MEIOTIC RECOMBINATION HOT SPOTS AND COLD SPOTS

Thomas D. Petes

Meiotic recombination events are distributed unevenly throughout eukaryotic genomes. This inhomogeneity leads to distortions of genetic maps that can hinder the ability of geneticists to identify genes by map-based techniques. Various lines of evidence, particularly from studies of yeast, indicate that the distribution of recombination events might reflect, at least in part, global features of chromosome structure, such as the distribution of modified nucleosomes.

GENE CONVERSION
The non-reciprocal transfer of information between homologous genes as a consequence of heteroduplex formation, followed by repair of mismatches in the heteroduplex. In current models of recombination, regions of potential gene conversion are associated with all crossovers.

HETERODUPLEX
A region of duplex DNA that contains strands derived from two different DNA molecules.

Curriculum in Genetics and Molecular Biology, Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA. e-mail: tompetes@email.unc.edu

In all eukaryotic organisms that have been analysed in detail, regions of high (hot spots) and low (cold spots) recombination have been noted[1,2]. As a result, there is no simple linear relationship between genetic and physical map distances. Understanding the rules that govern the distribution of recombination events will, therefore, be of great value to projects that are aimed at identifying genes on the basis of their position in a genetic map. In addition to this practical benefit, there are several other reasons why it is important to understand the mechanistic basis for recombination hot spots and cold spots. First, as described below, the mechanism of initiating recombination cannot be understood without knowledge of the factors that regulate hot spots and cold spots. Second, the number of recombination events per chromosome is relevant to the accuracy of chromosome segregation. Third, the distribution of exchanges influences the probability of assembling new configurations of physically linked genes during evolution. Finally, it is likely that an understanding of hot spots and cold spots will be relevant to comprehending other DNA-related processes that are affected by chromosome context, such as transcription and replication.

Recently, some of the important factors that regulate the probability of initiating recombination have been identified. In this review, I summarize the relevant observations, primarily derived from studies in yeast, although information concerning hot spots in mammals is also described. I then briefly describe the roles of post-translational histone modifications in various cellular processes, and discuss the possibility that the level of meiotic recombination is, in part, regulated by such modifications.

Hot spots and cold spots in yeast

In organisms such as fungi, in which the segregation of markers into four meiotic products can be analysed, there are two genetic criteria commonly used to measure recombination activity[3]. First, one can compare the level of reciprocal recombination (crossovers) for a region of known physical length — a process that requires at least two heterozygous markers. Alternatively, one can monitor the level of non-reciprocal recombination (GENE CONVERSION) for heterozygous single markers. If a diploid is heterozygous for alleles *A* and *a*, normal Mendelian segregation results in 2*A*: 2*a* segregation. Gene conversion results in 3*A*: 1*a* or 1*A*: 3*a* segregation.

In *Saccharomyces cerevisiae*, meiotic recombination is initiated by a double-stranded DNA break (DSB), the formation of which is catalysed by the DNA topoisomerase-II-related protein Spo11 (REFS 4,5); in addition to Spo11, DSB formation requires at least 11 other proteins[6]. The broken ends are processed by 5′ to 3′ degradation[7], and the resulting 3′ single-stranded ends invade the homologous chromosome, which results in the formation of a HETERODUPLEX (FIG. 1). Where the heteroduplex region includes DNA sequences that are derived from two different alleles, a DNA mismatch will be generated. Correction of the mismatch (when the invading strand is used as the template) results in gene conversion[3]. The frequency of gene conversion is therefore directly related to the frequency of local initiation of recombination. In *S. cerevisiae*, the frequency of gene conversion for different loci varies from 0.5 to 72%, with a median frequency of 4–5%[1].

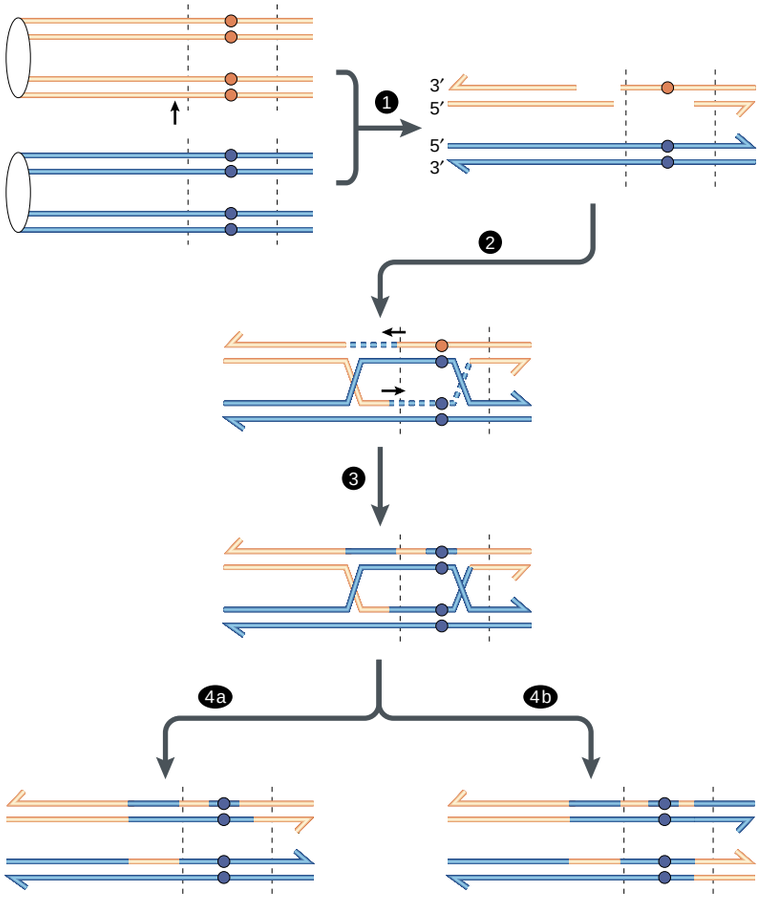

Figure 1 | **Double-stranded-break-repair model of recombination.** The Watson–Crick strands of the DNA duplexes are shown for each chromatid; ovals indicate the centromeres. Dashed vertical lines show the boundaries of a marker gene and the small red circles represent mutant subsitutions (the small blue circles represent the wild type). Recombination is initiated by a double-stranded break (indicated by an arrow) on one of the four paired chromatids. In subsequent panels of the figure, only two of the four chromatids are depicted. The ends of the broken DNA molecule are processed by 5′ to 3′ degradation (step 1). The 3′ terminus of the left-hand end invades the unbroken DNA molecule, and primes DNA synthesis (indicated by a dotted line), displacing the resident strand. The displaced strand pairs with the single strand that contains the mutant substitution, which results in a heteroduplex with a mismatch (step 2). The mismatched base is removed and the resulting gap is filled in (step 3) using the displaced strand as a template. These steps result in a gene conversion event (three meiotic products with the wild-type allele and only one with the mutant allele). The structure shown after step 3 has two HOLLIDAY JUNCTIONS. Cleavage of these junctions can lead to retention of the flanking sequences in the parental configuration (step 4a) or generation of a crossover (step 4b)[17].

Approximately ten recombination hot spots have been analysed in yeast, but the initiation of recombination has been examined in most detail in four: *ARG4*, *HIS4* and *HIS4LEU2* in *S. cerevisiae*, and *ade6-M26* in *Schizosaccharomyces pombe*. Unlike the other hot spots, the *HIS4LEU2* hot spot was artificially (and fortuitously) generated by an ectopic insertion of yeast *LEU2* and a 77-base-pair (bp) insertion of bacterial DNA immediately adjacent to *HIS4* (REF. 8). In the remainder of this section, I discuss generalizations about hot-spot function on the basis of analysis of these hot spots, as well as genome-wide studies of the distribution of recombination events.

HOLLIDAY JUNCTION
When two DNA molecules exchange DNA strands (producing a heteroduplex), the point of the exchange is called a 'Holliday junction.'

