 0014-4754/94/030234-08$1.50 + 0.20

Hotspots of homologous recombination

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Abstract. Homologous recombination occurs at higher than average frequency at and near hotspots. Hotspots are special nucleotide sequences recognized by proteins that promote, directly or indirectly, a rate limiting step of recombination. This review focuses on two well-studied examples, the Chi sites of the bacterium *Escherichia coli* and the M26 site of the fission yeast *Schizosaccharomyces pombe*. Chi, 5′ G-C-T-G-G-T-G-G 3′, is recognized by the RecBCD enzyme, which nicks the DNA near Chi and produces a 3′-ended single-stranded DNA 'tail'; this tail is a potent substrate for homologous pairing by RecA and single-stranded DNA binding proteins. M26, 5′ A-T-G-A-C-G-T 3′, is recognized by a heterodimeric protein and stimulates, by an as-yet-unknown mechanism, meiotic recombination at and near the *ade6* gene. Additional hotspots in bacteria, fungi, and mammals enhance recombination directly or indirectly via a variety of mechanisms. Although hotspots are widespread among organisms, the biological role of their localized enhancement of recombination remains a matter of speculation.
Key words. Homologous recombination; hotspots; nucleases; meiosis; *Escherichia coli*; Chi; *Schizosaccharomyces pombe*; M26.

Homologous recombination is frequently considered, incorrectly, to be a random process, since it can occur anywhere along homologous chromosomes. But the frequency at which it occurs varies widely from interval to interval along the genome. The frequency of recombination per 1000 base-pairs (kb) of DNA can vary as much as 1000-fold, but more typically 10- to 100-fold, in a given organism. This variation reflects either intervals with higher than average frequency (hotspots) or those with lower than average frequency (coldspots). Localized variation in frequency must be due to special nucleotide sequences that distinguish one interval from another. Nucleotide sequences have been determined for two well-studied hotspots discussed in detail here, the Chi sites of *E. coli* and the M26 site of *S. pombe*.

Hotspots are presumably activated by proteins that recognize these sites and enhance, directly or indirectly, rate-limiting steps of recombination. For some hotspots, such as Chi, the protein may directly promote recombination. For other hotspots the protein may be involved in another localized chromosomal process, such as transcription or replication, and only indirectly enhance recombination. Hotspots can also be manifest by particular, rare alleles of a gene; heteroduplex DNA encompassing such an allele contains a base mismatch that may be repaired by special enzymes to generate recombinants at high frequency with this allele but not with others in the same gene.

This review discusses two well-characterized hotspots. Other reviews discuss these and other examples (refs 6, 43, 49 and Nicolas and Petes in this issue). Radicella, Fox and Yamamoto (in this issue) discuss mismatch repair and its influence on recombination. Klar and Bonaduce[25] describe the genetic control of an especially strong meiotic coldspot between the *mat2* and *mat3* genes of *S. pombe*.

Chi sites of *E. coli*

Chi sites were first noted as mutations that enhance the growth (plaque size) of bacteriophage λ lacking its Red recombination genes[21]. Stahl and his colleagues showed that Chi enhances this growth by increasing the frequency of λ recombination promoted by the *E. coli* RecBCD pathway and that this increase is localized around the site of the Chi mutation[30,50]. The increase is greatest near Chi and diminishes exponentially, a factor of 2 for each 2–3 kb, to the left of Chi; there is little, if any, stimulation to the right[9,16,54] (fig. 1). ['Left' and 'right' in this review refer to the conventional genetic map of phage λ, in which most of the genetic characterization of Chi has been done.] An active Chi site is dominant to an inactive one; i.e., there is essentially as much increase in recombination with Chi in one parent as with Chi in both parents[30]. Chi in one parent opposite a large (several kb) heterology in the other parent increases recombination but only in the region of homology to its left[52]. These results established Chi as a site that increases homologous recombination at and to the left of itself.

