypersensitive sites and transcription factor-binding motifs within the mouse Eβ meiotic recombination hot spot. *Mol. Cell. Biol.* **11**, 1813–1819 (1991).
69. Mizuno, K. *et al.* The meiotic recombination hot spot created by the single-base substitution ade6–M26 results in remodeling of chromatin structure in fission yeast. *Genes Dev.* **11**, 876–886 (1997).
70. Yamashita, K., Shinohara, M. & Shinohara, A. Rad6–Bre1-mediated histone H2B ubiquitylation modulates the formation of double-strand breaks during meiosis. *Proc. Natl Acad. Sci. USA* **101**, 11380–11385 (2004).
71. Reddy, K. C. & Villeneuve, A. M. *C. elegans* HIM-17 links chromatin modification and competence for initiation of meiotic recombination. *Cell* **118**, 439–452 (2004).
72. Hunter, N., Valentin Borner, G., Lichten, M. & Kleckner, N. γ-H2AX illuminates meiosis. *Nature Genet.* **27**, 236–238 (2001).
This study describes evidence that sites of recombination in mouse chromosomes can be recognized by a phosphorylated version of the histone variant H2AX.
73. Shen, P. & Huang, H. V. Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. *Genetics* **112**, 441–457 (1986).
74. Giroux, C. N., Dresser, M. E. & Tiano, H. F. Genetic control of chromosome synapsis in yeast meiosis. *Genome* **31**, 88–94 (1989).
75. Thorne, L. W. & Byers, B. Stage-specific effects of X-irradiation on yeast meiosis. *Genetics* **134**, 29–42 (1993).
76. Peoples, T. L., Dean, E., Gonzalez, O., Lambourne, L. & Burgess, S. M. Close, stable homolog juxtaposition during meiosis in budding yeast is dependent on meiotic recombination, occurs independently of synapsis, and is distinct from DSB-independent pairing contacts. *Genes Dev.* **16**, 1682–1695 (2002).
77. Nag, D. K. & Petes, T. D. Physical detection of heteroduplexes during meiotic recombination in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 2324–2331 (1993).
78. Allers, T. & Lichten, M. Intermediates of yeast meiotic recombination contain heteroduplex DNA. *Mol. Cell* **8**, 225–231 (2001).
A study that uses physical methods to detect heteroduplex DNA and accompanying Holliday junctions. Some of the recombination intermediates detected are not easily accommodated by the canonical double-strand break repair model.
79. 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).
80. Sung, P. & Robberson, D. L. DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell* **82**, 453–461 (1995).
81. 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).
82. Symington, L. S. Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair. *Microbiol. Mol. Biol. Rev.* **66**, 630–670 (2002).
83. Hollingsworth, N. M. & Johnson, A. D. A conditional allele of the *Saccharomyces cerevisiae HOP1* gene is suppressed by overexpression of two other meiosis-specific genes: *RED1* and *REC104*. *Genetics* **133**, 785–797 (1993).
84. Hollingsworth, N. M. & Ponte, L. Genetic interactions between *HOP1*, *RED1* and *MEK1* suggest that *MEK1* regulates assembly of axial element components during meiosis in the yeast *Saccharomyces cerevisiae*. *Genetics* **147**, 33–42 (1997).
85. Wan, L., de los Santos, T., Zhang, C., Shokat, K. & Hollingsworth, N. M. Mek1 kinase activity functions downstream of *RED1* in the regulation of meiotic double strand break repair in budding yeast. *Mol. Biol. Cell* **15**, 11–23 (2004).
86. Schwacha, A. & Kleckner, N. Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. *Cell* **90**, 1123–1135 (1997).
87. West, S. C. Molecular views of recombination proteins and their control. *Nature Rev. Mol. Cell Biol.* **4**, 435–445 (2003).
88. 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).
89. Li, W. *et al.* The *Arabidopsis AtRAD51* gene is dispensable for vegetative development but required for meiosis. *Proc. Natl Acad. Sci. USA* **101**, 10596–10601 (2004).
90. Couteau, F. *et al.* Random chromosome segregation without meiotic arrest in both male and female meiocytes of a *dmc1* mutant of *Arabidopsis*. *Plant Cell* **11**, 1623–1634 (1999).
91. Lim, D. S. & Hasty, P. A mutation in mouse *rad51* results in an early embryonic lethal that is suppressed by a mutation in *p53*. *Mol. Cell. Biol.* **16**, 7133–7143 (1996).
92. Tsuzuki, T. *et al.* Targeted disruption of the *Rad51* gene leads to lethality in embryonic mice. *Proc. Natl Acad. Sci. USA* **93**, 6236–6240 (1996).
93. Pittman, D. L. *et al.* Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. *Mol. Cell* **1**, 697–705 (1998).
94. 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).
95. 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).

