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Discrete DNA sites regulate global distribution of meiotic recombination

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Abstract

Homologous recombination is induced to high levels in meiosis, is initiated by Spo11-catalyzed DNA double-strand breaks (DSBs), and is clustered at hotspots that regulate its positioning in the genome. Recombination is required for proper chromosome segregation in meiosis; defects in its frequency or positioning cause chromosome mis-segregation and, consequently, congenital birth defects such as Down's syndrome. Therefore elucidating how meiotic recombination is positioned is of fundamental and biomedical interest. Integration of historical and contemporary advances in the field, plus the re-analysis of published microarray data on the genome-wide distribution of recombination, support a unifying model for such regulation. We posit that discrete DNA sequence motifs position and regulate essentially all recombination across the genome, in much the same way that DNA sites position and regulate transcription. Moreover, we illustrate the use of overlapping mechanisms for the regulation of transcription and meiotic recombination. Bound transcription factors induce histone modifications that position recombination at hotspots.

Hallmarks of Meiosis

Most eukaryotes transition between alternating haploid and diploid states that are produced by meiosis and fertilization (or conjugation), respectively. Meiosis couples one round of DNA replication with two rounds of chromosome segregation to produce haploid meiotic products. Hallmarks that distinguish meiosis from mitosis include the pairing of homologous chromosomes, the induction of interhomolog recombination, and a reductional first division in which the individual homologs migrate to opposite poles. In the second, equational division the sister chromatids segregate from one another.

Meiotic recombination is initiated by Spo11-catalyzed, DNA double-strand breaks (DSBs) [1]. Recombination generates new combinations of alleles upon which natural selection can act and in most eukaryotes it is required for the faithful segregation of chromosomes in the first meiotic division [2]. Elucidating where and how recombination is positioned is important for understanding how chromosomes are partitioned faithfully in meiosis and how genomes evolve over time. To this end, attention has focused upon recombination hotspots, which are highly localized regions of the chromosome where the rate of recombination (per unit distance) is much higher than the average rate across the genome [1]. Here we place important recent discoveries on mechanisms within a historical context and we propose a

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unifying model. We posit that discrete DNA sequence motifs (DNA sites) regulate and position essentially all meiotic recombination in the genome.

Histone modifications regulate meiotic recombination

It has long been recognized that there is an association between the positions of meiotic recombination hotspots and regions of “open” chromatin structure [1]. A growing body of recent work, in diverse organisms, indicates that post-translational modifications of histones (*e.g.*, acetylation, ubiquitination, and methylation) help to position recombination [3–9]. For example, the trimethylation of histone H3 (H3K4-Me₃) is strongly implicated to regulate a subset of recombination hotspots in budding yeast, mice, and humans [5,10,11]. In mammals, orthologs of the histone H3K4 trimethylase Prdm9 apparently regulate those hotspots [10]. Similarly, the H3K4 trimethylase Set1 of budding yeast is known to regulate hotspot activity [5,11]. Thus the regulation of meiotic recombination by specific epigenetic marks might be broadly conserved, even though individual histone modifying enzymes such as Prdm9 can undergo rapid evolutionary divergence [12]. An emerging view in the field is that multiple histone modifications contribute to the formation of docking sites for, or sites of catalytic action by, meiotic recombination protein complexes. The question of what specific chromosomal features position such epigenetic marks remains largely unanswered. One answer involves the primary sequence of DNA.

DNA sites regulate meiotic recombination

Nearly twenty years ago Jürg Kohli's and Tom Petes' laboratories discovered DNA sequence-dependent regulation of meiotic recombination in two highly diverged organisms, fission yeast and budding yeast [13,14]. Additional regulatory DNA sites were identified subsequently in each yeast [15–17]. Those findings (and their broad implications) received surprisingly little attention or endorsement from many researchers in the field of recombination, perhaps because such regulatory motifs had not been demonstrated in metazoans.