To be active in λ, Chi must be properly oriented with respect to an available double strand (ds) DNA end[26]. In a normal λ infection this end is created by the λ terminase protein which cuts the *cos* site in the circular replicating DNA in preparation for packaging linear DNA molecules into virions. Because terminase and the prohead structure remain bound to the newly created left end, only the right end of λ is available for entry of

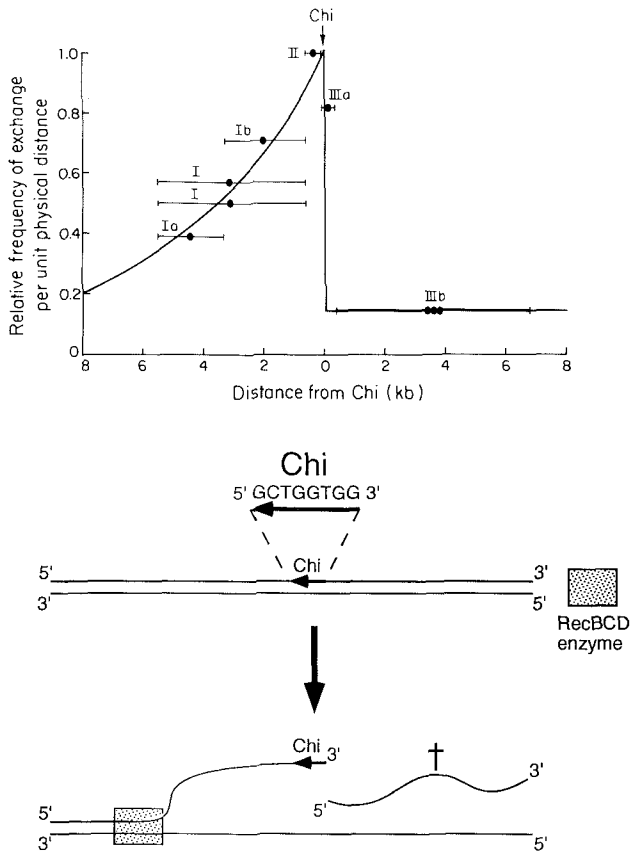

Figure 1. *Top* Stimulation of recombination by Chi in phage λ crosses. The curve summarizes the relative frequency of recombination per kb in genetic intervals, indicated by the line segments, around an actively oriented Chi in λ (data from ref. 9).
Bottom Cutting of DNA at Chi by RecBCD enzyme. The leftward pointing arrow above Chi symbolizes the direction of stimulation of recombination by an actively oriented Chi in λ. RecBCD enzyme (stippled box) initiates DNA unwinding at the right end of λ DNA and during unwinding cuts one strand four, five, or six nucleotides to the 3′ side of Chi. Continued unwinding releases a 'Chi tail' with the Chi sequence near its 3′ end. Under some reaction conditions (see text) the ss DNA fragment to the right of Chi (marked †) is degraded.

a factor that travels to Chi and recognizes it in one orientation but not the other. In a λ infection in which the *Eco*RI restriction enzyme is available to cut an unmodified *Eco*RI site in the infecting λ, a 'backwards' Chi is active[56]. These results showed that Chi is an asymmetric site, which is activated by a factor (presumably an enzyme) that enters a ds end, travels leftward, and stimulates recombination at and to the left of a properly oriented Chi site (fig. 1).

Chi is also active in one orientation but not the other in plasmids that replicate as a rolling circle[11]. The active orientation of Chi with respect to the end of the rolling circle tail is the same as that of an active Chi with respect to λ *cos*. In these plasmids an active Chi enhances the formation of multimeric plasmid forms by protecting the linear DNA from RecBCD enzyme's nucleolytic degradation (see below) or by stimulating plasmid-by-plasmid recombination, or both.

The nucleotide sequence required for Chi activity was elucidated by the analysis of seven Chi sites created or inactivated by mutations (see ref. 46 for a summary). Although wild-type λ and plasmid pBR322 lack Chi, mutations can create Chi at at least four loci in λ and three in pBR322[47,50]. Wild-type *E. coli* contains active Chi sites in many genes, including *lacZ*. Comparisons of the nucleotide sequences of seven active Chi sites revealed a common octamer 5′ G-C-T-G-G-T-G-G 3′; all of the mutations creating Chi generated this octamer. Further mutations inactivating a Chi site in (mutant) λ and (wild-type) *lacZ* are also confined to this octamer. Although only 5′ G-C-T-G-G-T-G-G 3′ has full Chi activity, 5′ G-C-T-A-G-T-G-G 3′ has about 40% activity, and 5′ A-C-T-G-G-T-G-G 3′ and 5′ G-T-T-G-G-T-G-G 3′ have about 10% and 5% activity, respectively[7].