nrg0605-hawle.indd 486 © 2005 Nature Publishing Group 17/5/05 5:21:07 pm

96. 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).
This study demonstrates that deletion of Hop2 leads to synapsis of non-homologous chromosomes.
97. Gerton, J. L. & DeRisi, J. L. Mnd1p: An evolutionarily conserved protein required for meiotic recombination. *Proc. Natl Acad. Sci. USA* **99**, 6895–6900 (2002).
98. 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).
99. Rabitsch, K. P. *et al.* A screen for genes required for meiosis and spore formation based on whole-genome expression. *Curr. Biol.* **11**, 1001–1009 (2001).
100. Zierhut, C., Berlinger, M., Rupp, C., Shinohara, A. & Klein, F. Mnd1 is required for meiotic interhomolog repair. *Curr. Biol.* **14**, 752–762 (2004).
101. Tsubouchi, H. & Roeder, G. S. The importance of genetic recombination for fidelity of chromosome pairing in meiosis. *Dev. Cell* **5**, 915–925 (2003).
102. Mahadevaiah, S. K. *et al.* Recombinational DNA double-strand breaks in mice precede synapsis. *Nature Genet.* **27**, 271–276 (2001).
103. Hawley, R. S. *et al.* There are two mechanisms of achiasmate segregation in *Drosophila* females, one of which requires heterochromatic homology. *Dev. Genet.* **13**, 440–467 (1992).
104. Hawley, R. S. Chromosomal sites necessary for normal levels of meiotic recombination in *Drosophila melanogaster*. I. Evidence for and mapping of the sites. *Genetics* **94**, 625–646 (1980).
105. Tsubouchi, H. & Roeder, G. S. The budding yeast Mei5 and Sae3 proteins act together with Dmc1 during meiotic recombination. *Genetics* **168**, 1219–1230 (2004).
106. 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).
107. Jinks-Robertson, S. & Petes, T. D. High-frequency meiotic gene conversion between repeated genes on nonhomologous chromosomes in yeast. *Proc. Natl Acad. Sci. USA* **82**, 3350–3354 (1985).
This study is a genetic demonstration that recombination can occur with high frequency between homologous sequences on non-homologous chromosomes during meiosis in *S. cerivisiae*.
108. Jinks-Robertson, S. & Petes, T. D. Chromosomal translocations generated by high-frequency meiotic recombination between repeated yeast genes. *Genetics* **114**, 731–752 (1986).
109. Haber, J. E., Leung, W. Y., Borts, R. H. & Lichten, M. The frequency of meiotic recombination in yeast is independent of the number and position of homologous donor sequences: implications for chromosome pairing. *Proc. Natl Acad. Sci. USA* **88**, 1120–1124 (1991).
110. Grell, R. F. Chromosome size at distributive pairing in *Drosophila melanogaster* females. *Genetics* **50**, 151–166 (1964).
111. Grell, R. F. Distributive pairing: the size-dependent mechanism for regular segregation of the fourth chromosomes in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **52**, 226–232 (1964).
112. Hawley, R. S. Chromosomal sites necessary for normal levels of meiotic recombination in *Drosophila melanogaster*. I. Evidence for and mapping of the sites. *Genetics* **94**, 625–646 (1980).
113. Hipeau-Jacquotte, R., Brutlag, D. L. & Bregegere, F. Conversion and reciprocal exchange between tandem repeats in *Drosophila melanogaster*. *Mol. Gen. Genet.* **220**, 140–146 (1989).
114. Burgess, S. M. Homolog pairing in *S. pombe*: the ends are the means. *Mol. Cell* **13**, 766–768 (2004).
115. Weiner, B. M. & Kleckner, N. Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. *Cell* **77**, 977–991 (1994).
116. Burgess, S. M. & Kleckner, N. Collisions between yeast chromosomal loci *in vivo* are governed by three layers of organization. *Genes Dev.* **13**, 1871–1883 (1999).
117. Peoples, T. L., Dean, E., Gonzalez, O., Lambourne, L. & Burgess, S. M. Close, stable homolog juxtaposition during meiosis in budding yeast is dependent on meiotic recombination, occurs independently of synapsis, and is distinct from DSB-independent pairing contacts. *Genes Dev.* **16**, 1682–1695 (2002).
118. Davis, L. & Smith, G. R. Dynein promotes achiasmate segregation in Schizosaccharomyces pombe. *Genetics* (in the press).
119. Albini, S. M. & Jones, G. H. Synaptonemal complex spreading in Allium cepa and A. fistulosum. I. The initiation and sequence of pairing. Chromosoma **95**, 324–338 (1987).
120. Petukhova, G. V. *et al.* The Hop2 and Mnd1 proteins act in concert with Rad51 and Dmc1 in meiotic recombination. *Nature Struc. Mol. Biol.* (in the press).

Acknowledgements

We thank S. Burgess for helpful comments and for sharing data prior to publication.

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez: http://www.ncbi.nlm.nih.gov/entrez
HIM-17 | *rec12* | Spo11

OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Down syndrome | Klinefelter syndrome | Turner syndrome

FURTHER INFORMATION

Stowers Institute Research Team web site:
http://www.stowers-institute.org/labs/ResearchTeam.asp
Access to this interactive links box is free online.

nrg0605-hawle.indd 487 © 2005 Nature Publishing Group 17/5/05 5:21:09 pm