The distribution of meiotic recombination crossovers in the human genome was recently mapped at high resolution [18]. Subsequently, Simon Myers and colleagues identified a consensus DNA sequence motif (5'-CCNCCNTNNCCNC-3') that is associated with 40% of hotspots [19]. Single nucleotide polymorphisms within such elements attenuate hotspot activity, thus providing compelling evidence that the DNA sequence motif regulates recombination. A similar inference was recently made for mice [10]. In each case, the methyltransferase (transcription factor) Prdm9 is implicated to help regulate the local induction of recombination, and this activity is thought to be targeted to the DNA motifs by the zinc finger DNA binding domain of Prdm9 [10,12]. These striking discoveries warrant comparison to what is known about DNA sequence-dependent regulation of recombination in budding yeast and fission yeast.

If any specific DNA sequence promotes (regulates) meiotic recombination, then base pair substitutions that ablate or create the DNA site should attenuate or promote recombination, respectively. These stringent criteria have been met for five different DNA sequence motifs of fission yeast [13,17]. The first hotspot defined at this resolution (in any eukaryote) was discovered by Herbert Gutz as a mutant allele of *ade6* [20]. A single base pair change [21] created fortuitously a cyclic-AMP responsive element (*CRE*)-like DNA site (*M26*; 5'-ATGACGT-3') that promotes recombination locally (Figure 1) [13]. Binding of an ATF/CREB-family transcription factor complex (Atf1-Pcr1 heterodimer) to the *M26* DNA site [22] promotes the catalysis of recombination-initiating DSBs by Rec12 (Spo11) [23,24]. A similar general mechanism (bound transcription factors promote recombination) has been

implicated to help position recombination at some hotspots in mammals and budding yeast [10,15,25].

Gerry Smith's group showed that a subset of *M26*-consensus DNA sites located elsewhere in the fission yeast genome are recombination hotspots (*e.g.*, [24,26–28]). In every case tested by base pair mutagenesis, the hotspot activity (whether defined physically by DSBs, genetically by elevated recombination, or both) requires the *M26* motif. Therefore *M26* DNA sites help to regulate the distribution of recombination across the genome. However, *M26* DNA sites cannot regulate all hotspots, because the majority of hotspots lack a detectable *M26* DNA sequence motif [29]. This is also true for the recently discovered, hotspot-associated DNA sequence motif of humans [19] and for recombination-promoting sequence motifs in budding yeast [30]. So, what entities position recombination at *M26*-independent hotspots?

In an insightful recent study, Walter Steiner and colleagues screened ~46,000 randomized DNA sequences for their ability to promote meiotic recombination [17]. Inspection of the hotspot-active DNA sequences revealed the presence of several motifs that were recovered at a frequency higher than expected by chance. Subsequent reconstruction of specific DNA sequence motifs by base pair substitutions in the genome confirmed that there are at least five discrete classes of hotspot-activating DNA sites in fission yeast (Box 1). Is it possible that all meiotic recombination—like transcription—is positioned and regulated by discrete DNA sites?

Global regulation of meiotic recombination by DNA sites

Genome-wide mapping of recombination in humans led to discovery of the hotspot-associated DNA sequence motif thought to help activate 40% of hotspots [19]. The distributions of recombination in the budding yeast and fission yeast genomes have also been mapped at high resolution (*e.g.*, [29,31–34]). Intriguingly, in contrast to the human studies, no hotspot-associated DNA sequence motifs were identified. Of course, the absence of evidence is not evidence for absence; recombination-promoting DNA sites clearly do exist in each yeast (*e.g.*, Figure 1) [13–17]. We reasoned that the failure to detect hotspot-associated DNA sequence motifs in the genome-wide studies could be a false-negative result imparted by the algorithms used to analyze the data. Indeed, this is demonstrably the case for the recombination-promoting DNA site *M26*. By comparing the published distribution of Rec12 (Spo11)-catalyzed, DSB peaks [29] to the published genomic DNA sequence [35], we discovered that DSB peaks (hotspots) are directed preferentially to *M26* DNA sites across the genome ($P \leq 7.3 \times 10^{-13}$) (Figure 2).

