Molecular features of meiotic recombination hot spots

K.T. Nishant[1] and M.R.S. Rao[1,2]*

Summary

Meiotic recombination occurs preferentially at certain regions called hot spots and is important for generating genetic diversity and proper segregation of chromosomes during meiosis. Hot spots have been characterized most extensively in yeast, mice and humans. The development of methods based on sperm typing and population genetics has facilitated rapid and high-resolution mapping of hot spots in mice and humans in recent years. With increasing information becoming available on meiotic recombination in different species, it is now possible to compare several molecular features associated with hot-spot loci. Further, there have been advances in our knowledge of the factors influencing hot-spot activity and the role that they play in structuring the genome into haplotype blocks. We review the molecular features associated with hot spots in terms of their properties and mechanisms underlying their function and distribution. A large number of these features seem to be shared among hot spots from different species suggesting common mechanisms for their formation and function. ***BioEssays*** **28:45–56, 2006.**

Introduction

Meiosis is a special type of cell division generating haploid gametes from diploid parental cells. Most of our understanding of the recombination process in meiosis have come from studies of lower eukaryotes, in particular *Saccharomyces cerevisiae*. The prophase stage of meiosis I is the defining stage of meiosis since it is characterized by the formation of double-strand breaks (DSBs) by the Spo11 endonuclease that initiates recombination,[1] homolog pairing through DSB-dependent and -independent mechanisms,[2] formation of the synaptonemal complex (SC) and chiasma between the homologs (Fig. 1). The temporal sequence of these events is likely to be different in *D. melanogaster* and *C. elegans* based on the study of mutants of spo11 homologs in these organisms that eliminate DSB formation but allow normal SC formation and synapsis. The repair of the DSBs that give rise to both conversion and crossover events through distinct pathways have been reviewed recently.[3] Meiotic homologous recombination serves two basic functions. It resorts the linkages between newly arising alleles to provide genetic diversity and it is required for the appropriate segregation of homologous chromosomes during meiosis.

Meiotic recombination occurs more frequently in some regions of the genome called hot spots because of the non-random distribution of DSBs that initiate the recombination events. The methods used to identify recombination hot spots in yeast, mice and humans are summarized in Fig. 2. While biochemical approaches have been used in yeast to map hot spots since synchronized meiosis can be induced in them in combination with mutant strains that arrest at certain steps of the recombination pathway, such approaches have been difficult in mammalian systems because of the asynchronous nature of gametogenesis and lack of a suitable in vitro cell culture model for the meiotic division process. Sperm typing and linkage disequilibrium mapping (See Box 1) have therefore been more successfully used to identify hot spots in mouse and humans. Meiotic recombination hot spots have been globally mapped in the yeast genome[4] by using DNA samples enriched for meiosis-specific DSBs as hybridization probes on DNA microarrays comprising 6200 yeast open reading frames (Fig. 3). While this has given invaluable knowledge regarding the distribution of hot-spot sites, detailed analysis of recombination activity has been examined in only a few hot spots. These are the *ARG4*,[5] *HIS4*,[6] *HIS4LEU2*,[7] *CYS3*,[8] and *HIS2*[9] hot spots in *S. cerevisiae*, and the *M26* hot spot in *Schizosaccharomyces pombe*.[10] Comparison of the properties of these yeast hot spots with those characterized in the mouse and human genomes has led to the understanding that many of the recombination pathways used in yeast might also be conserved in higher organisms.[11] Since meiotic recombination events almost exclusively occur

[1]Department of Biochemistry, Indian Institute of Science, Bangalore, India.
[2]Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India.
*Correspondence to: M.R.S. Rao, Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India.
E-mail: mrsrao@biochem.iisc.ernet.in
DOI 10.1002/bies.20349
Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: DSBs, double strand breaks; SC, synaptonemal complex; LD, linkage disequilibrium; DHSS, DNaseI hypersensitivity site; S/MAR, scaffold/matrix-associated region; HVMS, hypervariable minisatellite; LTR, long terminal repeat.

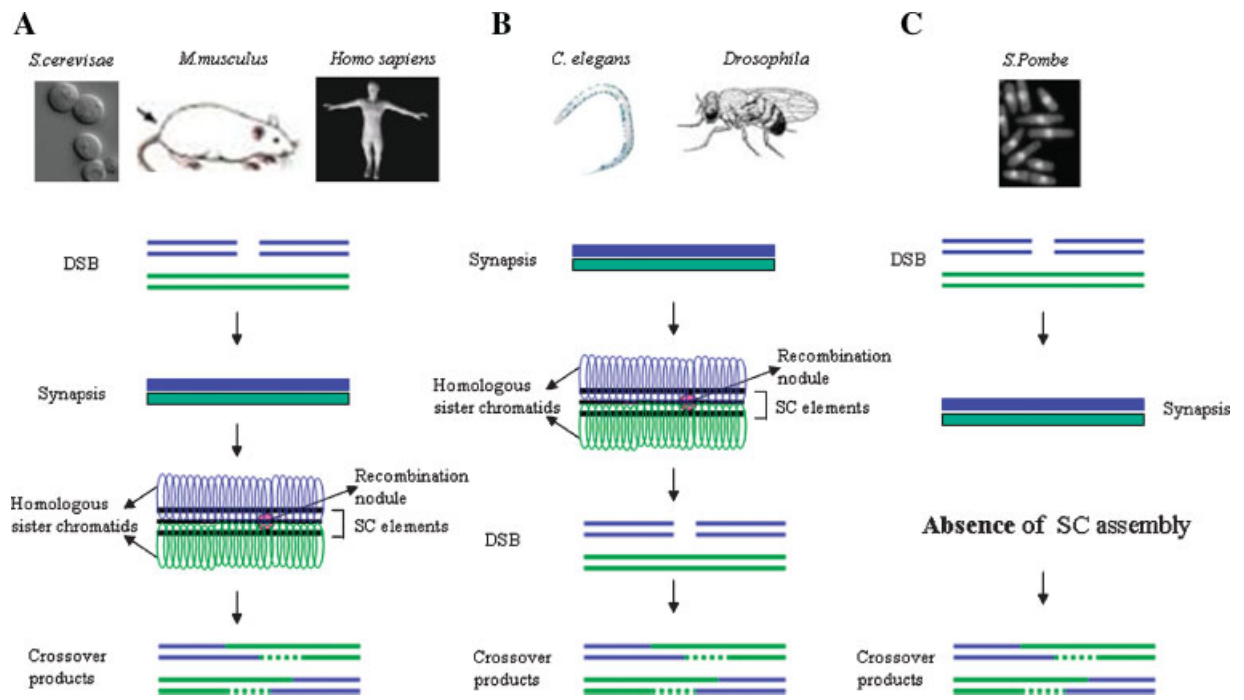

Figure 1. Sequence of events associated with recombination in meiotic prophase in different species. The formation of DSBs, synapsis of homologous chromosomes, assembly of the SC to further stabilize the pairing and generation of mature recombinants are seen to occur in a different temporal sequence in **A:** *S. cerevisae*,[94] *Mus musculus*,[95] *homo sapiens*, **B:** *C. elegans*,[96] *Drosophila*[97] and **C:** *S. pombe*.[98] These differences show that, although meiotic recombination pathways are conserved, diverged organisms utilize them in different ways.

inside hot spots, they offer a way to learn about processes associated with recombination. Furthermore, a comprehensive knowledge of the hotspots would enhance our ability to map disease alleles by their association with other markers since hot spots influence local patterns of linkage disequilibrium.

The recombination rates vary significantly in different species. The average recombination rate in yeast is 370 cM/Mb,[12] in rat it is 0.60 cM/Mb, in mouse 0.52 cM/Mb and in humans 1.13 cM/Mb.[13] This clearly shows that humans have about twice as many recombination events as rodents while the recombination rate for yeasts is substantially higher. The frequency of recombination at the hot spots in different species is also quite different. A 1–10% crossover frequency at hot spots is not unusual for yeasts[12] while for humans a 0.3% crossover frequency at the *SHOX* hot spot is the highest so far.[14] It is not clear at present how such diverse recombination rates are manifested in different organisms. Despite these differences in recombination rate and hot-spot strength, there is a striking similarity in the molecular features that are shared by all of them. The present review summarizes these features and shows how they may help us to understand factors responsible for the occurrence of hot spots.