Meiosis-specific double-stranded DNA breaks. As first shown at the *ARG4* locus[9], sequences that stimulate meiotic recombination are associated with local meiosis-specific DSBs. Such studies are usually conducted in strains that contain the *rad50S* mutation, which allows DSB formation but blocks subsequent processing of the DNA ends[8]. Deletions that reduce the frequency of DSBs in the hot spot simultaneously reduce the frequency of gene conversion[9–11]. Studies of various genomic locations in *S. cerevisiae* lead to the general conclusion that DSB sites occur between genes rather than within genes[12–14]. In addition, high levels of local DSBs are generally associated with regions of elevated recombination and *vice versa*. One exception to this generalization is that DSBs within heterozygous insertions sometimes fail to stimulate crossovers between homologues (H. Kearney, D. Kirkpatrick, J. Gerton and T. Petes, unpublished data). In strains that are heterozygous for a recombination hot spot, conversion events that result in loss of the hot spot are more common than events that result in duplication of the hot spot[15,16]. This result supports the view that hot spots usually act as a recipient during heteroduplex formation, which is in agreement with the DSB-repair model of recombination[17] (FIG. 1).

At the *ARG4* and *HIS4* loci, the frequency of gene conversion is highest for markers that are located near the 5′ end of the genes and declines for markers near the 3′ end of the gene[18]. Gradients in conversion frequencies (termed 'polarity gradients') have been interpreted as indicating that recombination events initiate in intergenic regions at the high end of the gradient. This interpretation is confirmed by the observation that deletions that remove sequences upstream of *ARG4* and *HIS4* reduce recombination and eliminate the gradient[15,16]. In contrast to the *ARG4* and *HIS4* hot spots, the *ade6-M26* hot spot is intragenic[19].

For several of the *S. cerevisiae* hot spots, the positions of the DSBs have been mapped to single-base-pair resolution[20–23]. In all hot spots, DSBs occur in many positions within a region of 100–500 bp. Deletions that remove the preferred sites of DSB formation do not eliminate DSB formation, which indicates that the sites of breakage might be position-specific and not sequence-specific[10]. Although no obvious consensus sequence for the break sites has been noted, Blumenthal-Perry *et al.*[24] reported a degenerate motif that might be associated with a subset of yeast hot spots. Meiosis-specific DSBs have also been detected in *S. pombe*[25,26]; preliminary evidence indicates that there are preferred sites for DSB formation located near the *ade6-M26* hot spot (G. Smith, W. Steiner, R. Schreckhise and E. Hartsuiker, personal communication).

Chromatin structure and transcription. Chromatin that is transcriptionally active often exhibits increased sensitivity, or hypersensitivity, to the action of nucleases. Similarly, the chromatin that contains recombination hot spots is hypersensitive to nucleases[12,27–30]. At some[27,30], but not all[28], loci, a meiosis-specific increase in nuclease sensitivity is detected before DSB

formation, and alterations of the hot-spot sequences that elevate or reduce hot-spot activity are correlated with increases or decreases, respectively, in the sensitivity of the hot-spot chromatin to nucleases. The available data indicate that nuclease-hypersensitive chromatin might be necessary, but not sufficient, for hot-spot activity. Furthermore, delaying meiotic DNA replication results in the delayed formation of DSBs[31,32]. This delay seems to be established locally on the chromosome, because restricting the delay in replication to a particular chromosomal region specifically delays DSB formation within that region[31]. It is possible that this delay reflects the time required to remodel chromatin to a meiosis-specific form.

At some hot-spot loci, the binding of specific transcription factors is required for hot-spot activity. The Bas1, Bas2, Gcn4 and Rap1 transcription factors bind to the upstream region of *HIS4*[33–35]. By deleting the transcription-factor-binding sites or the genes that encode the transcription factors, White and colleagues[36,37] showed that Bas1, Bas2 and Rap1 were required for hot-spot activity, but Gcn4 was not. However, deletion of the upstream TATAA sequence, which substantially reduces transcription, has no effect on the activity of the *HIS4* hot spot[38]. So, the *HIS4* hot spot requires the binding of transcription factors, but not a high level of transcription, although, to fully stimulate hot-spot activity, the transcription factors require an intact activation domain[39].

The *ade6-M26* allele differs from the wild type by a single-base-pair change[19]. This alteration allows binding of the heteromeric transcription factor Atf1/Pcr1 (Mts1/Mts2), and this binding is required for *ade6-M26* hot-spot activity[40]. As observed with *HIS4*, the elevated frequency of recombination is not a consequence of elevated levels of transcription[41]. We have called recombination hot spots that require the binding of transcription factors 'α'-hot spots[39].

Some hot spots seem to correspond to regions of nuclease-sensitive chromatin that are not associated with transcription-factor binding. For example, one type of recombination- and transcription-stimulating insertion consists of 12 tandem repeats of (CCGNN)[42]. These tandem repeats are poor substrates for nucleosome formation *in vitro*[43], and chromatin containing the $(CCGNN)_{12}$ tract is hypersensitive to DNase I (REF. 42). Because no known transcription factor is capable of binding the (CCGNN) repeats, it is likely that they stimulate recombination and gene expression by a mechanism that is different from that of the α-hot spots; hot spots created by nucleosome-excluding sequences have been termed 'β'-hot spots[42]. Another potential example of a β-hot spot is the tandem array of four (CGGATCCG) repeats that elevates activity of the *HIS4LEU2* hot spot[21].

Global regulation of recombination. Several observations indicate that hot-spot activity is not regulated exclusively at a local level. For example, the activity of some hot spots is context dependent; the level of recombination and DSB formation associated with the *ARG4* hot spot varies at least four-fold depending on the chromosomal location of the *ARG4* sequences[44], and the activity of *ade6-M26* of *S. pombe* is also context dependent[45]. In addition, in certain chromosomal contexts, although the *ARG4* promoter region is still nuclease hypersensitive, this region is not susceptible to DSB formation[44], which indicates that local changes in chromatin structure are not sufficient for hot-spot activity.

There is also evidence for interactions between hot spots. Wu and Lichten[44] found that deletions of regions that had high levels of DSB formation could stimulate DSB formation at other local sites. A related observation is that insertion of a hot spot next to a pre-existing hot spot often reduces the activity of both hot spots[21,44,46,47]. So, there is local competition for the ability of sequences to undergo DSB formation. It is likely that this competition involves the potential to form adjacent DSBs, rather than the direct suppression of one DSB on DSB formation nearby[48]. Although the strongest inhibitory interactions occur between hot spots that are located on the same chromosome, *trans* (interhomologue) inhibitory interactions between hot spots have also been observed[21,49]. As described above, 12 copies of (CCGNN) stimulate gene expression and hot-spot activity, but 48 copies of the same repeat stimulate gene expression and repress hot-spot activity[42]. The mechanism of this suppression is not understood, although it might be related to the competitive interactions between hot spots, because a tandem array of 48 (CCGNN) repeats could act as four $(CCGNN)_{12}$ competing hot spots[42].

A second argument that recombination activity is regulated, at least in part, by global features of chromosome structure is that DSB sites are often clustered. This has been shown using Southern blot analysis[13,50,51] and in a recent genome-wide survey[14]. Gerton *et al.*[14] measured DSB activity for all the yeast genes by using Spo11–DNA complexes isolated from meiotic cells[4] as a hybridization probe for yeast microarrays that contain all 6,200 yeast open reading frames (ORFs). In this analysis, non-random clustering of hot ORFs was evident[14]. Furthermore, many yeast hot spots are associated with genomic regions of high G + C base composition[14]. In FIG. 2, the recombination activities of each ORF on chromosome VI are shown. Chromosome VI has seven hot spots, containing a total of 18 hot ORFs; criteria for determining the statistical significance of these hot spots and for clustering hot ORFs into hot spots are described in Gerton *et al.*[14]. Although the spacing of hot spots seems random, there is a significant association between the location of hot spots and the base composition of the chromosome. When the base composition of yeast chromosomes is scanned using a 5-kb moving window, striking patterns of peaks of high G + C are observed[52]. As shown in FIG. 2, many of the recombination hot spots occur near or at peaks of high G + C. We refer to the hot spots that are associated with high G + C content as 'γ'-hot spots. No significant associations are observed between hot spots and repetitive DNA elements, such as replication origins, tRNA genes, or transposable elements.