Studies using λ transducing phages showed that Chi occurs throughout the *E. coli* genome approximately once per 5 kb of DNA[17,33]. In the approximately one-half of *E. coli* DNA now sequenced and reported in GenBank, the Chi octamer occurs about once per 5 kb (A. F. Taylor, pers. commun.). This frequency is about six times higher than that expected if the nucleotides of *E. coli* DNA were randomly associated. In fact, among the 65536 possible octamers, Chi is the tenth most abundant octamer present in the currently sequenced *E. coli* DNA[3a]. The partially active sequence 5′ G-C-T-A-G-T-G-G 3′ is present rarely, only once per 250 kb. Remarkably, about 85% of the Chi sites in a 136 kb region around the origin of replication are oriented such that they would stimulate recombination between themselves and the origin[5]. The biological implications of this bias remain unclear.

The preceding observations suggest that Chi is an element essential for *E. coli* recombination. Chi is indeed active in *E. coli* recombination following Hfr-mediated conjugation and phage P1-mediated transduction[14]. Chi's properties in these *E. coli* crosses parallel those in λ crosses. The suggestion that Chi is essential for recombination is bolstered by the calculation that the low level of recombination of Chi-less λ by the *E. coli* RecBCD pathway can be accounted for by the occurrence in λ of the weakly active Chi sites mentioned above[7]; in other words, in the absence of Chi and Chi-like sites there might be no RecBCD pathway recombination.

Genetic evidence indicated that Chi is recognized by the RecBCD enzyme of *E. coli*, which possesses three pathways of recombination, called RecBCD, RecE, and RecF[10,44]. Chi activates the RecBCD pathway but not the RecE or RecF pathway or the Red pathway of λ[53]. Since only RecBCD enzyme was known at the time of these studies to be unique to the RecBCD pathway, these results implicated RecBCD enzyme as the factor that recognizes Chi. Further evidence came from special mutations in the *recB* and *recC* genes, encoding two

subunits of the enzyme, that reduced or eliminated Chi activation but not RecBCD pathway recombination; these mutant enzymes may recognize an alternative site[42].

Direct evidence that RecBCD enzyme recognizes Chi came from the observation that purified RecBCD enzyme nicks DNA at high frequency near Chi and that extracts of *rec*$^+$ bacteria contain this nicking activity whereas extracts of *recBCD* mutants lacking Chi genetic activity do not[35,60]. Nicking occurs on the strand containing 5′ G-C-T-G-G-T-G-G 3′, four, five, or six nucleotides to the 3′ side (fig. 1). Nicking occurs during unwinding of the DNA by RecBCD enzyme, which starts at a ds end and proceeds unidirectionally[59]. Chi is cut only if it is properly oriented with respect to the ds end at which RecBCD enzyme enters the DNA; this orientation is that of an active Chi in λ with respect to *cos* (see above). These observations provided an enzymatic basis for the activation of Chi by a ds end in an orientation-dependent manner.

Genetic evidence indicates that RecBCD enzyme's nicking at Chi is essential for Chi's stimulation of recombination. Mutations altering either the enzyme or the Chi site coordinately reduce or abolish both nicking at Chi and Chi genetic activity[1,8,35]. For example, two Chi site mutations, 5′ G-C-T-A-G-T-G-G 3′ and 5′ A-C-T-G-G-T-G-G 3′, respectively produce about 40% and 10% as much enhancement of recombination and about 10% and 5% as much nicking as fully active Chi[7,8]. The *recB344* and *recC343* (Tex) mutations also co-ordinately reduce both activities[35,42]. Over one dozen mutations in the *recB*, *recC* or *recD* genes and four mutations in Chi render both activities undetectable[1,8,35]. Furthermore, *recBCD*-complementing genes from six enteric bacterial species confer to an *E. coli recBCD* deletion mutant both Chi hotspot activity in λ-infected cells and Chi nicking activity in extracts[34]. Similar genes from three Pseudomonas species confer neither activity, although they do complement the recombination deficiency and provide other RecBCD enzyme activities[34]. This strong correlation demonstrates that Chi nicking activity, or an as-yet-untested activity so far mutationally and evolutionarily inseparable from it, is required for Chi hotspot activity.

Upon nicking at Chi RecBCD enzyme is changed in a way that is important for recombination[61]. A single RecBCD enzyme cuts about 40% of the time that it passes a properly oriented Chi. If it cuts, it loses the ability to detectably cut at a second Chi on the same or a separate DNA molecule. Although RecBCD enzyme continues to unwind DNA after it cuts at Chi, it does not detectably initiate unwinding on a second DNA molecule. The non-specific (i.e., Chi-independent) ds exonuclease activity is only modestly reduced after cutting at Chi. In λ crosses Chi reduces the activity of another Chi to its left[55,64]. The constraint on RecBCD enzyme to cut at only a single Chi site near each end of a DNA molecule povides an enzymatic basis for assuring only a single recombinational exchange near each end of a linear DNA molecule (see below for further discussion).