Properties of meiotic recombination hot spots

The initiating sites for DSB formation in yeast and humans mediated through Spo 11 are not distributed randomly and occur in highly localized regions spread over 70–250 bp with no particular sequence preference,[15,16] unlike the case in bacteria where recombination occurs at a particular defined sequence '*chi*'.[17] Recent studies on the mechanism of removal of Spo11 in yeast and mouse has demonstrated an endonucleolytic cleavage action wherein the released Spo11 is attached to short oligonucleotides constituting DNA sequences in the vicinity of the DSB of less than 50 bp.[18] These oligonucleotides sequences comprise two distinct sized groups of 24–40 nucleotides and $\leq$15 nucleotides that are due to asymmetric nicks generated presumably by the Mre 11 endonuclease around the DSB. As a consequence, the biochemical distinction of the two ends of a single DSB is created at the initiation stage itself and constitutes an important revision in our understanding of the repair of meiotic DSBs. Although DNA sequence analysis has not revealed any conserved consensus sequence found at all DSB initiation sites, a common homology region (CoHR) of 50 bp with a poly(A) tract in the center has been identified in yeast, based on the DNA sequence analysis of six well-characterized DSB

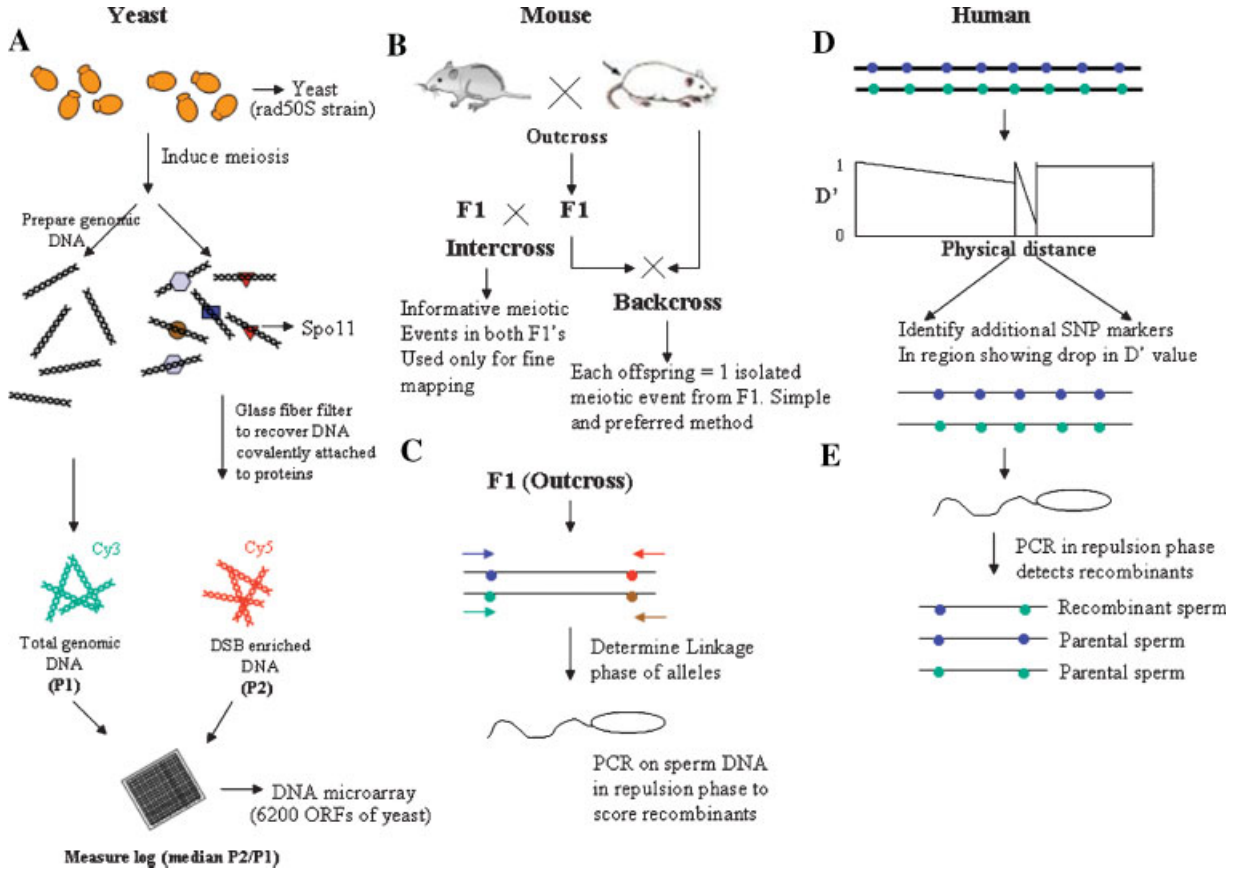

Figure 2. Mapping hot spots in yeast, mice and humans. Recombination hot spots have been rapidly characterized in simpler systems like yeast by biochemical approaches, while the identification of meiotic hot spots in mouse and humans has accelerated only recently due to the development of single molecule methods that allow recombinant DNA molecules to be recovered directly from sperm DNA[99] and the development of a number of statistical genetic analysis tools to detect recombination events from patterns of DNA sequence variation in populations.[100] **A:** Hot spots in yeast have been mapped used a ChIP (Chromatin immunoprecipitation) on CHIP (microarray) approach where DNA bound to proteins involved in recombination is immunoprecipitated and used as a probe on genomic microarrays to identify high frequency initiation sites. **B:** Hot spots in mice have traditionally been identified by setting up an outcross initially between strains to get heterozygous markers, followed by a backcross or intercross to identify meiotic exchange sites. **C:** A more recent approach involves use of sperm PCR on F_1 heterozygous mice. **D:** In humans, regions showing lack of association between polymorphic alleles ($D' \leq 1$) can be used to identify putative hot spots. **E:** These can be experimentally verified by performing PCR on sperm DNA obtained from individuals having heterozygous markers in these putative hot-spot regions and determining the number of recombinant sperms. If additional heterozygous markers are available in the region, then conversion events can also be examined.

initiation sites.[19] Though initially this gave a rational basis for the creation of DSB sites, more recently Haring et al.[20] have shown that deletion of the CoHR motif does not affect the frequency of DSB initiation. The non-random distribution of the initiating sites is also reflected in the highly clustered distribution of the hot spots. This has been reported in the yeast,[4,15] mouse and human MHC regions[16,21] and near the *ATH1* gene on mouse chromosome 1.[22] The significance of such a clustering of hot spots in terms of the mechanisms used in chromosome segregation and genomic diversity remains to be elucidated.

Size of the recombination hot spot

Crossovers at all the recombination hot spots identified so far in yeast, mice and humans have a small width of 1–3 kb. This has been observed at the *ARG4*,[5] *HIS4*[6] and *CYS3*[8] hot spots in yeast and at the *Psmb9*[23] and *Eb*[24] hot spots in mouse. The human hot spots in the MHC class II regions also show clustering of crossovers in narrow hot spots of 1–2 kb width[16] and so do the hot spots at the *SHOX* gene[14] and at the minisatellite MS32.[25] These common widths possibly imply common mechanisms of recombination initiation and resolution at all hot spots. As a consequence, many of the other

Box 1. Glossary

Linkage Disequilibrium (LD): Linkage disequilibrium is the non-random association of alleles at two or more loci on a chromosome where two alleles are observed together at a frequency greater than the product of their individual frequencies.

Conversion tract: Region inside the hot-spot interval wherein alleles on the chromosome that initiates double-strand breaks are replaced in a non-reciprocal manner by alleles from the homologous non-initiating chromosome.