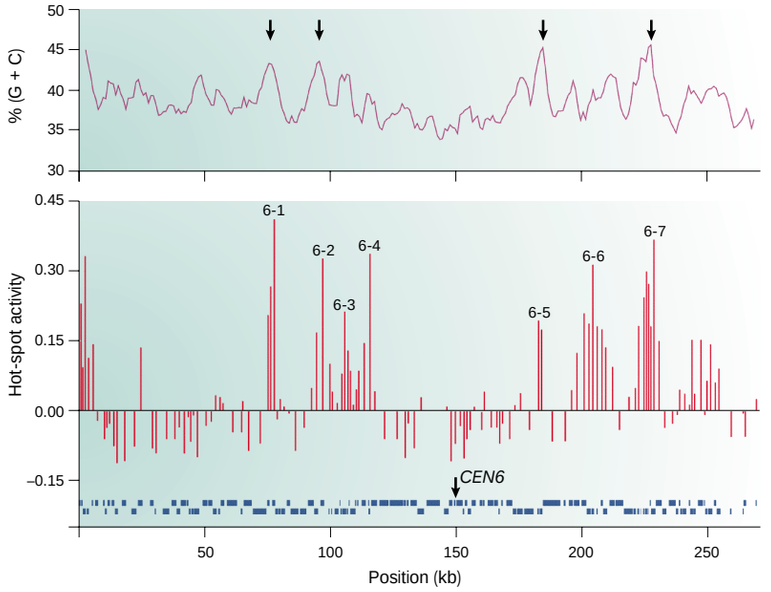

Figure 2 | **Global regulation of hot-spot activity.** Hot-spot activity of open reading frames (ORFs) relative to base composition on yeast chromosome VI (data from REF. 14). The ORFs are depicted as two lines of rectangles (Watson strand on the top line) at the bottom of the figure. Base composition (top of the figure) was measured in a 5-kb sliding window shifted at 1-kb intervals. The four peaks marked with arrows have a G + C content that is 3% or more higher than the average for the chromosome. Hot-spot activities are represented as lines above each ORF. These activities were calculated from seven experiments in which genomic microarrays were hybridized to two probes, one representing total genomic DNA (P1) and one enriched for double-stranded-break-associated sequences (P2). The P2/P1 ratios were calculated for each ORF and each microarray, and these ratios were used to rank the samples. ORFs were classified as 'hot' if they ranked in the top one-eighth in at least five of the seven experiments. Hot-spot activity was calculated as the $\log_{10}$ of the median P2/P1 ratio for each ORF[14]. All the hot spots in the figure, except 6-3 and 6-4, contain more than one ORF. For example, hot spot 6-7 includes five hot ORFs. ORFs were clustered into single hot spots if they were adjacent to, or separated by, a single non-hot ORF. Four (6-1, 6-2, 6-5 and 6-7) of the seven hot spots are within 2.5 kb of the marked peaks of high G + C. *CEN6* indicates the position of the centromere.

In summary, no unique property of DNA sequence or structure has been associated with yeast recombination hot spots. Significant associations have been established that relate hot spots to nuclease-hypersensitive intergenic regions and regions of high G + C base composition. Below, I argue that many of these properties might be related to patterns of the post-translational modification of histones.

Recombination cold spots. In *S. cerevisiae*, cold spots have not been studied intensively. As described above, the tandem array $(CCGNN)_{48}$ suppresses recombination[42]. In addition, Lambie and Roeder[53] showed that the centromere of chromosome III locally repressed both crossing-over and gene conversion. In *rad50S* strains, DSB formation is usually low near centromeres and telomeres[13,14,51]. In *RAD50* (wild-type) strains, however, DSB formation at the telomeres is repressed less than in *rad50S* strains[31], so one must be cautious in interpreting the distribution of recombination observed in this mutant background. Vegetative gene expression is also reduced at the telomeres, a property called 'telomere position effect'[54]. The relationship between this effect and the suppression of meiotic recombination near the telomere is not clear.

In *S. pombe*, meiotic recombination in the 15-kb region between *mat2* and *mat3* is very strongly suppressed[55]; gene expression at these loci is also suppressed. Mutations in several genes simultaneously elevate gene expression of *mat2* and *mat3*, and elevate meiotic recombination[56]. Two of the proteins that are required for silencing of gene expression in this region are Clr4, a homologue of a histone H3 methylase, and Swi6, a silencing protein that recognizes the Clr4-induced methylation of histone H3[57].

In summary, some 'cold' genomic regions (telomeres, centromeres and mating-type loci) seem to be chromosomal domains with modified histones that are recognized by proteins that silence both gene expression and recombination (α-cold spots). In contrast to these regions, in which recombination is actively suppressed, there might also be other regions that are cold because they lack hot spots (β-cold spots). One would expect that a hot-spot sequence inserted within an α-cold spot would be inactive for recombination, whereas a hot spot inserted into a β-cold spot would be active. By this criterion, the cold region near the centromere of chromosome III seems to be a β-cold spot[58]. It is also possible that the lack of G + C peaks near many yeast centromeres (for example, *CEN6*; FIG. 2) also contributes to the lack of recombination.

Hot spots in mammals

To what extent are the rules that govern hot spots and cold spots conserved between yeast and mammals? I briefly summarize conclusions derived from meiotic recombination studies done in mice and humans.

Hot-spot mapping. There are three methods that have been used to map hot spots in mammals. The classical method is to correlate physical distances with genetic distances established by standard (pedigree-based) linkage analysis[59]. The resolution of most such studies is limited by the density of markers and the number of individuals examined. Despite these limitations, several important conclusions concerning meiotic recombination have been made. First, the number of recombination events per kilobase of DNA is, in general, much smaller for mammals than for yeast. There is an average of 1 centiMorgan (cM) per 3 kb in *S. cerevisiae*[3]; the average in humans is about 1 cM per 10^3 kb (REF. 59). This huge difference might be related to the observation that human DNA is compacted in meiosis 20-fold more than is yeast DNA[60]. Second, in humans, females have significantly more meiotic recombination than males, with total map lengths of 44 and 28 Morgans, respectively[59] (FIG. 3); sex specificity for recombination activity has also been observed in mice[61]. Third, the frequency of recombination is affected by chromosome structural elements. In males, but not females, meiotic recombination near the centromere is substantially reduced. In both sexes, recombination near the telomeres is elevated, but the elevation is greater in males

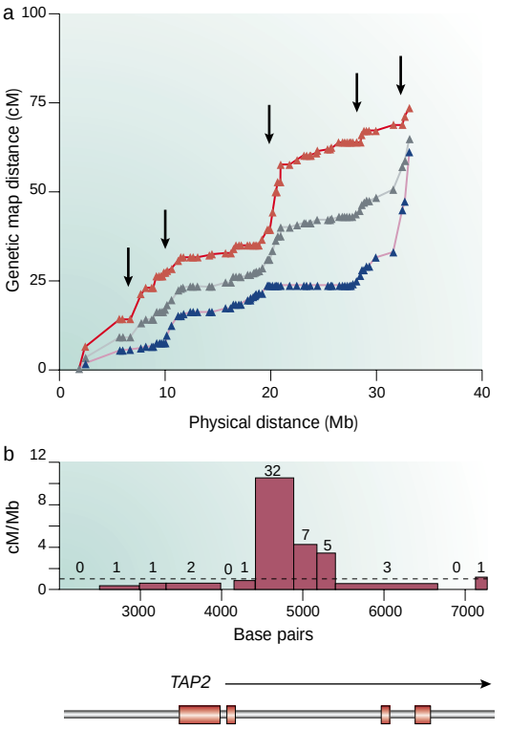

Figure 3 | **Genetic mapping in the human genome.** Mapping regions of high and low recombination in humans at two different levels of resolution. **a** | Linkage map of human chromosome 22q. These maps are based on genotyping short tandem repeat polymorphisms in eight Centre d'Étude Polymorphisme Humaine families[63] (see link to Marshfield Clinic). The *y* axis represents cumulative recombination distances measured from the centromere of chromosome 22 for females (red), males (blue), and the average of males and females (grey). The *x* axis represents physical distance from the centromere. Arrows indicate regions of elevated recombination. **b** | Mapping of the *TAP2* (ATP-binding cassette, sub-family B (MDR/TAP), member 3) recombination hot spot on human chromosome 6. Allele-specific PCR methods were used to identify crossovers in sperm DNA isolated from a male heterozygous for a large number of single-nucleotide polymorphisms near *TAP2* (REF. 66). The dotted line represents the average frequency of crossovers in the male meiosis. A clear recombination hot spot is observed within a large intron (black bar) of *TAP2* (red bars are exons). (Redrawn with permission from REF. 66 © (2000) Oxford University Press.)