Dixon and Kowalczykowski (ref. 13; Kowalczykowski, in this issue) have concluded that RecBCD enzyme is changed in a different way at Chi. They infer that the enzyme degrades DNA up to Chi (from the right end in figure 1) and pauses at Chi, where it makes a final cut. The enzyme, now changed, unwinds DNA past Chi (to the left of Chi in figure 1) without degrading the DNA. This change at Chi may be the basis for the accumulation of multimers by rolling circle plasmids containing a properly oriented Chi, as mentioned above[11]. The differences in the products observed and the conclusions drawn by Ponticelli et al.[35,60] and by Dixon and Kowalczykowski[13] presumably reflect differences in the reaction conditions used by the two groups. Further tests are needed to determine the reaction conditions most nearly reflecting those in the cell.

Regardless of the reaction conditions, a single-stranded (ss) DNA 'tail' extending to the left of Chi and with Chi near near its 3′ end is generated by RecBCD enzyme (fig. 1). This '3′ Chi tail' is a potent substrate for RecA and SSB proteins, which preferentially use homologous 3′ ss DNA ends, rather than 5′ ends, for pairing and strand exchange with supercoiled ds DNA molecules (refs 28, 29; Stasiak and Egelman, in this issue). Joint molecules are formed at high efficiency by RecBCD enzyme and RecA and SSB proteins when homologous supercoiled ds DNA and linear ds DNA molecules which Chi are used (ref. 12; Kowalczykowski, in this issue). Joint molecule formation is dependent upon each of the three proteins and is stimulated by Chi in the linear DNA. As expected from the view that RecA protein, RecBCD enzyme and Chi act 'early', i.e., before joint molecule formation, heteroduplex DNA (hDNA) in λ crosses is not detectable in *recA*, *recB*, or *recC* null mutants, and the level of hDNA is enhanced by a Chi site to the right of the region monitored[23]. Thus, the reactions promoted by purified components appear to accurately reflect recombination reactions in *E. coli* cells.

The preceding genetic and biochemical observations strongly support a previously proposed[48] model of recombination diagramed in figure 2. Steps A, B, and C illustrate the unwinding of ds DNA with the production of ss DNA loops which move and grow as RecBCD enzyme travels along DNA; these steps were established by electron microscopy of partially unwound DNA molecules[59]. At step D RecBCD enzyme generates a 3′ ss DNA tail with Chi near its end, as discussed above. This tail is elongated in steps E and F by continued unwinding by RecBCD enzyme. Step G is the joint molecule formed by RecA and SSB proteins, discussed

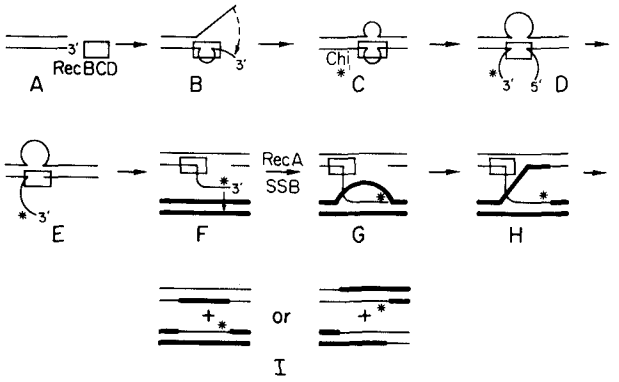

Figure 2. Model for Chi-stimulated recombination by the RecBCD pathway (modified from ref. 48). The parental DNAs are represented by thin and thick lines, RecBCD enzyme by an open box, and Chi by an asterisk. For explanation, see the text.

above (Kowalczykowski, in this issue). Cleavage of the DNA strand displaced from the supercoiled ds molecule ('D-loop') allows it to pair with the gap in the linear molecule to form a Holliday junction (step H). Cleavage of the 'crossed' DNA strands in the Holliday junction produces a pair of 'patch' type recombinants (I, left), while cleavage of the 'uncrossed' strands produces a pair of 'splice' type recombinants (I, right). Resolution of the Holliday junction likely occurs by the action of either the RuvABC proteins or the RecG protein (probably in conjunction with one or more other proteins). RecBCD pathway recombination requires either $ruvA^+B^+C^+$ or $recG^+$ (ref. 31); RuvAB proteins and RecG protein promote branch migration of Holliday junctions, and RuvC cleaves them (refs 15, 24, 32, 63; Müller and West, in this issue). Three additional proteins are needed for the RecBCD pathway[16]: DNA gyrase, presumably to provide one supercoiled substrate for RecA protein, and DNA polymerase I and DNA ligase, presumably to fill gaps and seal nicks remaining after resolution.