Histone acetytransferases (HATs): Histone acetytransferases are a family of proteins that catalyze the formation of an amide bond between the epsilon-amino group of an amino-terminal lysine residue of one of the core histone proteins (H2A, H2B, H3 and H4) and a few non-histone substrates with acety CoA. They can be Type A HATs, which are present in the nucleus, or Type B HATs, which are found in the cytoplasm.

Scaffold/matrix-associated region (S/MAR): DNA elements ranging from a few hundred bases to a few kilo bases found at the base of chromatin loops that mediate attachment of the 30 nm chromatin fiber to the nuclear matrix (at interphase) or scaffold (at M phase when chromosomes condense).

shared properties of hot spots in several species include similar lengths of DNA lost by resection of the 5′ end of the DSB, gradients of gene conversion extending from localized initiation sites, preferential conversion by recombination suppressing alleles and the ability of single base changes to alter recombination frequencies. The crossover break points at most of these hot spots are also distributed symmetrically.

Crossover hot spots are also gene conversion hot spots

The recombination hot spots are sites for initiation and resolution of both crossovers and conversion events.[23,26] Decreasing gradients of gene conversion on both sides of the DSB initiation site and co-conversion of markers flanking the initiation site suggesting the extension of conversion tracts in both directions from the DSB site were initially experimentally demonstrated for the *ARG4*[5] and *HIS4*[27] hot spots in yeast. Conversion tracts have also been experimentally observed at the *Psmb9* hot spot in mice with an average length of 480 bp[23] while analysis of three human hot spots *DNA3, DMB2* and *SHOX* again showed presence of short conversion tracts ($\leq$300 bp in average). In mammals, there has been no evidence so far for kilobase-long conversion tracts as seen often in yeast,[28] caused presumably by extensive migration of the Holliday junction. Most of the conversion tracts observed both in mouse and humans are short and of the same length as the hot spot itself.[3] The coincidence of crossover hot spots with gene conversion hot spots also explains the paradoxical observation of low LD between markers at short distances ($\leq$5 kb) in the midst of regions showing high LD over longer distances ($\leq$100 kb).[29]

Hot spots are evolutionarily transient

The presence of heterozygous alleles at the hot-spot interval that vary in initiation rates can cause crossover asymmetry and transmission distortion. The reason for this is that the majority of the crossovers will arise from initiations on the active haplotype. This will result in the replacement of markers from the initiating chromosome with those from the non-initiating chromosome during DSB repair, resulting in their overtransmission and eventual loss of the hot spot.[30] This suggests that hot spots would be short lived on evolutionary time scales; this is supported by the lack of congruence seen in hot-spot activity and position in closely related species.[31–35] Recent studies have provided mechanistic support for this hypothesis wherein SNPs has been found at the *DNA2*[11] and *NID1*[36] hotspots in humans whose alternative alleles show differences in the frequency of crossing over, which could over a period of time result in loss of the hot spot.

Meiotic hot spots and chromatin structure

A chromosomal region of open or accessible chromatin structure has been shown to be necessary to potentiate the initial interaction between the chromosome domain and the recombination machinery. The influence of chromatin structure on hot-spot activity was suggested initially for the *HIS4LEU2* hot spot where a tandem array of four (CGGATCCG) repeats supposedly creates a nuclease-sensitive chromatin by being a poor region for nucleosome formation and stimulates recombination.[37] Hot spots that function as a consequence of such open chromatin structures, which usually show DNase I hypersensitivity (DHSS), have been termed as β hot spots[12] (Table 1). In *S. cerevisiae*, a close correlation between the location of DHSSs and the distribution of recombination hot spots has been observed. It has also been reported that DHSSs are established before entry into meiosis and that DHSSs associated with hot spots are preserved constitutively in mitotic and meiotic cells.[38] Moreover, the chromatin at hot spots have been shown to display a meiosis-specific increase in nuclease hypersensitivity before DSB formation.[39] However, not all nuclease hypersensitive sites are DSB hot spots and there is no correlation between the degree of nuclease hypersensitivity and the frequency of DSB formation.[40] In mice, the presence of DHSSs close to the *Eb* hot spot has been observed in spleen, liver and pachytene nuclei.[41] The *Psmb9* hot spot also contains

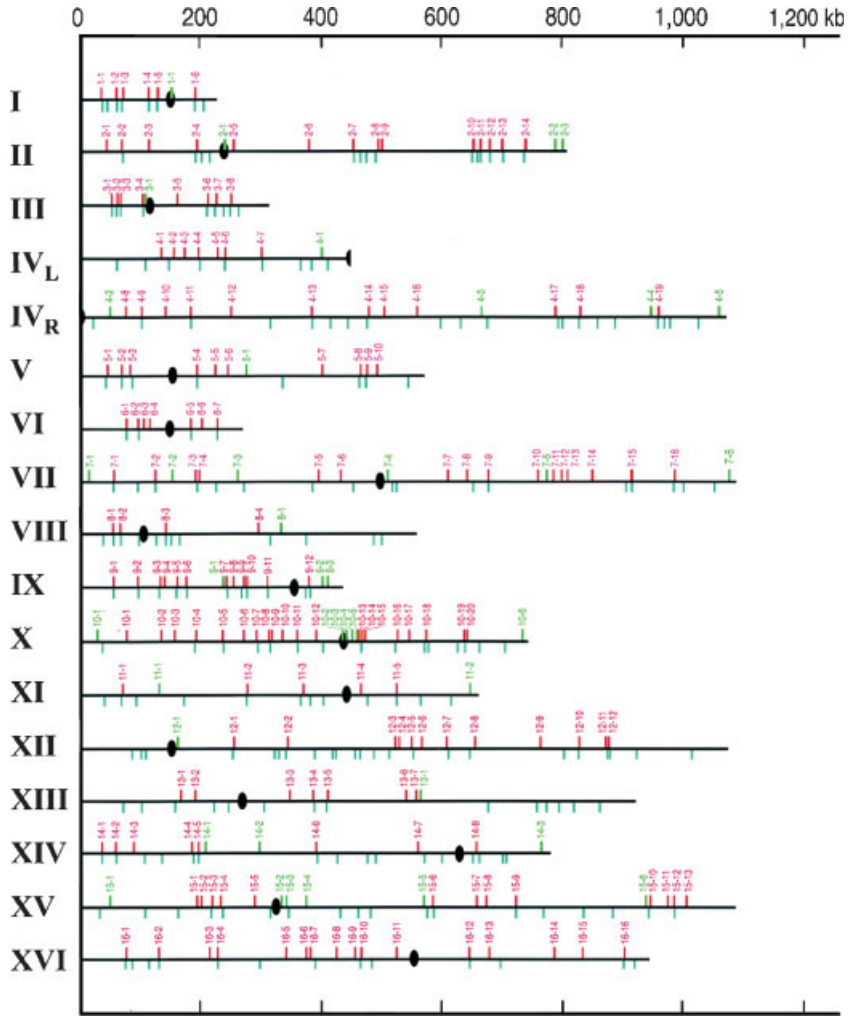

Figure 3. Chromosomal map positions of 177 hot spots (red) and cold spots (green) in *S. cerevisae*. Ovals indicate centromeres. The X axis shows nucleotide distance in kilobases while the Y axis indicates chromosome number. Reproduced with permission from Gerton JL, DeRisi J, Shroff R, Lichten M, Brown PO et al. 2000 Proc Natl Acad Sci USA 97:11383–11390.