Initial estimates suggested that *M26* DNA sites regulate about 50% of all meiotic recombination [23]. In support of this possibility, 10 out of 15 (67%) of the *M26*-consensus DNA sequences examined by Southern blotting have an associated DSB peak [27]. As all *M26*-associated DSB peaks examined to date by mutagenesis strictly require the *M26* DNA site, one can make a strong inference about the overall contribution of *M26* DNA sites to DSB-initiated recombination across the genome from the extent of colocalization (Figure 2) and the peak area integrals [29]. By this measure, *M26* DNA sites regulate and position 20% of meiotic recombination. This value is an underestimate, because it is based upon perfect matches to the seven base pair *M26* DNA site [13] and does not take into account the fact that some *M26*-variant sites can also promote recombination [27,36].

The most conservative calculation indicates that *M26* DNA sites regulate 20% of meiotic recombination. Hence, the recent discovery that at least four additional DNA sequence motifs of fission yeast also activate hotspot recombination (Box 1) [17] has profound implications. If each class of DNA site regulates on average the same fraction of meiotic

recombination as that regulated by the *M26* class, then together the five already identified, recombination-promoting DNA site motifs could account for the regulated positioning of all meiotic recombination in the genome. Furthermore, it is likely that there are additional recombination-promoting DNA sequence motifs yet to be identified [17].

Single-site insufficiency, multi-site synergy

Some *M26* DNA sites in the fission yeast genome promote meiotic recombination, whereas others (at least 74%) do not (Figure 2b). We refer to this phenomenon as “single-site insufficiency”. Single-site insufficiency has also been documented at the genome-wide level for 84% of the DNA sites occupied by the budding yeast transcription factor Bas1 [30]. Similarly, the human DNA sequence motif that is thought to activate 40% of meiotic recombination hotspots is insufficient to promote recombination for at least 90% of its locations in the genome [19]. So in each of three, highly diverged eukaryotes, the ability of any given recombination-promoting DNA sequence motif to activate hotspot recombination is somehow dependent upon the context within which that DNA site is embedded. The accessibility of the DNA sites within local chromatin structure [8, 30], presence of chromosomal domains that are relatively (but not absolutely) “hot” or “cold” for recombination [31–33], and higher order features of chromosome architecture [37] might provide such context.

We propose that an alternative, more-reductionist explanation for single-site insufficiency of recombination-promoting DNA sequence motifs is that hotspot activity might require a synergistic interaction between two or more *cis*-linked DNA sites. We call this “multi-site synergy”. Jürg Kohi’s group found that at least one additional DNA sequence element within a 510 base pair region of the *ade6* locus must be linked in *cis* to the *M26* DNA site to render the hotspot active [38]. There is some flexibility to this requirement, because the *M26* DNA site can be moved within, or inverted at, the *ade6* locus and retain hotspot activity [26]. Thus in two regards the *M26* DNA site recapitulates for the regulation of meiotic recombination the way that enhancer elements help to regulate transcription. These are a requirement for two or more *cis*-linked DNA sequence elements, and some degree of distance- and orientation-independent synergism between those DNA elements.

A second example of multi-site synergism for the regulation of meiotic recombination can be inferred from deletion studies in budding yeast, where DNA regions that contain binding sites for the transcription factors Rap1, Bas1, and Bas2 each contribute to activation of the *HIS4* hotspot [14–16]. Moreover, functional redundancy exists between the DNA regions with binding sites for Bas1 and Bas2. In both humans [19] and mice [39,40], additional chromosomal elements located in *cis* to defined hotspots help to regulate hotspot activity and the hotspot-associated DNA sequence motif of humans is functional at only 10% (or less) of its locations in the genome [19]. We suggest that at least one additional, yet-unidentified, *cis*-linked DNA sequence element is required to help activate those hotspots (*i.e.*, as occurs in fission yeast and budding yeast). Of course, it is also possible that yet-unidentified, DNA sequence-independent factors contribute to the regulation of DNA sequence-dependent hotspots.