Although aspects of this model are strongly supported by the available genetic and biochemical evidence, several points remain to be established. A major question is whether Holliday junctions occur in the RecBCD pathway. The strongest genetic support for a Holliday junction in recombination would come from reciprocality, the production of both reciprocal recombinant types in one recombination event (fig. 2, I left or I right). The inability to examine, with high assurance, all of the substrate and product molecules accompanying recombination in bacteria precludes determining unambiguously whether recombination is reciprocal. Using indirect methods, various investigators have concluded that the RecBCD pathway is either reciprocal[22,27,38,51] or non-reciprocal[54,55]. The high specificity of purified RuvC protein for Holliday junctions[15] and its requirement in the RecBCD pathway, albeit in a *recG* mutant[31], argue that Holliday junctions are intermediates in the RecBCD pathway. Nevertheless, the low activity of RuvC protein on Holliday junctions[15] leaves doubt.

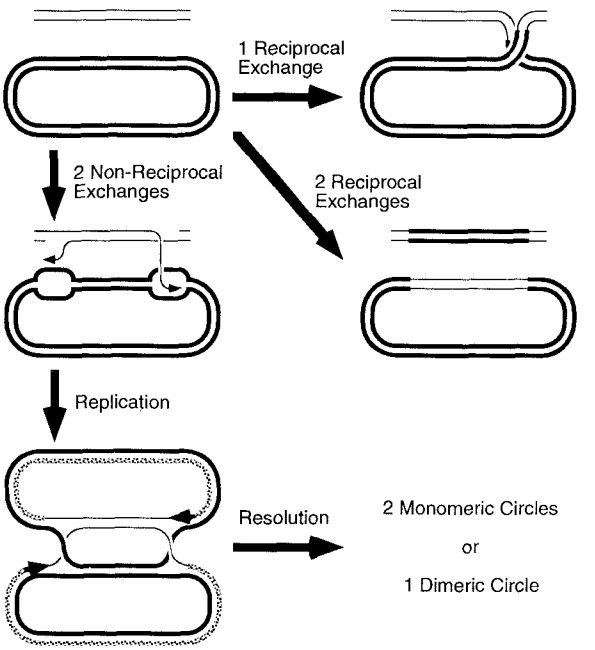

Figure 3. Recombination between a linear chromosomal fragment (thin lines) and a circular complete chromosome (thick lines). One reciprocal exchange, or any odd number of reciprocal exchanges, (*upper right*) produces a linear chromosome with a terminal duplication. Two, or any even number of, reciprocal exchanges (*middle right*) produce a recombinant circular chromosome and a recombinant linear fragment. Two non-reciprocal exchanges (*lower left*), coupled with replication primed by two invading 3' Chi tails (fig. 2, step G) at Chi sites near each end of the linear fragment, produce monomeric circles joined by two Holliday junctions, which can be resolved to produce two separate monomeric circles or one dimeric circle. For further explanations see ref. 45.

Further genetic and biochemical experiments are needed to answer this central question.

The model in figure 2 was developed to account for the RecBCD pathway acting on phage λ, in which complete chromosomes recombine with each other. In this situation any number of exchanges produces complete, viable chromosomes. In *E. coli* conjugational and transductional crosses a linear chromosomal fragment injected into the recipient cell recombines with the recipient's circular chromosome (for a review see 45). In this situation only even numbers of exchanges produce a circular, viable chromosome; odd numbers would produce a broken chromosome with a terminal repeat (fig. 3).

How does *E. coli* assure even numbers of exchanges? In other words, how are the exchanges coordinated to occur in pairs? This problem is compounded by the length of the linear fragment over which the exchanges might occur – 100 kb for P1-mediated transduction and up to a few million base-pairs for conjugation – and the number of Chi sites they are likely to contain – on the average 20 for transduction and several hundred for conjugation. If an exchange occurred at each Chi site, about half of the time odd numbers of exchanges, and hence chromosomal inviability, would result.