DHSSs in somatic nuclei from liver and spleen but not in pachytene nuclei.[42]

The formation of an open chromatin structure can also arise as a consequence of modification of histone tails through methylation, acetylation or ubquitylation. This hypothesis has received considerable support in recent literature and is also implicated in modulating transcription levels through a combinatorial effect of such modifications that constitute a histone code.[43] For example, mutants of the *HIM-17* gene in *C. elegans*, which is required for methylation of histone H3, are defective for meiotic recombination.[44] Two other studies in *S. cerevisiae* have also implicated histone modifications such as methylation on histone H3 and ubiquitylation on histone H2B in DSB formation.[45,46] It has also been demonstrated that histones H3 and H4 in the vicinity of the *M26* hot spot are hyperacetylated in *S. pombe* during meiosis by the HAT Gcn5.[47] These studies clearly establish correlations between meiotic DSB formation and certain histone modifications. Histone modifications are speculated to promote DSB formation either through effects on higher order chromatin compaction[44] or by providing local 'marks' at the chromatin level that recruit proteins involved in DSB formation.[12] Future experiments using chromatin immunoprecipitation techniques should help clarify the role of a possible histone code in modulating hotspot activity.

The effect of chromatin structure on hot-spot activity in yeast is also substantiated from the observation that a recombination reporter placed at different positions in the genome assumes the properties of its location: insertions into cold regions give low DSB levels and insertion into hot regions

Table 1. Distribution of well characterized meiotic hot spots in yeast, mice and humans based on mechanisms known to promote hot spot activity

α hot spots	β hot spots	γ hot spots
HIS4 (yeast)[7]	*HIS4LEU2* (yeast)[8]	Chromosome 6 hot spots 6-1;6-2;6-5;6-7 (yeast)[6,13]
M26 (yeast)[81]	*CYS3* (yeast)[9]	16p13.3/MS205 (human)[52]
ARG4 (yeast)[1]	*M26* (yeast)[81]	*MS32* (human)[24]
Psmb9 (mouse)[22]	*Psmb9* (mouse)[22]	
Pb (mouse)[61]	*Eb* (mouse)[23]	
17.2 kb (mouse) (Nishant et al., 2004)	*Ea* (mouse)[77]	
MS32 (human)[24]	*Pb* (mouse)[61]	
TAP2 (human)[62]	17.2 kb (mouse)[101]	
	PGM1 (human)[51]	
	16p13.3/*MS205* (human)[52]	
	β globin (human)[31]	
	IGF2-H19 (human)[47,48]	
	15q11-q13 (human)[47,48]	

Many hot spots can be seen to be present in more than one class indicating multiple mechanisms involved in initiating recombination.
α hot spots: hot spots associated with transcriptionally active regions, **β hot spots**: hot spots associated with nucleosome excluding sequences, **γ hot spots**: hot spots associated with GC rich DNA sequences. While α and β hot spots represent open chromatin permitting access to the recombination machinery, the γ hot spots have been suggested to represent sequences that result in transiently stalled replication forks. This stalling would cause histones to be modified to allow replication to proceed and such modified histones could be recognized as a signal by the recombination machinery.[20]

give high DSB levels.[48] In mammals, the effect of imprinting on recombination frequencies gives another example of the role of chromatin structure. Differential imprinting between males and females often results in the formation of hot spots that are sex-specific due to differential accessibility of the recombination machinery for the DNA.[49,50] One way this could arise is through methylation of CpG sequences that can suppress crossing over as has been demonstrated in the fungus *Ascobolus immerses*.[51] This type of methylated DNA may suppress recombination by being refractory to meiotic endonucleases, by alterations in chromatin structure and transcription levels, by perturbation of proper pairing between homologs or by impairing the normal processing of recombination intermediates. Modifications in chromatin structure that enhance recombination activity can therefore occur through several mechanisms.

Matrix-associated regions and meiotic hot spots

Scaffold/matrix-associated regions (S/MARs) are DNA elements 300 bp to several thousand base pairs long that mediate attachment of the chromatin to the nuclear scaffold and are separated by loops of approximately 5–100 kb. S/MARs are proposed to have a role in meiotic pairing since they are associated with sites of open chromatin and unwind easily in response to superhelical stress,[52] which facilitates homology testing without requiring DNA breaks.[53] Experimental demonstration of the association of S/MAR elements with meiotic loci has been found to be true for the *Ea, Pb* and *Psmb9* mouse MHC hotspots and a 17.2 kb hot spot on chromosome 8 C-D.[101] Predicted S/MAR regions are also observed at the *PGM1* hot spot in humans.[54] Sequence analysis of a 15 kb region surrounding the 3 kb hot spot at 16p13.3 also shows association of DNA unwinding elements, which is one of the properties associated with MAR elements.[55] These observations suggest that a subset of S/MARs associated with the meiotic chromosome cores are converted into crossover regions, for at least some recombination hot spots, which is a distinct possibility considering that they are capable of undergoing stress-induced duplex destabilization. Since the mechanisms through which MAR elements may influence hot-spot function have not been experimentally elucidated, it is also entirely possible that, given the similar density in the distribution of meiotic hot spots and S/MAR elements in the genome, the overlap observed between the two at many of the hot spots is purely coincidental with no functional relationship. However, since other DNA transaction processes like replication and transcription are associated with the nuclear matrix, there is a distinct possibility that the recombination process is also facilitated through association with the nuclear matrix.

Mechanisms of meiotic hot-spot function

Timing of hot-spot activity

The mechanism of selection of potential DSB sites by Spo 11 or the pre-DSB complex leading to activation of specific hot spots is not clear, though it is hypothesized that this may be mediated through interactions with unidentified components of the replication machinery. The timing of replication and recombination events are seen to be correlated in yeast with premeiotic DNA replication being necessary prior to initiation of meiotic recombination.[56] It has also been suggested that

DSB-forming factors may be loaded onto chromosomes during premeiotic DNA replication in a way similar to that seen for establishment of sister chromatid cohesion. This hypothesis is supported by the observation that, when replication is blocked, DSBs do not form and, when replication of a certain chromosomal region is delayed, DSB formation is accordingly delayed.[57] The relationship between replication disruption and absence of recombination is not linked to a replication–checkpoint-dependent regulatory block to meiotic progression[58] and depends instead on a direct mechanistic link between the two processes. This is also supported by the observation that Spo 11 is required for normal progression through premeiotic S phase.[59]

Transcription and hot-spot activity

The relationship between transcription and hot-spot activity was first observed for the *HIS4* and *M26* hot spots. The *HIS4* hot spot requires the binding of transcription factors Bas1, Bas2 and Rap1 at the upstream region for hot-spot activity,[60] while the *M26* hot spot requires the binding of the heterodimeric transcription factor Mts1/Mts 2 at the heptameric sequence ATGACGT for hot-spot activity.[61] Similarly deletion of the poly(AT) tract located near the TATA element of the *ARG4* gene confers a three-fold reduction in recombination.[62] It is also observed that most naturally occurring hot spots in *S. cerevisae* lie in promoter regions of genes.[63] However, transcription per se may not be required for DSB formation, since deletion of the TATA box at *HIS4* markedly diminishes transcription without affecting recombination activity.[64] Genome-wide identification of recombination hot spots in yeast also reveals that, out of 20 DSBs that were mapped to intergenic regions, 13 were located at the 5′ end of two genes, six were located between the 3′ end of one gene and 5′ end of another gene, while only two were found between the 3′ end of two genes.[4] These results suggest that recombination initiation in yeast occurs preferentially at promoter elements that are associated with transcription factor binding and such hot spots are referred to as α hot spots (Table 1). The binding of transcription factors may stimulate recombination in two ways. They may recruit chromatin remodeling factors such as HATs that alter chromatin structure and make it nuclease hypersensitive[65] or they may recruit the recombination machinery to the DSB sites. The mouse MHC hot spots notably *Psmb9* and *Pb* are also found to be associated with transcription-factor-binding sites. Transcripts from the *Psmb9* gene are, however, detected in spleen cells but not in the testis suggesting that the transcriptional state is not directly related to the recombinational activity at the *Psmb9* hot spot. The mouse *Pb* gene is not transcribed indicating that it is a pseudogene.[66] Presence of a non-coding RNA transcript expressed in somatic cells as well as in the testis has also been observed near a 17.2 kb hot spot in mice.[101] In humans, the transcription factor AP-1 binding sites are observed at the *MS32*[25] and *TAP2* hot spots.[67] The human MHC hot spots analyzed to date are also seen to be present near transcribed regions and have not been observed in non-coding regions. A recent study based on the population genetics of an European population using the Seattle SNP database has found evidence for the existence of human α recombination hot spots similar to those in yeast.[68] A correlation between hot spots and gene density has also been observed in two recent studies on fine-scale recombination rate variation in humans,[69,70] although it must be stressed that there is no obvious pattern of hot-spot location within a gene: they could lie in introns, exons or promoter regions.