than in females[59]. Fourth, in addition to the effects of chromosome structure, regional differences in recombination frequencies are observed. For example, on chromosome 22, there are several non-telomeric regions with elevated recombination frequencies[62] (FIG. 3). The hot regions shown in FIG. 3a do not contain single, small hot spots, but represent 1-Mb areas of elevated recombination. Similar regions are observed on other human chromosomes, described by Yu *et al.*[63] as recombination 'jungles'.

Two other methods, analysis of LINKAGE DISEQUILIBRIUM and single-sperm DNA typing, allow more precise hot-spot mapping. Linkage disequilibrium between two physically distant markers might reflect reduced recombination between the markers and, conversely, if two nearby markers are in linkage equilibrium, there might be a recombination hot spot between the two markers[64]. This method was used to localize a hot spot near the human *TAP2* (ATP-binding cassette, sub-family B (MDR/TAP), member 3) locus to a resolution of about 1 kb (REFS 65,66) (FIG. 3). It should be pointed out that the recombination hot spots might represent a complicating factor in the use of linkage disequilibrium studies to look for candidate disease genes[65]. Caveats in using linkage disequilibrium to map hot spots are discussed by Hedrick[64].

The more direct method of high-resolution mapping of hot spots is to use sperm DNA as a PCR template. In one study, which involved the examination of 602 individual sperm from a single donor, 29 recombinants were mapped at 7 polymorphic loci in a 280-kb interval[67]. In a second study, batches of DNA that contain about 10,000 sperm samples were first screened by PCR to identify those with a crossover in a 5-kb interval at the *TAP2* hot spot[66]. The position of the crossovers was then mapped more precisely using additional polymorphic markers. Most occurred in a 1.2-kb interval (FIG. 3b). In the male, recombination rates for this interval are elevated about tenfold relative to the average[66]. In females, the activity of the *TAP2* hot spot is even more striking: it is 20- to 40-fold more active than in males[65,66].

Hot-spot mechanisms in mammals. Because only a few hot spots have been mapped to high resolution in mammals, it is difficult to make generalizations. A meiotic recombination hot spot in the mouse MHC locus (the Eβ-hot spot) is associated with transcription-factor-binding sites and regions of DNase I hypersensitivity[68]; this hot spot seems to resemble a yeast α-hot spot. Three human hot spots have been mapped to a resolution of 1–2 kb: the MINISATELLITE-associated hot spot MS32 (REF. 69), a hot spot for unequal crossovers that are associated with deletions that give rise to hereditary neuropathies[70,71], and the major histocompatibility complex (MHC)-associated *TAP2* hot spot[66]. None of these hot spots are associated with promoter sequences and there are no significant shared motifs[66].

Although no shared sequence has been observed among all the hot spots, there is evidence to indicate that some mammalian hot spots might be associated with G + C-rich repetitive DNA sequences. For example, several human minisatellites, such as CEB1 and MS205, are unstable and are extremely G + C rich (79 and 74%, respectively)[72–74]. These observations indicate that γ-hot spots might exist in mammals. In addition, the common poly(GT) repeat, which stimulates recombination in yeast[75,76], is correlated with regions of high recombination in a low-resolution analysis of human chromosome 22 (REF. 77); an analysis of other human chromosomes indicates that this correlation is weak[63]. There is also a positive, but weak, correlation between high G + C content and regions of high recombination[63,78].

LINKAGE DISEQUILIBRIUM
The condition in which the frequency of a particular haplotype for two loci is significantly greater than that expected from the product of the observed allelic frequencies at each locus.

MINISATELLITE
Regions of DNA in which repeat units of 10–50 base pairs are tandemly arranged in arrays 0.5–30 kb in length.

Although the DNA lesion that initiates meiotic recombination has not been defined in mammalian cells, it is likely to be a DSB, because proteins that are homologous to yeast Spo11 exist in both mice and humans[79–81]. Furthermore, mice that are homozygous for a *Spo11* mutation are sterile, which is consistent with the Spo11 protein having an important role in the initiation of recombination[82,83].

It is likely that in mammals, as in yeast, recombination rates are regulated at two levels: first, at the level of gross chromosome structures (suppressed recombination at the centromeres in males and enhanced recombination at the telomeres); and second, at the local level (locally open chromatin or properties of local DNA sequences). In humans, unlike yeast, the hot spots analysed so far are not associated with promoters.

Histone post-translational modifications

One factor that is likely to be relevant to hot-spot activity is the accessibility of DNA to the recombination machinery. The packaging of DNA into nucleosomes reduces the accessibility of the DNA to sequence-specific DNA-binding proteins, such as those involved in the activation of transcription[84]. The amino-terminal 'tails' of histones, particularly histones H3 and H4, are susceptible to various post-translational modifications, including acetylation, methylation, phosphorylation and ubiquitylation[85–87]. Before considering a possible role for these modifications in recombination, I first review some of the connections between histone modifications and cellular processes, such as gene expression, chromosome condensation and DNA repair.

Histone acetylation, primarily on several lysines in the amino-terminal tails of H3 and H4, has been studied more extensively than other modifications. Acetylation is often associated with the activation of transcription[88]. One strong line of evidence that supports this association is the finding that several transcriptional activators are histone acetyltransferases (HATs) and several repressors of transcription are histone deacetylases (HDACs). Histone acetylation promotes transcription by allowing a more open chromatin configuration. One complication is that certain acetylation events, such as acetylation of Lys 12 of H4, are associated with transcriptional silencing rather than activation[89]. So, the position of the acetylation or the combination of acetylation with other modifications (such as phosphorylation) might determine which biological function is affected[86]. The level of histone acetylation for different promoters is expected to reflect the way in which various transcription factors interact with HATs and HDACs of various specificities. One important interaction is likely to be between BROMODOMAIN motifs of transcriptional activators and repressors with modified histones. For example, the bromodomain of the HAT co-activator P/CAF interacts specifically with a lysine-acetylated H4 peptide[90]. In addition to affecting gene expression, acetylation of histones is associated with chromatin assembly after DNA replication[86].

Another histone modification that can be associated with changes in gene expression in mammalian cells is methylation. The transcriptional activator CARM1 (coactivator-associated arginine methyltransferase-1) has a methyltransferase activity specific for arginine residues in H3 (REF. 91). By contrast, methylation of Lys 9 of H3 by the murine SUV39H methyltransferase seems to be associated with transcriptional silencing[92]. This methylation results in the binding of a specific type of silencing protein (the CHROMODOMAIN-containing HP1 class)[57,93].

Phosphorylation of histones is also linked to many cellular events[87]. Phosphorylation of H3 on Ser 10 is associated with both transcriptional activation[94] and chromosome condensation[95]. In response to DNA damage, in mammalian cells, a variant H2A histone (H2A.X) is phosphorylated on a serine residue that is located near the carboxyl terminus[96]. In yeast, the comparable phosphorylation event on H2A requires the Mec1 kinase[97].

The last class of histone modification that will be discussed is ubiquitylation. Robzyk *et al.*[98] recently showed that H2B undergoes Rad6-dependent ubiquitylation in yeast. Strains with a mutation of the target residue (Lys 123) grow slowly and fail to undergo meiosis. It is not clear whether the failure to undergo meiosis represents the loss of expression of genes that are required for this cellular process or the loss of a particular type of chromatin that is required for meiosis.