The constraint on RecBCD enzyme to cut at a single Chi site[61], as discussed above, provides a simple solution to this problem. A single RecBCD enzyme initiating unwinding at a ds DNA end produces, about 40% of the time, a cut at the first Chi[61] and (presumably) a subsequent single exchange to the 'downstream' side of Chi. With decreasing probability these events will occur not at the first Chi but at other Chi sites farther from the end: about 24% at the second Chi, about 14% at the third Chi, etc. But in each case only a single exchange results. A second RecBCD enzyme will similarly produce a single exchange near the other end of the linear fragment. Because a linear molecule has precisely two ends, there will be precisely two exchanges, the requisite for chromosomal integrity.

If the two exchanges were reciprocal, a linear fragment would be regenerated (fig. 3). This fragment should recombine with the circular recombinant chromosome just as the initial linear fragment did. In the absence of some other event, such as degradation of the linear fragment, this process would continue indefinitely. A solution to this problem is non-reciprocality of the exchanges. In one proposal[45] the two invading 3′ Chi tails prime bidirectional DNA replication to convert the linear fragment into a circle attached to the initial circle by two Holliday junctions (fig. 3). Resolution of these Holliday junctions produces two monomer circles or one dimeric circle that could be converted to monomers by *dif* site-specific recombination[3]. An essentially identical set of reactions could account for ds gap repair by the RecBCD pathway[45]. The primary biological role of Chi-stimulated, RecBCD pathway-dependent recombination may in fact be the repair of damaged chromosomes by recombination with their intact sisters.

The M26 site of *Schizosaccharomyces pombe*

The M26 site is created by the *ade6-M26* mutation, one of the 394 *ade6* mutations isolated and studied by Gutz[19]. In crosses between *ade6* mutations, the *M26* mutation stands out: it recombines with nearly all other *ade6* mutations at a 3- to 15-fold higher frequency than does the nearby *M375* mutation. Analysis of meiotic spore tetrads from crosses with *ade6*$^+$ strain shows that the *M26* mutation undergoes gene conversion about 10 times more frequently than does *M375*; 5% vs. 0.5% of the tetrads contain a convertant. Although *M375* converts with near parity, *M26* shows a marked disparity: *M26* produces about eight times as many tetrads with three *ade6*$^+$ spores and one *ade6-M26* spore ($3^+:1^-$ tetrads) as $1^+:3^-$ tetrads. In other words, *M26* is preferentially a recipient, rather than a donor, of genetic information. *M26* also increases the frequency of conversion of other *ade6* mutations, either to its right or to its left or both in a single meiosis. Conversion of the other mutation accompanies that of *M26*, a phenomenon called co-conversion, and is in the same direction as conversion of *M26*. Co-conversion is nearly 100% for a close marker (*ade6-M216*) but only 50% for a more distant one (*ade6-52*). These properties led Gutz[19] to propose that the *ade6-M26* mutation created a site, called M26, that stimulates recombination near itself and, less frequently, at a distance. Further analysis supports this proposal.

A more complete analysis of co-conversion tracts and crossing-over at *ade6* was recently accomplished with strains bearing nine markers within or flanking *ade6*[18]. Crosses were made between *ade6-M26* (or the nearby *ade6-706* mutation as a non-hotspot control) and *ade6-469* parents that differed at the seven additional markers, and Ade$^+$ recombinants were selected. Those with the flanking markers of the *ade6-M26* (or *ade6-706*) parent were classified as covertants at the *ade6-M26* (or *ade6-706*) site, whereas Ade$^+$ recombinants with a single or triple exchange were classified as convertants with an accompanying cross-over. With this classification M26 stimulates both conversion and crossing-over about 13-fold. About 65% of the convertants, for either *ade6-M26* or *ade6-706*, have an associated cross-over. In nearly all cases the convertants inherit the selected *ade*$^+$ marker allelic to *ade6-M26* (or *ade6-706*) and additional markers as an uninterrupted block, taken to be a conversion tract. One end of each tract was selected to fall between *ade6-M26* (or *ade6-706*) and *ade6-469*, to produce an Ade$^+$ recombinant. The other ends of the tracts fall at variable points between the additional markers. The frequency at which a marker is included in a conversion tract falls exponentially, a factor of about 4 per kb from either *ade6-M26* or *ade6-706*; mean tract lengths from *ade6-M26* (or *ade6-706*) and the variable (unselected) end points are 510 or 720 bp, respectively. From these observations Grimm et al.[18] concluded that M26 stimulates the initiation, rather than the resolution, of conversion tracts and that these initiations occur at the same site(s) in the presence or absence of M26. In other words, M26 appears to stimulate the 'background' recombination rather than creating a new initiation site.