Sex-dependent activation of hot spots

In addition to sex-specific variation in recombination rates,[71] activity of some of the hot spots in mice and humans have been shown to be sex dependent and function only in males or females. These include male-specific hot spots in the subtelomeric region of chromosome 16p13.3[55] and at the *PAR1*[72] locus. Both male- and female-specific meiotic recombination hot spots have been reported in the Prader–Willi/Angelman syndrome imprinted region on chromosome 15q11-q13.[50] Hot spots that function only in male meiosis have been reported at the *IGF2–H19* region on chromosome 11p 15.5.[49] In humans, males show higher recombination rates towards telomeres while females show higher rates towards centromeres,[73] which could be a consequence of different densities/intensities of hot spots in males and females in these chromosomal regions. These sex-specific hot spots arise for several reasons. For example, the *SHOX* hot spot at the *PAR1* locus is likely to be male specific due to the requirement of a obligatory crossover between X and Y chromosomes during male meiosis. In contrast, male and female hot spots on 15q11–q13 and 11p 15.5 are a consequence of imprinting. However, the basis for the presence of male-specific hotspots on subtelomeric regions, for example on chromosome 16p13.3 is unclear. In mice, the MHC hot spots also show sex-specific differences in hot-spot activity suggesting the involvement of *cis*-acting factors. For example, recombination at the *Psmb9* hot spot in the genetic crosses involving the *wm7* haplotype is observed only in female meiosis. This has been suggested to be as a result of a *cis*-acting element in the *wm7* haplotype distal to the hot spot, which acts as a suppressor of recombination in male meiosis, and another *cis* element proximal to the hot spot, which facilitates recombination in females.[74] More recently, based on comparisons of recombination in meiocytes from XY sex-reversed and XO females with that in meiocytes from XX female and XY male mice, it has been suggested that, rather than the genotype, the sex-specific differences in the pairing and synapsis of homologous chromosomes cause the

observed differences in the pattern of meiotic exchanges seen between males and females.[75]

Haplotype-dependent activation of hot spots

A unique feature of the mouse MHC hot spots is that their activity depends on the haplotype of the MHC locus, in the genetic crosses. Recombination in crosses between *d*, *b*, *k*, *s* and *f* haplotypes occurs exclusively at the *Eb* hot spot. Recombination in crosses between *k* and *p* haplotypes occurs at the *Ea* hot spot. Crosses involving the *cas3* and *wm7* haplotypes show hot-spot activity at *Psmb9* and genetic crosses that include the *cas4* haplotype have recombination at the *Pb* hot spot. The influence of haplotype on hot-spot activity was also recently shown in an high-resolution study of the *Eb* hot spot by sperm PCR in mice hybrid strains carrying the *b, d, k* or *p* haplotypes crossed with the *s* haplotype.[24] Due to the presence of the *s* haplotype, which is most active in initiating recombination, the *Eb* hot spot showed crossover asymmetry and overtransmission of markers from the recombination-suppressed non-*s* haplotypes into the crossover progeny. Based on the study, the initiation efficiency of the different haplotypes was seen to decrease in the order $s > k > (b, d) > p$.[24] Haplotype-specific recombination activity has also been observed at the human HLA locus[76] though it has not been as well characterized as in the mouse. Multiple sequence differences including nucleotide substitutions, insertions or deletions are likely to be responsible for the differences in hot-spot activity observed on different haplotype backgrounds. For example, in the case of the *Psmb9* hot spot, the active *cas3* haplotype contains six copies of a TCTG tetramer while the inactive *b* haplotype contains only four copies.[77] Such differences between the haplotypes need not be confined to the hot-spot interval itself and may reside some distance away and influence hot-spot activity as enhancers or suppressors as speculated for the mouse *Psmb9* hot spot.[74]

Repetitive DNA sequences in hot-spot activity

A weak association of GT repeats with three hot spots identified in the human MHC has been observed[21] and they have been shown to be concentrated in regions showing high recombination rates on chromosome 22.[78] Hot spots identified in the *PGMI* gene in humans also show presence of $(GT)_{21}$ and $(GT)_{26}$ repeats.[54] A role for GT repeats in meiotic recombination also comes from their enrichment in rat synaptonemal complex associated DNA.[79] Several tetranucleotide repeats have been shown to be present near some meiotic hot spots. For example, the $(CAGA)_6$ repeat has been observed at the *Psmb9* hot spot in the mouse MHC while the *Ea*, *Eb* and *Pb* hot spots show the presence of $(CAGG)_5$, $(AGGC)_{10}$, and $(CCTG)_2$ repeats, respectively.[80] The most-well-studied class of repetitive DNA with respect to meiotic hot-spot activity is the hypervariable minisatellite DNA sequence (HVMS). In humans, hot-spot activity has been observed at the *MS32, CEB1* and *NID1* minisatellite loci while minisatellite DNA motifs are found at the *Ea, Eb, Psmb9* and *Pb* hot spots in the mouse MHC[80] and also at the 17.2 kb hot spot on mouse chromsosome 8 C-D.[101] They are also highly enriched at subtelomeric regions that exhibit high recombination rates. Another class of repetitive sequences that has shown a weak association with hot spots is the LTR elements. Solo LTR elements have been observed at the *Eb* and *Psmb9* hot spots in the mouse MHC and the mouse LTR-IS sequences were seen to be weakly associated with three of the hot-spot segments (*DPB1* to *RING 3; BAT2* to *LTA* and *HLA-F*-telomere) identified in a high-resolution study of recombination in the human MHC.[21] LTR elements are also present at the human *MS32* hot spot.[25] The middle repetitive mouse transposable (MT) repeats represent another class of repeats for which a role in meiotic hot-spot activity has been suggested. They have been observed at the *Eb, Psmb9* and *Pb* hot spots in the mouse MHC.[80] In addition to the associations seen between these repeat classes and meiotic hot spots, occurrence of DSBs during meiosis has also been reported near CAG repeat tracts and palindromic repeats in *S. cerevisae*.[81,82] The association of some of the hot spots with repeat sequences is paradoxical since it has the potential to be detrimental for genome stability through ectopic recombination with other members of these dispersed repeat families and through expansion and contraction of repeat tracts during DSB repair. It is therefore not surprising that, in many eukaryotes, methylation, which suppresses crossing over as discussed earlier, is triggered by DNA repeats as a result of a process called methylation induced premeiotically induced methylation.[83]

Distribution of meiotic recombination hot spots

Effect of chromosome position and size

Some hot spots in yeast have been shown to be active even when moved to different genomic locations without altering the local chromatin structure.[84] Changes in the chromatin structure at different chromosomal positions can, however, alter hot-spot function. For example, placing the *ARG4* gene in a novel chromosomal context with altered chromatin structure has been shown to result in loss of hot-spot activity.[85] High-resolution mapping of hot spots on yeast chromosome III and genome-wide mapping of hot spots in yeast have shown that chromosomes can be subdivided into large hot or cold domains for DSB formation.[4,15] While chromosomal hot domains can show loss of hot-spot activity with changes in chromatin structure,[85] it has also been demonstrated that chromosomal cold domains can show presence of hot-spot activity at defined DNA sequences by specific targeting of Spo11 through fusion to the DNA-binding domain of Gal4 protein.[86] Hotspot activity is also affected by the presence or

absence of other adjacent hot spots.[38] It is interesting to note that there is a significant correlation between chromosome size, the number of hot spots and hot-spot strength. The larger chromosomes in yeast have more hot spots than the smaller chromosomes, while the hot spots for the smaller chromosomes exhibit higher frequency of DSB formation than those on the larger chromosomes.[4] Similarly, in humans, there is a strong inverse correlation between the length of each chromosome and its average recombination rate.[71] It is also interesting to note that presence of a homologous chromosome, interhomologue pairing and sensing of DNA homology is not necessary for DSB formation in *S. cerevisae*, though the repair of these DSBs using the sister chromatid is inefficient in haploid meiosis.[87]