Many types of histone modifications have been described. Several (although not all) of these modifications seem to loosen chromatin structure, which results in increased levels of transcription. One complication in understanding the roles of these modifications is that some modifications seem coupled. For example, some HATs preferentially acetylate H3 that has been phosphorylated at Ser 10 (REF. 99).

Histone modifications and recombination

As discussed above, there is evidence for three different types of recombination hot spot: α (which requires transcription-factor binding), β (which contains nucleosome-excluding DNA sequences) and γ (which is associated with regions of high G + C base composition). These categories are not necessarily mutually exclusive. Although it is possible that these three types of hot spot involve intrinsically different mechanisms, I suggest that all three might be mechanistically related. Below, I argue that hot spots have two properties: first, a chromosomal region with highly modified histones, which potentiate the initial interactions between the chromosome and the recombination machinery; and second, intergenic DNA sequences in this region that are unbound by nucleosomes or transcription factors (naked DNA) and are susceptible to the action of Spo11.

I suggest that an early step in the initiation of recombination is an interaction of the recombination complex with a region of open chromatin that has highly modified histones. The α-hot spots use transcription factors to attract the complexes (HAT complexes or others) that are required to modify the histones in the hot-spot region (FIG. 4). The β-hot spots are hot for two different reasons. First, their nucleosome-excluding properties allow entry of the histone-modifying complexes in the absence of transcription factors. This hypothesis is supported by the

BROMODOMAIN
A protein motif (originally defined in the *Drosophila* Brahma protein) that is involved in binding certain acetylated histones; often associated with transcriptional activation.

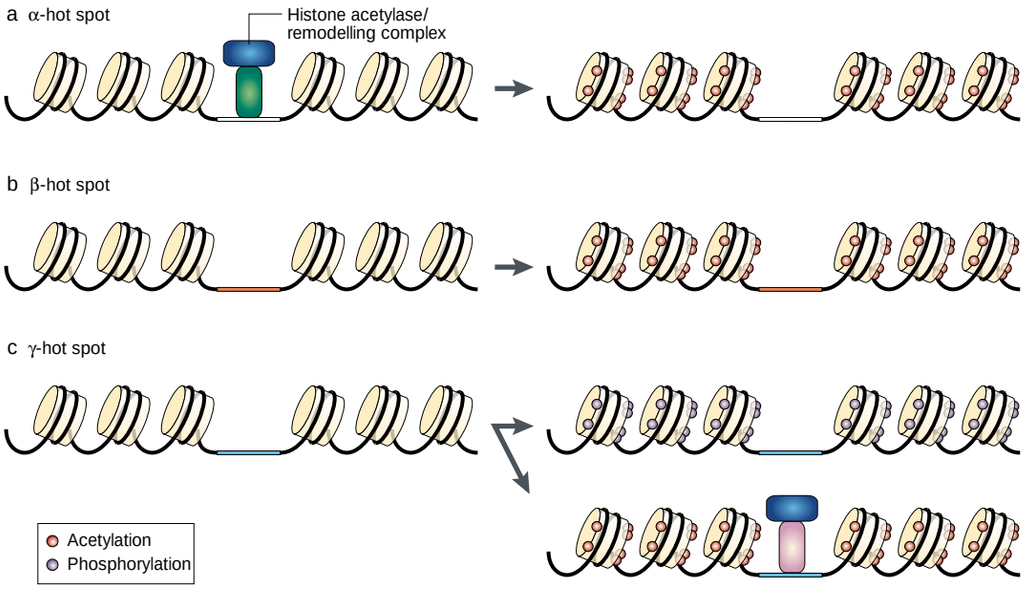

Figure 4 | **Three types of recombination hot spot.** Nucleosomes are indicated as yellow cylinders, and intergenic regions as white and coloured bars. Post-translational modifications of nucleosomes are shown as small red balls (acetylation) or blue balls (phosphorylation). **a** | α-hot spots: their activity is dependent on transcription-factor (green) binding. The transcription factor tethers a histone acetyltransferase (HAT) or other chromatin-loosening complex (blue) to the region. **b** | β-hot spots: the intergenic DNA sequence (orange bar) is intrinsically 'open'. Consequently, HAT complexes are able to acetylate the flanking nucleosomes in the absence of a transcription factor. **c** | γ-hot spots: these have high levels of G + C (blue bar). Two possibilities are presented: stalled replication forks in the high G + C region result in Mec1-mediated phosphorylation of histones in the flanking nucleosomes (top), or a DNA-binding protein (pink; with affinity for G + C-rich sequences) stabilizes the interaction of a HAT complex to the region (bottom).

observation that nucleosome-excluding sequences, such as $(CCGNN)_{12}$ and poly(A), stimulate both transcription and recombination[42,100]. Second, the nucleosome-free region of DNA that is associated with β-hot spots is unlikely to be bound by transcription factors and is, therefore, accessible to Spo11.

In contrast to the α- and β-hot spots, the γ-hot spots would not be expected to represent 'open' chromatin. I suggest that high G + C regions might represent DNA sequences that result in transiently stalled DNA-replication forks. Modification of histones in the region of the stalled forks might be required to allow replication to proceed. Although there is no direct evidence in favour of post-translational modification of histones at stalled replication forks, several observations are consistent with this possibility. First, strains with mutations in *MEC1*, which encodes a CHECKPOINT protein with protein kinase activity[101,102], are sensitive to drugs that reduce the rate of DNA synthesis[103,104]. It has been suggested that the essential function of Mec1 is to prevent replication fork blockage by elevating nucleotide pools[105]. Alternatively (or in addition), Mec1-dependent phosphorylation of histones might loosen the chromatin, allowing continued synthesis from blocked replication forks; Downs *et al.*[97] found Mec1-mediated phosphorylation of H2A as part of the response of yeast cells to DNA damage. In summary, I suggest that high G + C regions might result in replication fork blockage and subsequent local modification of histones. This modification allows DNA replication to proceed and, in addition, is one of the signals recognized by the recombination machinery.

An alternative possibility is that the yeast cell contains proteins that recognize and bind G + C-rich DNA. These proteins could directly interact with the recombination machinery or result in histone modifications that encourage interactions with the recombination complex. One argument for the possibility of such proteins is the recent report of mammalian receptors that specifically recognize bacterial DNA with a high frequency of CpG pairs[106]. One evolutionary advantage for a mechanism that would target the recombination machinery to G + C-rich regions is to rid the yeast cell of foreign DNA. As discussed previously, in recombination events that involve heterozygous hot spots, these hot spots are preferentially lost. So, in cells that are heterozygous for a G + C-rich insertion, a mechanism that targets the recombination machinery to G + C-rich DNA would result in loss of the insertion.

Once chromatin has been modified through the mechanisms proposed, I suggest that the recombination machinery can bind to the region and initiate DSB formation. The efficiency of binding might simply reflect the total number of chromatin-loosening histone modifications or the recognition of a specific pattern of histone modifications[86]. It is also possible that chromatin re-modelling that is independent of histone modifications, such as that done by the Swi/Snf complexes[84], is recognized by the recombination complexes.

CHROMODOMAIN
A protein motif that is involved in binding certain methylated histones; often associated with transcriptional repression.

CHECKPOINT
A position in the cell cycle at which progression can be arrested to complete a cellular function, such as the repair of DNA damage.

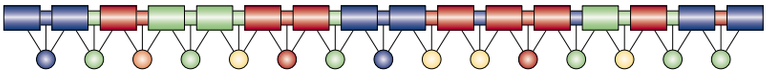

Figure 5 | **Hot-spot activity in a model chromosome.** Genes are shown as large rectangles and intergenic regions as small rectangles. Hot-spot activity is represented by the small circles under each intergenic region. Colour code from 'hottest' to 'coldest': red, orange, yellow, green and blue. The activity of each hot spot represents an integration of the activities of both the intra- and intergenic regions.