Genetic evidence indicates that hybrid DNA is frequently formed very near the M26 site. In *S. pombe*, as in other organisms, C-C mismatches are corrected at a lower frequency than are other mismatches (ref. 39; Radicella, Fox and Yamamoto, in this issue); this is deduced from the frequent post-meiotic segregation (PMS) of markers that can produce C-C mismatches by hybrid DNA formation during recombination. G→C transversion mutations, which when crossed with wild-type can produce C-C mismatches, show frequent PMS even when located 2 bp or 6 bp to the 5′ or 3′ side of M26, respectively (P. Schär and J. Kohli, personal communication). (5′ and 3′ are with respect to the direction of transcription of *ade6*). In fact, the fre-

quency of PMS among all aberrant segregations in tetrads is the same (27%) for these transversions and for G→C transversions analyzed at three other sites in the *S. pombe* genome. These observations suggest that hybrid DNA is formed frequently, perhaps in all M26-stimulated events, at the M26 site itself and argue against the formation of ds DNA gaps at M26.

Further analysis of the data indicates that the transcribed strand of *ade6* is preferentially, and perhaps always, transferred to the M26 containing chromatid (P. Schär and J. Kohli, pers. commun.). This bias in strand transfer contrasts with the preferential transfer of the non-transcribed strand at the *ARG4* and *HIS4* loci of *S. cerevisiae* (Nicolas and Petes, in this issue). The basis of the biases and their differences in the two organisms is unknown.

The M26 site is active in meiosis but not in mitosis[36,40]. It stimulates recombination between two other *ade6* mutations even when the *M26* mutation is homozygous[36]. This result shows that the M26 hotspot does not simply form a base mismatch that is corrected at high frequency, although it does not rule out M26 stimulating the correction of neighboring mismatches.

Nucleotide sequence analysis of the wild-type and mutant *ade6* genes showed that the *M26* mutation is a single bp change, G:C→T:A, changing a 5′ G-G-A 3′ codon to a 5′ T-G-A 3′ translational stop[36,58]. The *M375* mutation is located just 3 bp away from *M26* and also changes 5′ G-G-A 3′ to 5′ T-G-A 3′[58]. Thus, *M375* has served as an excellent non-hotspot control for studies of M26.

Site-directed mutagenesis showed that the hotspot activity of M26 requires the heptanucleotide sequence 5′ A-T-G-A-C-G-T 3′[41]. The underlined T is G in *ade6*$^+$. When this nucleotide is A, C, or G, there is no detectable hotspot activity; i.e., the frequency of recombinants is the same as that produced by *M375*. Similar changes to all three possible nucleotides at each of the six other positions in the M26 heptamer essentially abolish activity, whereas changes outside the heptamer have little effect. These results establish M26 as a unique nucleotide sequence, presumably a binding site for a protein.

A protein in *S. pombe* extracts that binds the M26 site with high specificity has been detected using a 'gel mobility shift' assay (W. P. Wahls and G. R. Smith, manuscript submitted). This protein binds a DNA fragment with the M26 heptamer but does not detectably bind otherwise identical fragments with single bp changes in the heptamer; changes outside the heptamer have little effect on binding. The strong correlation between the binding of this protein to mutant DNAs and the genetic hotspot activities of these sites indicates that this protein is essential for M26 hotspot activity.

Biochemical studies have elucidated some properties of this protein (W. P. Wahls and G. R. Smith, manuscript submitted). Although M26 has hotspot activity only during meiosis[36,40], the M26 binding protein is present in both mitotic and meiotic cells; both the amount and behavior of the protein appear to be the same in the two types of cells. Presumably some additional factor present only in meiotic cells allows the binding protein to activate the M26 site. The protein binds directly to the M26 site: mutations within the heptamer or methylation of any of the three G residues on the two strands of the site block binding, whereas mutations or methylations outside the heptamer have no detectable effect. The protein has been purified about 40,000-fold to near homogeneity and contains two polypeptides with masses of about 70 and 28 kDa. These polypeptides, called Mts1 and Mts2, are present in solution as a heterodimer. There are about 200 copies of the protein per cell. After isolation from an SDS-polyacrylamide gel, Mts1 and Mts2 can be renatured and reassociated to reconstitute strong binding activity. Individually Mts1 and Mts2 have weak, but nevertheless M26-specific, binding activity, presumably as homodimers. These properties are reminiscent of heterodimeric transcriptional activators[2].