Meiotic hot spots and haplotype block boundaries

Recent studies have demonstrated that the human genome is organized into regions of high LD (haplotype blocks) separated by short discrete segments of very low LD that are ascribed to recombination hot spots. Experimental proof that these LD/haplotype blocks result from the localization of recombination to irregularly spaced hot spots can be seen from the high-resolution analysis of LD in 216 kb of the MHC class II region[16] and in a 206 kb interval on chromosome 1q42.3.[88] However, haplotype blocks have also been shown to arise as a consequence of natural selection, population bottlenecks or population admixture all of which can create long-range LD.[89] In such cases, the haplotype blocks will usually be specific to the population studied. However, several studies have also demonstrated conserved positions of hot-spot activity in different populations at some of the loci studied.[54,89–91] This in turn results in conserved patterns of haplotype blocks across populations through the overriding influence of hot spots on population history in shaping LD patterns. Although the role of recombination hot spots in shaping haplotype structure has now been validated by the correlation observed between sperm crossover hot spots and regions showing LD breakdown on two different chromosomal locus,[16,88] a few exceptions to this paradigm have also emerged recently. This involves the identification of two sperm crossover hot spots (*NID1* and *MS32*) in regions showing strong LD on chromosome 1q42.3[88] and another observation of the absence of a sperm crossover hot spot in the MHC class II locus at *DPB1*, which shows LD breakdown.[92] The mechanistic basis for these exceptions is not yet clear, and it has been suggested that the presence of sperm crossover hot spots in regions of strong LD is most likely to be a consequence of the hot spot being relatively too young to have influenced patterns of allelic association. In contrast, the absence of sperm crossover hot spots in regions that show LD breakdown has been suggested to be due to the presence of extinct hot spots in the region. These exceptions support the argument that hot spots are very fluid features of the genome on evolutionary time scales and also reflect our lack of understanding of the mechanisms involved in hot-spot formation.

Perspectives

It is clear from the present overview of the molecular features of meiotic recombination hot spots that they are extensively shared between yeast, mice and humans and that the DNA sequence, DNA structure and chromatin configuration have overlapping roles to play in the establishment of a hot spot. However, DNA sequence may have a principle role in formation of hot spots since an open chromatin structure does not necessarily result in formation of a hot spot.[38] This can be best seen in the very different recombination landscapes of the two sibling species of *Drosophila, D. melanogaster* and *D. simulans*.[93] This is also reflected in a number of recent studies of syntenic regions in closely related species,[31–34] which share an overall high degree of 95–99% nucleotide similarity but not the polymorphisms within the hot-spot interval. For example, the *TAP2* hot spot, which is active in humans, was seen to be inactive in Chimpanzees and it was also observed that two sequence motifs implicated in hot-spot formation varied between the two species at the hot-spot interval. This includes a Pur binding motif found in humans but absent in the Chimpanzee due to a single base pair difference and two S/MAR elements that were in different positions in the two species.[32] In the β globin hot spot which is active in humans but not in macaques, the helical stability of a $(RY)_n$ repeat at the hot-spot interval implicated in hot-spot function is seen to be higher in rhesus macaques and may be responsible for the difference in hot-spot activity seen.[31] The ability of single base changes to significantly alter initiation of DSBs is also seen at allele specific hot spots like the *DNA2* and *NID1* hot spots in humans.[11,36] In addition, hot-spot activity differs widely among yeast strains that show sequence differences.[4] Together with the observation that crossing over frequencies can differ between haplotypes, all these data suggest that, instead of any unique single DNA sequence determinant of hot-spot activity, there exist multiple fuzzy DNA sequence determinants that influence recombination activity based on the nature of the allele present.[11,24,33] These nucleotide variations present inside the hot-spot interval could effect significant changes in DSB initiation frequency through alterations in DNA and chromatin structure, epigenetic mechanisms and recognition of the *trans* acting recombination machinery components. Unfortunately, we do not have sufficient information yet on differences in hot-spot distributions in different populations, which would be very useful in determining sequence variants that influence hotspot activity. Since linkage-disequilibrium-based approaches may not be helpful in picking up such differences in recombination rates in extant populations and sperm typing is not feasible across large genomic regions in several populations, the recent finding of oligonucleotides surrounding DSB sites being complexed with

Spo11[18] opens up new methods of very high-resolution identification of meiotic hot spots through their purification and subsequent use as probes to identify homologous sequences in the genome. The development of such techniques to discover global genomic patterns of hot-spot distribution can help us understand the influence of the primary DNA sequence on recombination rate variation. In contrast to the knowledge available on the nature of the DNA sequence and chromatin features of other processes like transcription or replication, relatively little is known about their role in initiation of meiotic recombination. Comparison of the DNA sequence and chromatin properties associated with large numbers of meiotic hot spots in closely related species or populations would be required in future to determine the general principles that operate to direct recombination events preferentially to certain chromosomal regions.

Note added in proof

Since the acceptance of this review for publication, a report by Myers et al. (Science 310, 321–324, 2005) describing the genome wide identification of 25,000 human meiotic hotspots has been published.