We propose that a synergistic interaction between two or more *cis*-linked DNA sites is the primary determinant for the regulated positioning of meiotic recombination at hotspots along chromosomes, in much the same way that multiple DNA sites function together to position and regulate transcription. Furthermore, we suggest that there is some degree of DNA site redundancy, in much the same way that different enhancer elements can each regulate transcription. This evidence-based, reductionist model is not mutually exclusive with models

in which other features (*e.g.*, permissive or restrictive chromosome domains) help to regulate recombination.

Transcription factors, but not transcription

There are now at least eight discrete DNA sequence motifs demonstrated by base pair mutagenesis (five) or implicated by deletion analyses (three) to promote meiotic recombination. These include five in fission yeast that are essential for hotspot activity (Box 1) [13,17] and three in budding yeast which contribute to hotspot activity (binding sites for Bas1, Bas2, and Rap1) [14–16]. These DNA sequence motifs are each known or putative binding sites for transcription factors. In every case tested, the DNA sequence-dependent stimulation of recombination requires the corresponding DNA binding protein(s) or, in the case of one protein that is essential for viability (Rap1), exhibits a protein dose-dependent response (*e.g.*, Figure 1) [14,17,23,30].

Although DNA sequence-dependent recombination hotspots are activated by transcription factors, the regulation of transcription and recombination are separable. First, in budding yeast some mutations that reduce transcription do not reduce recombination to the same extent [15,41,42]. Second, steady-state transcription levels in fission yeast are similar for the *M26* hotspot and *M375* control alleles of *ade6* [43]; removal of the *M26* DNA site-binding, hotspot-activating transcription factor (Atf1–Pcr1) does not substantially change these levels [23]. Third, the region of Atf1 that is sufficient to activate hotspot recombination when bound to the chromosome is distinct from the region required for transcription-dependent stress responses [36]. Fourth, in both yeasts the global mapping studies revealed that, on a population scale, the frequency of recombination at hotspots is largely independent of steady-state mRNA levels of protein-coding genes located at (or near to) hotspots [11,29,31].

If discrete DNA sites and bound transcription factors regulate meiotic recombination in a transcription rate-independent fashion, then what is their mechanism of action? The answer can be found in transcription factor-induced modifications of chromatin structure.

DNA sites establish “histone codes” for recombination

The acetylation, ubiquitination, and methylation of histones are each implicated in meiotic recombination. Kunihiro Ohta’s laboratory revealed that *M26* DNA sites can establish such epigenetic marks [3]. Furthermore, they delineated a causal, sequential relationship between *M26* DNA sites, the heterodimeric transcription factor Atf1–Pcr1, multiple histone modifying enzymes and their target modifications, the catalysis of DSBs by Rec12 (Spo11), and local recombination hotspot activity (Figure 3) [3,8,44]. In a nutshell, the availability of good *cis*-acting controls (plus and minus *M26* DNA sites at *ade6* and elsewhere) allowed the authors to demonstrate unambiguously that specific epigenetic changes in local chromatin structure are required to position recombination at hotspots. These seminal findings solidified a hypothesis on epigenetic regulation that is supported by a growing body of data from diverse organisms including fungi, nematodes, and mammals [3–9,11]. Looking forward, the use of similar controls for *cis*-acting specificity at other hotspots will help to define which histone modifications regulate recombination directly in *cis*, which function indirectly in *trans*, and which constitute “background noise”.

The histone modifications at *M26* hotspots are central components of a pathway with identified steps ranging from the action of a regulatory p38 protein kinase cascade to the catalysis of recombination-initiating DSBs by Rec12 (Spo11) (Figure 3a) (*e.g.*, [3, 8, 22–24, 45, 46]). This pathway provides a prototypical mechanism by which discrete DNA sites regulate the frequency and distribution of meiotic recombination across the genome (*e.g.*,

Figure 2). A similar mechanism is likely employed by other recombination-promoting DNA sites, which in every case tested require the corresponding site-specific transcription factor(s) for hotspot activity [14, 17, 23, 30].

Multiple DNA sequence motifs of fission yeast, budding yeast, and humans help to regulate meiotic recombination; and the five already identified, recombination-promoting DNA site motifs of fission yeast could position all recombination in the genome. Within that context, we emphasize that in each organism and in every case tested the recombination-promoting DNA site motifs often display single-site insufficiency (*e.g.*, Figure 2b) [19, 27, 30], they can function redundantly at the same locus [16, 17], and they work synergistically or additively with other *cis*-linked DNA sites [15, 38].