Although an interaction between the recombination machinery and modified chromatin might be necessary for hot-spot activity, it is not sufficient. DSBs are formed primarily in nuclease-hypersensitive intergenic regions[12]. The preferred target of Spo11 is likely to be DNA sequences that are free of nucleosomes and unbound by transcription factors. When DSBs were mapped at a hot spot resulting from insertion of telomeric repeats that contain three Rap1-binding sites, breaks occurred immediately adjacent to, but not within, the telomeric repeats, as expected if transcription-factor binding prevents cleavage in the telomeric sequences[23]. In addition to being free of nucleosomes or transcription factors, another important factor might be the susceptibility of the DNA to Spo11 cleavage. From the analysis of *in vivo* cleavage sites, although there is no pronounced specificity, certain sequences (such as poly(A))[20] are avoided.

From the considerations described above, cold spots could lack recombination for several reasons. It is likely that some cold spots are in chromosomal regions (such as the telomeres and centromeres of many organisms) that lack a sufficient level of histone modifications to attract the recombination machinery. Alternatively, they might represent regions with the 'wrong' type of modification (those that lead to silenced, inaccessible chromatin); methylation of histones, for example, is connected with the silencing of gene expression in *Drosophila* and *S. pombe*[92]. It is also possible that a cold spot represents a promoter in which all DNA sequences are bound to transcription factors or other proteins and, therefore, is inaccessible to the recombination complex. Finally, some DNA sequences, such as poly(A), might not be a good substrate for Spo11. As in the case of hot spots, the level of recombination for each gene is likely to reflect an integration of all these factors.

Conclusions

The level of meiotic recombination hot-spot activity, as measured by monitoring DSBs, is likely to reflect the integration of regional chromosome modifications and local characteristics of the DNA sequence in the intergenic regions (FIG. 5). This model has several appealing features. First, it is consistent with the observation that hot-spot activity is associated with transcription-factor binding (and, presumably, recruitment of chromatin-modifying complexes), but not with transcription *per se*. Second, the model agrees with the observation that intergenic nuclease-hypersensitive chromatin is necessary, but not sufficient, for hot-spot activity. Third, because histones can be acetylated in large regions comprising many genes[107], the clusters of hot spots, as observed in yeast[13,14], are expected. Fourth, the model is consistent with the context specificity of hot spots that are observed both in *S. cerevisiae*[44,58] and *S. pombe*[45]. Fifth, Borde *et al.*[31] showed that delaying meiotic DNA replication also delayed DSB formation; the delay in DSB formation could represent the time necessary to modify histones after DNA replication.

There are several possible tests of the model. One would expect that mutations in HATs and HDACs would affect meiotic recombination hot-spot activity. For genes that are activated by histone acetylation, mutations in the relevant acetyltransferase would be expected to reduce gene expression and simultaneously reduce hot-spot activity. Changes in genome-wide gene expression profiles have been examined in strains with mutations that affect various HATs, HDACs, or chromatin remodelling factors[108–110]. A useful test of the model would be to do a global analysis of hot-spot activity[14] in strains with these mutations. An alternative approach is to measure the level of histone modifications for hot-spot and non-hot-spot sequences by using chromatin immunoprecipitation with antibodies that are directed against modified histones. Given the plethora of histone modifications, this type of analysis will have to be done with many antibodies with different specificities. Finally, I have suggested that G + C-rich sequences might result in blocked replication forks, and subsequent local Mec1-dependent phosphorylation of histones. Regions of stalled replication can be visualized by two-dimensional agarose gel electrophoresis, and histone phosphorylation can be examined by chromatin immunoprecipitation experiments.

In conclusion, the distribution of meiotic recombination events is one example of a problem in which investigations on a genome-wide basis are likely to be particularly informative. Although many of the details of the model described above are almost certainly incorrect, it is unlikely that models focused exclusively on the local details of chromatin structure and DNA sequence will successfully explain the mechanism of hot spots and cold spots. An understanding of such mechanisms is crucial not only for understanding the recombination process, but also for the maximal use of recombination-based methods for identifying disease-associated human genes.

Links

DATABASE LINKS Spo11 | *ARG4* | *HIS4* | *ade6* | *LEU2* | *rad50S* | Bas1 | Bas2 | Gcn4 | Rap1 | Atf1 | Mts1 | *RAD50* | Swi6 | *CEN6* | *TAP2* | *Spo11* | CARM1 | Mec1 | Rad6 | Swi | Snf