Although the M26 site appears to be a relatively simple protein binding site, it appears to act only in conjunction with another chromosomal site. This was shown by moving to the distant *ura4* locus a 3 kb DNA fragment bearing the *ade6-M26* or *ade6-M375* mutation 1.0 kb from one end of the fragment and crossing these alleles with the *ade6-469* allele similarly transplaced; the endogenous *ade6* locus was deleted, so that *ade*$^+$ recombinants arose from only recombination between the homologous transplaced *ade6* genes[37]. In these crosses the frequency of recombinants is not greater with *ade6-M26* than with *ade6-M375*, although it is about 16-fold higher when the alleles are at the endogenous *ade6* locus. In similar experiments with the *ade6* alleles transplaced to three other loci the M26 hotspot is also inactive (J. Virgin, pers. commun.). These results imply that the M26 hotspot is active only when it is near, or correctly oriented with respect to, another chromosomal element found at *ade6* but not at the other loci tested. This second element might be either a discrete site, as M26 is, or a more diffuse region of the genome that provides the proper chromatin structure for M26 to be active. Further analysis may reveal whether this second element contains a discrete site or a diffuse region and how it activates M26.

Other hotspots of homologous recombination: biological significance

The available evidence indicates that Chi, and probably M26, act at the initiation of strand exchange, but other hotspots act as several other stages of recombination. Some of these hotspots are briefly summarized here (see

43 for additional references and a more extensive review). In *Haemophilus influenzae* an 11 bp sequence, found frequently in *H. influenzae* DNA but rarely in other DNA, specifies high-frequency uptake of DNA into the cell during transformation, in which the linear exogenous DNA recombines with the chromosome. The packaging of host DNA by certain phages, such as P22 of *Salmonella typhimurium*, occurs preferentially at certain sites, producing higher frequencies of transduction for some markers than for others. The pairing of homologous chromosomes may be initiated at special sites inferred in *Drosophila melanogaster* and *Caenorhabditis elegans*[4,20]. Some recombination-promoting enzymes can enter ds DNA only at special sites, as exemplified by RecBCD enzyme entering the lambda *cos* site (see above). Enzymes of the lambda Red and *E. coli* RecBC(D-) (also called ‡), RecE, and RecF pathways also appear to enter this site, for lambda's *cos* is a hotspot in each of these pathways (ref. 62 and references therein). The M26 site (see above), the *cog* site of *Neurospora crassa*, and the YS17 site in the *buff* locus of *Sordaria brevicollis* have genetic properties similar to those of Chi and may, like Chi, stimulate the initiation of strand exchange. One can imagine hotspots acting at the resolution of strand exchange, though to my knowledge there are no evident cases.

Recombination can be influenced by other localized chromosomal processes. For example, hotspots of recombination occur at the origins of replication of phages T4 (ref. 65) and ϕX174, probably because nicked or ss DNA intermediates of replication are recombinogenic. Similarly, the mitotic hotspot *HOT1* of *Saccharomyces cerevisiae* requires transcription promoted by the rRNA promoter-enhancer complex; presumably, the ss DNA formed during transcription is recombinogenic (ref. 57; Gangloff, Lieber and Rothstein, in this issue).

These examples demonstrate that recombination is not isolated from other chromosomal processes. The coupling of recombination to another process may provide a clue to answering the puzzling question of why there are hotspots of recombination. In some cases the coupling may be 'unintentional'. For example, RecBCD enzyme's primary role may be the repair of ds breaks in the *E. coli* chromosome and, to limit its action, it enters only ds DNA ends. As a consequence it enters and potentially recombines any DNA with ds ends, such as chromosomal fragments injected during conjugation or transduction and lambda DNA linearized at *cos* in preparation for packaging. Alternatively, recombination may be 'unintentionally' coupled to another process. For example, recombination stimulated by the T4, ϕX174, and *HOT1* hotspots may be a by-product of replication and transcription. The variety of recombination mechanisms found within and among organisms may reflect the cells' ability to use to their advantage the intermediates and products of one metabolic process, such as replication and transcription, for another, such as recombination. In this view hotspots of recombination may be, initially or primarily, hotspots of another process.

Acknowledgments. I am grateful to S. Amundsen, J. Virgin, and W. Wahls for helpful comments on the manuscript, to W. Wahls for preparing the figures, to A. Taylor and J. Virgin for unpublished information, and to C. Grimm, J. Bähler, J. Kohli, and P. Schär for their manuscripts before publication. Research on recombinational hotspots in my laboratory is supported by a grant (GM31693) from the National Institutes of Health.

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