We note that essentially identical characteristics are manifest for the epigenetic marks which help to regulate recombination. First, multiple different histone modifications are implicated [3–8,11,47]. Second, the individual modifications display insufficiency (where tested) [11]. And third, multiple modifications are proposed to function synergistically [3,8] or sequentially [6,11] at the same locus. Thus there are instructive parallels between the regulation of meiotic recombination by discrete DNA sites and by histone modifications. In each case, there is evidence that combinatorial “codes” are at work. Moreover, at the *ade6-M26* hotspot, synergistic interactions occur both between two or more *cis*-linked DNA sequence elements [13,38] and between two or more histone modifications [3,8]. Therefore individual hotspots can apparently be regulated by at least two combinatorial codes, one for DNA sites and one for histone modifications. In the fission yeast paradigm, the DNA codes [13,38] seed the histone codes [3,8,44] (Figure 3a). Notably, this provides a unifying and guiding principle, because this type of mechanism is consistent with—and can explain—available data on the regulated positioning of meiotic recombination in diverse eukaryotes ranging from fungi to humans.

Evolutionary origin of regulatory DNA sites

The regulation of hotspot recombination by Prdm9 is conserved in mice and humans [10], even though the DNA binding domain of Prdm9 (and hence its DNA site specificity) is evolving rapidly [12]. The sequences of other regulatory DNA sites are more highly and broadly conserved (Box 1). For example, the conserved CCAAT-box and *M26* (*CRE*-like) DNA sites are each bound by conserved proteins [36,48]; and in each case the protein-DNA complex can regulate transcription (this function is conserved) [48,49] or promote meiotic recombination (conservation of function remains untested) [17,23]. In summary, the data suggest that fundamental mechanisms, including at least some regulatory DNA sites, are evolutionarily ancient and have been retained during the radiation of eukaryotic lineages. This hypothesis awaits further testing. For example, it will be interesting to see whether broadly conserved factors known to regulate recombination in fission yeast (*e.g.*, the CCAAT motif and its binding proteins) also regulate recombination in other eukaryotes.

Concluding remarks

Despite important discoveries in one area (*e.g.*, regulation by DNA sites) or another (*e.g.*, regulation by epigenetics), a unified understanding of what regulates hotspot meiotic recombination has long been elusive. In our opinion a conceptually straightforward, evidence-based, unifying model explains how meiotic recombination is regulated locally and globally along chromosomes. We posit that discrete, *cis*-acting DNA sites regulate and position essentially all meiotic recombination. Binding of transcription factors provides a direct, mechanistic link between DNA sites and epigenetic changes of chromatin structure which help to position recombination events. As is the case for transcription, no single type of DNA site, transcription factor, or histone modification can account for the regulated

positioning of all recombination. Instead, these elements function combinatorially (with potential for synergism, antagonism, and redundancy) to establish preferential sites of action by meiotic recombination protein complexes. This model brings a bold new perspective to the field and, more broadly, begs reconsideration of classical views on meiotic recombination. Looking forward, the regulatory DNA sites provide a key to unlock the composition, regulation, and mechanisms by which histone codes position meiotic recombination.

Conserved DNA sites that regulate meiotic recombination

A gain-of-function genetic screen identified five distinct classes of DNA sequence motifs that promote meiotic recombination in fission yeast [17]. Their ability to induce recombination locally, and hence to regulate the positioning of recombination events within the genome, might be broadly conserved in other eukaryotes.

The *M26/CRE* group

Sequences in this group contain perfect or near-perfect matches to the *CRE*-like DNA site *M26* (5'-ATGACTG-3') [13], to which the heterodimeric, basic-leucine-zipper transcription factor Atf1-Pcr1 binds and promotes recombination [22,23]. This finding validated the utility of the genetic screen. The sequences of the *M26* DNA site and the small domain of Atf1-Pcr1 heterodimer sufficient to promote recombination are conserved in other eukaryotes [36].