FURTHER INFORMATION Marshfield Clinic | Thomas Petes' lab

1. Lichten, M. & Goldman, A. S. H. Meiotic recombination hotspots. *Annu. Rev. Genet.* **29**, 423–444 (1995).
This review lucidly summarizes information derived from studies of hot spots in a large variety of organisms.
2. Wahls, W. P. Meiotic recombination hot spots: shaping the genome and insights into hypervariable minisatellite DNA change. *Curr. Top. Dev. Biol.* **37**, 37–75 (1998).
3. Petes, T. D., Malone, R. E. & Symington, L. S. in *The Molecular and Cellular Biology of the Yeast* Saccharomyces Vol. 1 (eds Broach, J. R., Jones, E. W. & Pringle, J. R.) 407–521 (Cold Spring Harbor Laboratory Press, New York, 1991).
4. 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).
5. Bergerat, A. *et al.* An atypical topoisomerase II from archaea with implication for meiotic recombination. *Nature* **386**, 414–417 (1997).
References 4 and 5 describe the evidence that Spo11 — a topoisomerase-related protein — is responsible for double-stranded break formation in yeast.
6. Roeder, G. S. Meiotic chromosomes: it takes two to tango. *Genes Dev.* **11**, 2600–2621 (1997).
7. Sun, H., Treco, D. & Szostak, J. W. Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination intiation site. *Cell* **64**, 1155–1161 (1991).
8. 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).
9. Sun, H., Treco, D., Schultes, N. P. & Szostak, J. W. Double-strand breaks at an initiation site for meiotic gene conversion. *Nature* **338**, 87–90 (1989).
This study showed that double-stranded breaks are the DNA lesions that initiate meiotic recombination in yeast.
10. de Massy, B. & Nicolas, A. The control in *cis* of the position and amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae*. *EMBO J.* **12**, 1459–1466 (1993).
11. Fan, Q.-Q., Xu, F. & Petes, T. D. Meiosis-specific double-strand DNA breaks at the *HIS4* recombination hot spot in the yeast *Saccharomyces cerevisiae*: control in *cis* and *trans*. *Mol. Cell. Biol.* **15**, 1679–1688 (1995).
12. Wu, T.-C. & Lichten, M. Meiosis-induced double-strand break sites determined by yeast chromatin structure. *Science* **263**, 515–518 (1994).
This analysis showed that double-stranded breaks occur in nuclease-sensitive regions of chromatin.
13. Baudat, F. & Nicolas, A. Clustering of meiotic double-strand breaks on yeast chromosome III. *Proc. Natl Acad. Sci. USA* **94**, 5213–5218 (1997).
14. Gerton, J. L. *et al.* Global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **97**, 11383–11390 (2000).
A microarray-based study that describes mapping of double-stranded breaks throughout the yeast genome.
15. Nicolas, A., Treco, D., Schultes, N. P. & Szostak, J. W. An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. *Nature* **338**, 35–39 (1989).
16. Detloff, P., White, M. A. & Petes, T. D. Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. *Genetics* **132**, 113–123 (1992).
17. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. The double-strand-break-repair model for recombination. *Cell* **33**, 25–35 (1993).
18. Nicolas, A. & Petes, T. D. Polarity of gene conversion in fungi: contrasting views. *Experientia* **50**, 242–252 (1994).
19. Szankasi, P., Heyer, W. D., Schuchert, P. & Kohli, J. DNA sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*: wild-type and mutant alleles including the recombination hot spot allele *ade6-M26*. *J. Mol. Biol.* **204**, 917–925 (1988).
20. Liu, J., Wu, T.-C. & Lichten, M. The location and structure of double-strand DNA breaks induced during yeast meiosis: evidence for a covalently linked DNA–protein intermediate. *EMBO J.* **14**, 4599–4608 (1995).
21. Xu, L. & Kleckner, N. Sequence non-specific double-strand breaks and interhomology interactions prior to double-strand break formation at a meiotic recombination hotspot in yeast. *EMBO J.* **16**, 5115–5128 (1995).
22. de Massy, B., Rocco, V. & Nicolas, A. The nucleotide mapping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *Saccharomyces cerevisiae*. *EMBO J.* **14**, 4589–4598 (1995).
23. Xu, F. & Petes, T. D. Fine-structure mapping of meiosis-specific double-strand DNA breaks at a recombination hotspot associated with an insertion of telomeric sequences upstream of the *HIS4* locus in yeast. *Genetics* **143**, 1115–1125 (1996).
24. Blumenthal-Perry, A., Zenvirth, D., Klein, S., Onn, I. & Simchen, G. DNA motif associated with meiotic double-strand break regions in *Saccharomyces cerevisiae*. *EMBO Rep.* **1**, 232–238 (2000).
25. Cervantes, M. D., Farah, J. A. & Smith, G. R. Meiotic DNA breaks associated with recombination in *S. pombe*. *Mol. Cell* **5**, 883–888 (2000).
26. Zenvirth, D. & Simchen, G. Meiotic double-strand breaks in *Schizosaccharomyces pombe*. *Curr. Genet.* **38**, 33–38 (2000).
27. Ohta, K., Shibata, T. & Nicolas, A. Changes in chromatin structure at recombination initiation sites during yeast meiosis. *EMBO J.* **13**, 5754–5763 (1994).
28. 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).
29. Keeney, S. & Kleckner, N. Communication between homologous chromosomes: genetic alterations at a nuclease-hypersensitive site can alter meiotic chromatin structure at that site both in *cis* and in *trans*. *Genes Cells* **1**, 475–489 (1996).
30. Mizuno, K.-i. *et al.* The meiotic recombination hotspot created by the single-base substitution *ade6-M26* results in remodeling of chromatin structure in fission yeast. *Genes Dev.* **11**, 876–886 (1997).
31. Borde, V., Goldman, A. S. H. & Lichten, M. Direct coupling between meiotic DNA replication and recombination initiation. *Science* **290**, 806–809 (2000).
32. Smith, K. N., Penkner, A., Ohta, K., Klein, F. & Nicolas, A. B-type cyclins *CLB5* and *CLB6* control the initiation of recombination and synaptonemal complex formation in yeast meiosis. *Curr. Biol.* **11**, 88–97 (2001).
33. Arndt, K. T., Styles, C. & Fink, G. R. Multiple global regulators control *HIS4* transcription in yeast. *Science* **237**, 874–880 (1987).
34. Tice-Baldwin, K., Fink, G. R. & Arndt, K. T. BAS1 has a Myb motif and activates *HIS4* transcription only in combination with BAS2. *Science* **246**, 931–935 (1989).
35. Devlin, C., Tice-Baldwin, K., Shore, D. & Arndt, K. T. RAP1 is required for BAS1/BAS2- and GCN4-dependent transcription of the yeast *HIS4* gene. *Mol. Cell. Biol.* **11**, 3642–3651 (1991).
36. 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).
37. 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).
This reference describes evidence for α-hot spots — recombination hot spots that require transcription-factor binding.
38. White, M. A., Detloff, P., Strand, M. & Petes, T. D. A promoter deletion reduces the rate of mitotic, but not meiotic, recombination at the *HIS4* locus in yeast. *Curr. Genet.* **21**, 109–116 (1992).
39. Kirkpatrick, D. T., Fan, Q.-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).
40. 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).
41. Grimm, C., Schaer, P., Munz, P. & Kohli, J. The strong *ADH1* promoter stimulates mitotic and meiotic recombination at the *ADE6* gene of *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **11**, 289–298 (1991).
42. Kirkpatrick, D. T., Wang, Y.-H., Dominska, M., Griffith, J. D. & Petes, T. D. Control of meiotic recombination and gene expression in yeast by a simple repetitive DNA sequence that excludes nucleosomes. *Mol. Cell. Biol.* **19**, 7661–7671 (1999).
43. Wang, Y.-H. & Griffith, J. D. The [(G/C)3NN]n motif: a common DNA repeat that excludes nucleosomes. *Proc. Natl Acad. Sci. USA* **93**, 8863–8867 (1996).
44. Wu, T.-C. & Lichten, M. Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. *Genetics* **140**, 55–66 (1995).
45. Virgin, J. B., Metzger, J. & Smith, G. R. Active and inactive transplacement of the M26 recombination hotspot in *Schizosaccharomyces pombe*. *Genetics* **141**, 33–48 (1995).
46. Fan, Q.-Q., Xu, F., White, M. A. & Petes, T. D. Competition between adjacent meiotic recombination hotspots in the yeast *Saccharomyces cerevisiae*. *Genetics* **145**, 661–670 (1997).
47. Zahn-Zabal, M., Lehmann, E. & Kohli, J. Hot spots of recombination in fission yeast: inactivation of the *M26* hot spot by deletion of the *ade6* promoter and the novel hotspot *ura3-aim*. *Genetics* **140**, 469–478 (1995).
48. Ohta, K., Wu, T.-C., Lichten, M. & Shibata, T. Competitive inactivation of a double-strand DNA break site involves parallel suppression of meiosis-induced changes in chromatin configuration. *Nucleic Acids Res.* **27**, 2175–2180 (1999).
49. Rocco, V. & Nicolas, A. Sensing of DNA non-homology lowers the initiation of meiotic recombination in yeast. *Genes Cells* **1**, 645–661 (1996).
50. Zenvirth, D. *et al.* Multiple sites for double-strand breaks in whole meiotic chromosomes of *Saccharomyces cerevisiae*. *EMBO J.* **11**, 3441–3447 (1992).
51. Klein, S. *et al.* Patterns of meiotic double-strand breakage on native and artificial yeast chromosomes. *Chromosoma* **105**, 276–284 (1996).
52. Blat, Y. & Kleckner, N. Cohesins bind to preferential sites along yeast chromosome III with differential regulation along arms versus the centric region. *Cell* **98**, 249–259 (1999).
53. Lambie, E. J. & Roeder, G. S. A yeast centromere acts in *cis* to inhibit meiotic gene conversion of adjacent sequences. *Cell* **52**, 863–873 (1988).
54. Gottschling, D. E., Aparicio, O. M., Billington, B. L. & Zakian, V. A. Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell* **63**, 751–762 (1990).
55. Egel, R. Two tightly-linked silent cassettes in the mating-type region of *Schizosaccharomyces pombe*. *Curr. Genet.* **8**, 199–203 (1984).
56. Thon, G., Cohen, A. & Klar, A. J. S. Three additional linkage groups that repress transcription and meiotic recombination in the mating-type region of *Schizosaccharomyces pombe*. *Genetics* **138**, 29–38 (1994).
57. Bannister, A. J. *et al.* Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124 (2001).
In this reference, and in references 92 and 93, evidence that links the silencing of gene expression and methylation of histones is described.
58. Borde, V., Wu, T.-C. & Lichten, M. Use of a recombination reporter insert to define meiotic recombination domains on chromosome III of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**, 4832–4842 (1999).
59. Broman, K. W., Murray, J. C., Sheffield, V. C., White, R. L. & Weber, J. L. Comprehensive human genetic maps: individual and sex-specific variation in recombination. *Am. J. Hum. Genet.* **63**, 861–869 (1998).
60. Loidl, J., Scherthan, H., Den Dunnen, J. T. & Klein, F. Morphology of a human-derived YAC in yeast meiosis. *Chromosoma* **104**, 183–188 (1995).
61. Lindahl, K. His and hers recombinational hotspots. *Trends Genet.* **7**, 273–276 (1991).
62. Dunham, I. *et al.* The DNA sequence of human chromosome 22. *Nature* **402**, 489–495 (1999).
63. Yu, A. *et al.* Comparison of human genetic and sequence-based physical maps. *Nature* **409**, 951–953 (2001).
In references 59 and 63, the characteristics of human linkage maps are described.
64. Hedrick, P. W. Inference of recombination hotspots using gametic disequilibrium values. *Heredity* **60**, 435–438 (1988).
65. Cullen, M. *et al.* Characterization of recombination in the HLA class II region. *Am. J. Hum. Genet.* **60**, 397–407 (1997).
66. Jeffreys, A., Ritchie, A. & Neumann, R. High resolution analysis of haplotype diversity and meiotic crossover in the human *TAP2* recombination hotspot. *Hum. Mol. Genet.* **9**, 725–733 (2000).
This study examines a single human meiotic recombination hot spot at high resolution using linkage disequilibrium and sperm genotyping.
67. Hubert, R., MacDonald, M., Gusella, J. & Arnheim, N. High resolution localization of recombination hot spots using sperm typing. *Nature Genet.* **7**, 420–424 (1994).
68. Shenkar, R., Shen, M. & Arnheim, N. DNase I-hypersensitive sites and transcription factor-binding motifs within the mouse Eβ meiotic recombination hot spot. *Mol. Cell. Biol.* **11**, 1813–1819 (1991).
69. Jeffreys, A., Murray, J. & Neumann, R. High-resolution mapping of crossovers in human sperm defines a minisatellite-associated recombination hotspot. *Mol. Cell* **2**, 267–273 (1998).