References

1. Keeney S, Giroux CN, Kleckner N. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell 88:375–384.
2. Page SL, Hawley RS. 2003. Chromosome choreography: the meiotic ballet. Science 301:785–789.
3. de Massy B. 2003. Distribution of meiotic recombination sites. Trends Genet 19:514–522.
4. Gerton JL, DeRisi J, Shroff R, Lichten M, Brown PO, et al. 2000. Global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 97:11383–11390.
5. Schultes NP, Szostak JW. 1990. Decreasing gradients of gene conversion on both sides of the initiation site for meiotic recombination at the *ARG4* locus in yeast. Genetics 126:813–822.
6. Fan Q, Xu F, Petes TD. 1995. 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.
7. Cao L, Alani E, Kleckner N. 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell 61:1089–1101.
8. de Massy B, Rocco V, Nicolas A. 1995. The nucleotide mapping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *S. cerevisiae*. EMBO J 14:4589–4598.
9. Malone RE, Bullard S, Lundquist S, Kim S, Tarkowski T. 1992. A meiotic gene conversion gradient opposite to the direction of transcription. Nature 359:154–155.
10. Gutz H. 1971. Gene conversion: remarks on the quantitative implications of hybrid DNA models. Genet Res 17:45–52.
11. Jeffreys AJ, Neumann R. 2002. Reciprocal crossover asymmetry and meiotic drive in a human recombination hot spot. Nat Genet 31:267–271.
12. Petes TD. 2001. Meiotic recombination hot spots and cold spots. Nat Rev Genet 2:360–369.
13. Jensen-Seaman MI, Furey TS, Payseur BA, Lu Y, Roskin KM, et al. 2004. Comparative recombination rates in the rat, mouse, and human genomes. Genome Res 4:528–538.
14. May CA, Shone AC, Kalaydjieva L, Sajantila A, Jeffreys AJ. 2002. Crossover clustering and rapid decay of linkage disequilibrium in the Xp/Yp pseudoautosomal gene *SHOX*. Nat Genet 31:272–275.
15. Baudat F, Nicolas A. 1997. Clustering of meiotic double-strand breaks on yeast chromosome III. Proc Natl Acad Sci USA 94:5213–5218.
16. Jeffreys AJ, Kauppi L, Neumann R. 2001. Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. Nat Genet 29:217–222.
17. Smith GR, Amundsen SK, Dabert P, Taylor AF. 1995. The initiation and control of homologous recombination in *E. coli*. Philos Trans R Soc Lond B Biol Sci 347:13–20.
18. Neale MJ, Pan J, Keeney S. 2005. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. Nature 436:1053–1057.
19. Blumental-Perry A, Zenvirth D, Klein S, Onn I, Simchen G. 2000. DNA motif associated with meiotic double-strand break regions in Saccharomyces cerevisiae. EMBO Re 3:232–238.
20. Haring SJ, Lautner LJ, Comeron JM, Malone RE. 2004. A test of the CoHR motif associated with meiotic double-strand breaks in Saccharomyces cerevisiae. EMBO Re 5:41–46.
21. Cullen M, Perfetto SP, Klitz W, Nelson G, Carrington M. 2002. High-resolution patterns of meiotic recombination across the human major histocompatibility complex. Am J Hum Genet 71:759–776.
22. Kelmenson PM, Petkov P, Wang X, Higgins DC, Paigen BJ, et al. 2005. A torrid zone on mouse chromosome 1 containing a cluster of recombinational hotspots. Genetics 169:833–841.
23. Guillon H, de Massy B. 2002. An initiation site for meiotic crossing-over and gene conversion in the mouse. Nat Genet 32:296–299.
24. Yauk CL, Bois PR, Jeffreys AJ. 2003. High-resolution sperm typing of meiotic recombination in the mouse MHC *E beta* gene. EMBO J 22:1389–1397.
25. Jeffreys AJ, Murray J, Neumann R. 1998. High-resolution mapping of crossovers in human sperm defines a minisatellite-associated recombination hot spot. Mol Cell 2:267–273.
26. Jeffreys AJ, May CA. 2004. Intense and highly localized gene conversion activity in human meiotic crossover hot spots. Nat Genet 36:151–156.
27. Nicolas A, Petes TD. 1994. Polarity of meiotic gene conversion in fungi: contrasting views. Experientia 50:242–252.
28. Borts RH, Haber JE. 1989. Length and distribution of meiotic gene conversion tracts and crossovers in *Saccharomyces cerevisiae*. Genetics 123:69–80.
29. Pritchard JK, Przeworski M. 2001. Linkage disequilibrium in humans: models and data. Am J Hum Genet 69:1–14.
30. Boulton A, Myers RS, Redfield RJ. 1997. The hotspot conversion paradox and the evolution of meiotic recombination. Proc Natl Acad Sci USA 94:8058–8063.
31. Wall JD, Frisse LA, Hudson RR, Di Rienzo A. 2003. Comparative linkage-disequilibrium analysis of the beta-globin hotspot in primates. Am J Hum Genet 73:1330–1340.
32. Ptak SE, Roeder AD, Stephens M, Gilad Y, Paabo S, et al. 2004. Absence of the *TAP2* human recombination hotspot in chimpanzees. PLoS Biol 2:0849–0845.
33. Winckler W, Myers SR, Richter DJ, Onofrio RC, McDonald GJ, et al. 2005. Comparison of fine-scale recombination rates in humans and chimpanzees. Science 308:107–111.
34. Ptak SE, Hinds DA, Koehler K, Nickel B, Patil N, et al. 2005. Fine-scale recombination patterns differ between chimpanzees and humans. Nat Genet 37:429–434.
35. Kauppi L, Jeffreys AJ, Keeney S. 2004. Where the crossovers are: recombination distributions in mammals. Nat Rev Genet 5:413–424.
36. Jeffreys AJ, Neumann R. 2005. Factors influencing recombination frequency and distribution in a human meiotic crossover hotspot. Hum Mol Genet 14:2277–2287.
37. Xu L, Kleckner N. 1995. Sequence non-specific double-strand breaks and interhomolog interactions prior to double-strand break formation at a meiotic recombination hot spot in yeast. EMBO J 14:5115–5128.
38. Wu TC, Lichten M. 1995. Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics 140:55–66.
39. Ohta K, Shibata T, Nicolas A. 1994. Changes in chromatin structure at recombination initiation sites during yeast meiosis. EMBO J 13:5754–5763.