The CCAAT group

Members of this group share a common core motif (5'-CCAAT-3') which is a broadly conserved DNA sequence element that, like *M26/CRE*, can regulate transcription. The heterotetrameric, histone-fold, CCAAT-binding transcription factor is also broadly conserved [48]. Notably, the removal of protein subunits Php2, Php3, and Php5 each abolishes the CCAAT motif-dependent hotspot activity [17]. These findings suggest that the binding of CCAAT transcription factor to the CCAAT DNA site promotes recombination, much as binding of the Atf1-Pcr1 transcription factor to the *M26* DNA site promotes recombination.

The oligo-C group

Sequences in this group share a consensus core (5'-CCCCGCA-3') that might constitute a binding site for the conserved, zinc-finger transcription factors Scr1, Rsv1, Hsr1, and Rst2 [17]. The role of those proteins in oligo-C-promoted recombination has not been determined. The oligo-C motif is similar [17] to a human DNA sequence motif that is implicated to activate 40% of meiotic recombination hotspots and to trigger mitotic genome instability [19]. It is also similar to the core of tandemly repetitive, hypervariable minisatellite DNA sequences known to promote homologous recombination in mitotically dividing human cells [52] and thought to do so in meiosis [53].

The ade6-4095 and motif-8-6 groups

Members of the ade6-4095 group share the motif 5'-GGTCTRGAC-3'; and members of the motif-8-6 group are represented by the motif 5'-WTCGGCCGA-3'. These elements are presumably bound by DNA sequence-specific, hotspotactivating proteins yet to be identified.

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Figure 1. DNA sequence-dependent regulation of meiotic recombination. (a) At the *ade6-M26* hotspot, two *cis*-linked DNA sequence elements function synergistically to promote the initiation of recombination. One of these elements (the *M26* DNA site) resides within the 5' end of the *ade6* open reading frame and has been defined at single-nucleotide resolution. The other element maps within a 510 base pair region of the *ade6* promoter. After phosphorylation of Atf1 by the protein kinase Spc1, the Atf1-Pcr1 heterodimer binds to the *M26* DNA site and promote DSB through Rec12 (Spo11). (b) Example of DNA sequence-dependent, protein-dependent activation; adapted with permission from ref. [45] (copyright © American Society for Microbiology).

Figure 2.

M26 DNA sites regulate initiation of recombination across the *S. pombe* genome. We identified 285 *M26* DNA sites in the genome sequence [35] and compared their distribution to the published distributions of 194 “prominent” (prom.) and 159 “weak” DSB hotspots [29]. **(a)** Example distribution of *M26* DNA sites and DSB peaks. A DSB peak shown experimentally to require the *M26* DNA sequence is indicated (*). **(b)** Non-random, genome-wide colocalization of DSB peaks with *M26* DNA sites. Expected and observed fractions of *M26* DNA sites present in DSB peaks are compared for weak, prominent and all peaks found in the genome. **(c)** Proportion of DSB peaks which contain *M26* DNA sites. Experimental procedures and statistical tests are described in detail elsewhere [51]; primary data on colocalization are available upon request. DSB peak distributions and classifications (“weak” vs. “prominent”) in panel “a” were adapted from ref. [29] with permission under the Creative Commons Attribution License.

Figure 3.

Pathway mechanisms elucidated for the *ade6-M26* recombination hotspot. **(a)** Schematic diagram of pathway components. Sequential functions are depicted horizontally; vertically stacked factors function together at the same step or the order of function is uncertain. **(b)** Model for recruitment of meiotic recombination proteins, after ref. [45]. The Atf1-Pcr1-M26 protein-DNA complex triggers the reversible, post-translational modifications of histones and nucleosome repositioning. These factors, perhaps in conjunction with bound proteins such as Atf1-Pcr1 heterodimer, promote the recruitment of meiotic recombination protein Rec12 (Spo11). Present data cannot distinguish whether Rec12 complexes bind preferentially to DNA within "open" chromatin, or dock to specific histone modifications, or both.