70. Reiter, L. T. *et al.* A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nature Genet.* **12**, 288–297 (1996).
71. Lopes, J. *et al.* Fine mapping of *de novo* CMT1A and HNPP rearrangements within CMT1A-REPs evidences two distinct sex-dependent mechanisms and candidate sequences involved in recombination. *Hum. Mol. Genet.* **7**, 141–148 (1998).
72. Jeffreys, A., Neil, D. L. & Neumann, R. Repeat instability at human minisatellites arising from meiotic recombination. *EMBO J.* **17**, 4147–4157 (1998).
73. Buard, J. & Vergnaud, G. Complex recombination events at the hypermutable minisatellite CEB1 (D2S90). *EMBO J.* **13**, 3203–3210 (1994).
74. Armour, J. A. L., Harris, P. C. & Jeffreys, A. J. Allelic variation at minisatellite MS205 (D16S309): evidence for polarized variability. *Hum. Mol. Genet.* **2**, 1137–1145 (1993).
75. Treco, D. & Arnheim, N. The evolutionarily conserved repetitive sequence d(TG.AC)n promotes reciprocal exchange and generates unusual recombinant tetrads during yeast meiosis. *Mol. Cell. Biol.* **6**, 3934–3947 (1986).
76. Gendrel, C. G., Boulet, A. & Dutreix, M. (CA/GT)(n) microsatellites affect homologous recombination during yeast meiosis. *Genes Dev.* **14**, 1261–1268.
77. Majewski, J. & Ott, J. GT repeats associated with recombination on human chromosome 22. *Genome Res.* **10**, 1108–1114 (2000).
78. Eisenbarth, I., Vogel, G., Krone, W., Vogel, W. & Assum, G. An isochore transition in the *NF1* gene region coincides with a switch in the extent of linkage disequilibrium. *Am. J. Hum. Genet.* **67**, 873–880 (2000).
79. Keeney, S. *et al.* A mouse homolog of the *Saccharomyces cerevisiae* meiotic recombination DNA transesterase Spo11p. *Genomics* **61**, 170–182 (1999).
80. Metzler-Guillemain, C. & de Massy, B. Identification and characterization of a *SPO11* homolog in the mouse. *Chromosoma* **109**, 133–138 (2000).
81. Romanienko, P. J. & Camerini-Otero, R. D. Cloning, characterization and localization of mouse and human SPO11. *Genomics* **61**, 156–169 (1999).
82. 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* **65**, 989–998 (2000).
83. Romanienko, P. J. & Camerini-Otero, R. D. The mouse *spo11* gene is required for meiotic chromosome synapsis. *Mol. Cell* **6**, 975–987 (2000).
84. Workman, J. L. & Kingston, R. E. Alteration of nucleosome structure as a mechanism of transcriptional activation. *Annu. Rev. Biochem.* **67**, 545–579 (1998).
85. Grunstein, M. Histone acetylation in chromatin structure and transcription. *Nature* **389**, 349–352 (1997).
86. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41–45 (2000).
A review that discusses how different histone modifications result in different biological 'read-outs'.
87. Cheung, P., Allis, C. D. & Sassone-Corsi, P. Signaling to chromatin through histone modifications. *Cell* **103**, 263–271 (2000).
88. Struhl, K. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.* **12**, 599–606 (1998).
89. Braunstein, M., Sobel, R. E., Allis, C. D., Turner, B. M. & Broach, J. R. Efficient transcriptional silencing in *Saccharomyces cerevisiae* requires a heterochromatin histone acetylation pattern. *Mol. Cell. Biol.* **16**, 4349–4356 (1996).
90. Dhalluin, C. *et al.* Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491–496 (1999).
91. Chen, D. *et al.* Regulation of transcription by a protein methyltransferase. *Science* **284**, 2174–2177 (1999).
92. Rea, S. *et al.* Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593–599 (2000).
93. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001).
94. Mahadevan, L. C., Willis, A. C. & Barratt, M. J. Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* **65**, 775–783 (1991).
95. Koshland, D. & Strunnikov, A. Mitotic chromosome condensation. *Annu. Rev. Cell Dev. Biol.* **12**, 305–333 (1996).
96. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* **273**, 5858–5868 (1998).
97. Downs, J. D., Lowndes, N. & Jackson, S. P. A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* **408**, 1001–1004 (2000).
98. Robzyk, K., Recht, J. & Osley, M. A. Rad6-dependent ubiquitination of histone H2B in yeast. *Science* **287**, 501–504 (2000).
99. Cheung, P. *et al.* Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol. Cell* **5**, 905–915 (2000).
100. Iyer, V. & Struhl, K. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. *EMBO J.* **14**, 2570–2579 (1995).
101. Paciotti, V., Clerici, M., Lucchini, G. & Longhese, M. P. The checkpoint protein Ddc2, functionally related to *S. pombe* Rad26, interacts with Mec1 and is regulated by Mec1-dependent phosphorylation in budding yeast. *Genes Dev.* **14**, 2046–2059 (2000).
102. Mallory, J. C. & Petes, T. D. Protein kinase activity of Tel1p and Mec1p, two *Saccharomyces cerevisiae* proteins related to the human ATM protein kinase. *Proc. Natl Acad. Sci. USA* **97**, 13749–13754 (2000).
103. Weinert, T. A., Kiser, G. L. & Hartwell, L. H. Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. *Genes Dev.* **8**, 652–665 (1994).
104. Allen, J. B., Zhou, Z., Siede, W., Friedberg, E. C. & Elledge, S. J. The *SAD1/RAD53* protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. *Genes Dev.* **8**, 2416–2428 (1994).
105. Desany, B. A., Alcasabas, A. A., Bachant, J. B. & Elledge, S. J. Recovery from DNA replication stress is the essential function of the S-phase checkpoint pathway. *Genes Dev.* **12**, 2956–2970 (1998).
106. Hemmi, H. *et al.* A Toll-like receptor recognizes bacterial DNA. *Nature* **408**, 740–745 (2000).
107. Vogelauer, M., Wu, J., Suka, N. & Grunstein, M. Global histone acetylation and deacetylation in yeast. *Nature* **408**, 495–498 (2000).
In this paper, the authors use chromatin immunoprecipitation methods to examine histone acetylation and deacetylation in large chromosomal domains.
108. Wyrick, J. J. *et al.* Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. *Nature* **402**, 418–421 (1999).
109. Hughes, T. R. *et al.* Functional discovery via a compendium of expression profiles. *Cell* **102**, 109–126 (2000).
110. Bernstein, B. E., Tong, J. K. & Schreiber, S. L. Genomewide studies of histone deacetylase function in yeast. *Proc. Natl Acad. Sci. USA* **97**, 13708–13713 (2000).

Acknowledgements

I thank J. Gerton, C. D. Allis, F. Winston, J. Kohli, G. Smith and M. Lichten for comments and suggestions on the manuscript. I was supported by the National Institutes of Health.