40. Fan QQ, Petes TD. 1996. Relationship between nuclease-hypersensitive sites and meiotic recombination hot spot activity at the *HIS4* locus of *Saccharomyces cerevisiae*. Mol Cell Biol 16:2037–2043.
41. Shenkar R, Shen MH, Arnheim N. 1991. DNase I-hypersensitive sites and transcription factor-binding motifs within the mouse *E beta* meiotic recombination hot spot. Mol Cell Biol 11:1813–1819.
42. Mizuno K, Koide T, Sagai T, Moriwaki K, Shiroishi T. 1996. Molecular analysis of a recombinational hotspot adjacent to *Lmp2* gene in the mouse MHC: fine location and chromatin structure. Mamm Genome 7:490–496.
43. Jenuwein T, Allis CD. 2000. Translating the histone code. Science 293:1074–1080.
44. Reddy KC, Villeneuve AM. 2004. *C. elegans* HIM-17 links chromatin modification and competence for initiation of meiotic recombination. Cell 118:439–452.
45. Sollier J, Lin W, Soustelle C, Suhre K, Nicolas A, et al. 2004. Set1 is required for meiotic S-phase onset, double-strand break formation and middle gene expression. EMBO J 23:1957–1967.
46. Yamashita K, Shinohara M, Shinohara A. 2004. Rad6-Bre1-mediated histone H2B ubiquitylation modulates the formation of double-strand breaks during meiosis. Proc Natl Acad Sci USA 101:11380–11385.
47. Yamada T, Mizuno KI, Hirota K, Kon N, Wahls WP, et al. 2004. Roles of histone acetylation and chromatin remodeling factor in a meiotic recombination hotspot. EMBO J 23:1792–1803.
48. Borde V, Wu TC, Lichten M. 1999. Use of a recombination reporter insert to define meiotic recombination domains on chromosome III of *Saccharomyces cerevisiae*. Mol Cell Biol 19:4832–4842.
49. Paldi A, Gyapay G, Jami J. 1995. Imprinted chromosomal regions of the human genome display sex-specific meiotic recombination frequencies. Curr Biol 5:1030–1035.
50. Robinson WP, Lalande M. 1995. Sex-specific meiotic recombination in the Prader–Willi/Angelman syndrome imprinted region. Hum Mol Genet 4:801–806.
51. Maloisel L, Rossignol JL. 1998. Suppression of crossing-over by DNA methylation in Ascobolus. Genes Dev 12:1381–1389.
52. Bode J, Benham C, Ernst E, Knopp A, Marshaled R, et al. 2000. Fatal connections: when DNA ends meet on the nuclear matrix. J Cell Biochem 35:3–22.
53. McKee BD. 2004. Homologous pairing and chromosome dynamics in meiosis and mitosis. Biochim Biophys Acta 1677:165–180.
54. Rana NA, Ebenezer ND, Webster AR, Linares AR, Whitehouse DB, et al. 2004. Recombination hotspots and block structure of linkage disequilibrium in the human genome exemplified by detailed analysis of *PGM1* on 1p31. Hum Mol Genet 13:3089–3102.
55. Badge RM, Yardley J, Jeffreys AJ, Armour JA. 2000. Crossover breakpoint mapping identifies a subtelomeric hotspot for male meiotic recombination. Hum Mol Genet 9:1239–1244.
56. Rattray AJ, Symington LS. 1993. Stimulation of meiotic recombination in yeast by an ARS element. Genetics 134:175–188.
57. Borde V, Goldman AS, Lichten M. 2000. Direct coupling between meiotic DNA replication and recombination initiation. Science 290:806–809.
58. Smith KN, Penkner A, Ohta K, Klein F, Nicolas A. 2001. B-type cyclins CLB5 and CLB6 control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr Biol 11:88–97.
59. Cha RS, Weiner BM, Keeney S, Dekker J, Kleckner N. 2000. Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev 14:493–503.
60. White MA, Dominska M, Petes TD. 1993. Transcription factors are required for the meiotic recombination hotspot at the *HIS4* locus in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 90:6621–6625.
61. Kon N, Krawchuk MD, Warren BG, Smith GR, Wahls WP. 1997. 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.
62. Schultes NP, Szostak JW. 1991. A poly(dA.dT) tract is a component of the recombination initiation site at the *ARG4* locus in *Saccharomyces cerevisiae*. Mol Cell Biol 11:322–328.
63. Nicolas A. 1998. Relationship between transcription and initiation of meiotic recombination: towards chromatin accessibility. Proc Natl Acad Sci USA 95:87–89.
64. White MA, Detloff P, Strand M, Petes TD. 1992. A promoter deletion reduces the rate of mitotic, but not meiotic, recombination at the *HIS4* locus in yeast. Curr Genet 21:109–116.
65. Utley RT, Ikeda K, Grant PA, Cote J, Steger DJ, et al. 1998. Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature 394:498–502.
66. Isobe T, Yoshino M, Mizuno K, Lindahl KF, Koide T, et al. 2002. Molecular characterization of the *Pb*recombination hotspot in the mouse major histocompatibility complex class II region. Genomics 80:229–235.
67. Jeffreys AJ, Ritchie A, Neumann R. 2000. High resolution analysis of haplotype diversity and meiotic crossover in the human *TAP2* recombination hotspot. Hum Mol Genet 9:725–733.
68. Zhang J, Li F, Li J, Zhang MQ, Zhang X. 2004. Evidence and characteristics of putative human alpha recombination hotspots. Hum Mol Genet 13:2823–2828.
69. McVean GA, Myers SR, Hunt S, Deloukas P, Bentley DR, et al. 2004. The fine-scale structure of recombination rate variation in the human genome. Science 304:581–584.
70. Crawford DC, Bhangale T, Li N, Hellenthal G, Rieder MJ, et al. 2004. Evidence for substantial fine-scale variation in recombination rates across the human genome. Nat Genet 36:700–706.
71. Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, et al. 2002. A high-resolution recombination map of the human genome. Nat Genet 3:241–247.
72. Lien S, Szyda J, Schechinger B, Rappold G, Arnheim N. 2000. Evidence for heterogeneity in recombination in the human pseudoautosomal region: high resolution analysis by sperm typing and radiation-hybrid mapping. Am J Hum Genet 66:557–566.
73. Broman KW, Murray JC, Sheffield VC, White RL, Weber JL. 1998. Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63:861–869.
74. Shiroishi T, Sagai T, Hanzawa N, Gotoh H, Moriwaki K. 1991. Genetic control of sex-dependent meiotic recombination in the major histocompatibility complex of the mouse. EMBO J 10:681–686.
75. Lynn A, Schrump S, Cherry J, Hassold T, Hunt P. 2005. Sex, Not Genotype, Determines Recombination Levels in Mice. Am J Hum Genet 77:(Epub ahead of print].
76. Thomsen M, Neugebauer M, Arnaud J, Borot N, Sevin A, et al. 1994. Recombination fractions in the HLA system based on the data set 'provinces Francaises': indications of haplotype-specific recombination rates. Eur J Immunogenet 21:33–43.
77. Uematsu Y, Kiefer H, Schulze R, Fischer-Lindahl K, Steinmetz M. 1986. Molecular characterization of a meiotic recombinational hotspot enhancing homologous equal crossing-over. EMBO J 5:2123–2129.
78. Majewski J, Ott J. 2000. GT repeats are associated with recombination on human chromosome 22. Genome Res 10:1108–1114.
79. Pearlman RE, Tsao N, Moens PB. 1992. Synaptonemal complexes from DNase-treated rat pachytene chromosomes contain (GT)n and LINE/SINE sequences. Genetics 130:865–872.
80. Shiroishi T, Koide T, Yoshino M, Sagai T, Moriwaki K. 1995. Hot spots of homologous recombination in mouse meiosis. Adv Biophys 31:119–132.
81. Nasar F, Jankowski C, Nag DK. 2000. Long palindromic sequences induce double-strand breaks during meiosis in yeast. Mol Cell Biol 20:3449–3458.
82. Jankowski C, Nasar F, Nag DK. 2000. Meiotic instability of CAG repeat tracts occurs by double-strand break repair in yeast. Proc Natl Acad Sci USA 97:2134–2139.
83. Rossignol JL, Faugeron G. 1994. Gene inactivation triggered by recognition between DNA repeats. Experientia 50:307–317.
84. Fox ME, Virgin JB, Metzger J, Smith GR. 1997. Position- and orientation-independent activity of the *Schizosaccharomyces pombe* meiotic recombination hot spot *M26*. Proc Natl Acad Sci USA 94:7446–7551.
85. Rocco V, de Massy B, Nicolas A. 1992. The *Saccharomyces cerevisiae ARG4* initiator of meiotic gene conversion and its associated double-

strand DNA breaks can be inhibited by transcriptional interference. Proc Natl Acad Sci USA 89:12068–12072.
86. Pecina A, Smith KN, Mezard C, Murakami H, Ohta K, et al. 2002. Targeted stimulation of meiotic recombination. Cell 111:173–184.
87. Gilbertson LA, Stahl FW. 1994. Initiation of meiotic recombination is independent of interhomologue interactions. Proc Natl Acad Sci USA 91:11934–11937.
88. Jeffreys AJ, Neumann R, Panayi M, Myers S, Donnelly P. 2005. Human recombination hot spots hidden in regions of strong marker association. Nat Gene 37:601–606.
89. Phillips MS, Lawrence R, Sachidanandam R, Morris AP, Balding DJ, et al. 2003. Chromosome-wide distribution of haplotype blocks and the role of recombination hot spots. Nat Genet 33:382–387.
90. De La Vega FM, Isaac H, Collins A, Scafe CR, Halldorsson BV, et al. 2005. The linkage disequilibrium maps of three human chromosomes across four populations reflect their demographic history and a common underlying recombination pattern. Genome Res 15:454–462.
91. Kauppi L, Sajantila A, Jeffreys AJ. 2003. Recombination hotspots rather than population history dominate linkage disequilibrium in the MHC class II region. Hum Mol Genet 12:33–40.
92. Kauppi L, Stumpf MP, Jeffreys AJ. 2005. Localized breakdown in linkage disequilibrium does not always predict sperm crossover hot spots in the human MHC class II region. Genomics 86:13–24.
93. True JR, Mercer JM, Laurie CC. 1996. Differences in crossover frequency and distribution among three sibling species of *Drosophila*. Genetics 142:507–523.
94. Roeder GS. 1997. Meiotic chromosomes: it takes two to tango. Genes Dev 11:2600–2621.
95. Mahadevaiah SK, Turner JM, Baudat F, Rogakou EP, de Boer P, et al. 2001. Recombinational DNA double-strand breaks in mice precede synapsis. Nat Genet 27:271–276.
96. Dernburg AF, McDonald K, Moulder G, Barstead R, Dresser M, et al. 1998. Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. Cell 94:387–398.
97. McKim KS, Green-Marroquin BL, Sekelsky JJ, Chin G, Steinberg C, et al. 1998. Meiotic synapsis in the absence of recombination. Science 279:876–878.
98. Bahler J, Wyler T, Loidl J, Kohli J. 1993. Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. J Cell Biol 121:241–256.
99. Hubert R, MacDonald M, Gusella J, Arnheim N. 1994. High resolution localization of recombination hot spots using sperm typing. Nat Genet 7:420–424.
100. Stumpf MP, McVean GA. 2003. Estimating recombination rates from population-genetics data. Nat Rev Genet 4:959–968.
101. Nishant KT, Ravishankar H, Rao MRS. 2004. Characterization of a mouse recombination hotspot encoding a novel non-protein coding RNA. Mol Cell Biol 24:5620–5634.