

# HOMOLOGOUS RECOMBINATION NEAR AND FAR FROM DNA BREAKS: Alternative Roles and Contrasting Views

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■ **Abstract** Double-strand breaks and other lesions in DNA can stimulate homologous genetic recombination in two quite different ways: by promoting recombination near the break (roughly within a kb) or far from the break. Recent emphasis on the repair aspect of recombination has focused attention on DNA interactions and recombination near breaks. Here I review evidence for recombination far from DNA breaks in bacteria and fungi and discuss mechanisms by which this can occur. These mechanisms include entry of a traveling entity (“recombination machine”) at a break, formation of long heteroduplex DNA, priming of DNA replication by a broken end, and induction of recombination potential in *trans*. Special emphasis is placed on contrasting views of how the RecBCD enzyme of *Escherichia coli* promotes recombination far (tens of kb) from a double-strand break. The occurrence of recombination far from DNA breaks and of correlated recombination events far apart suggests that “action at a distance” during recombination is a widespread feature among diverse organisms.

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## INTRODUCTION

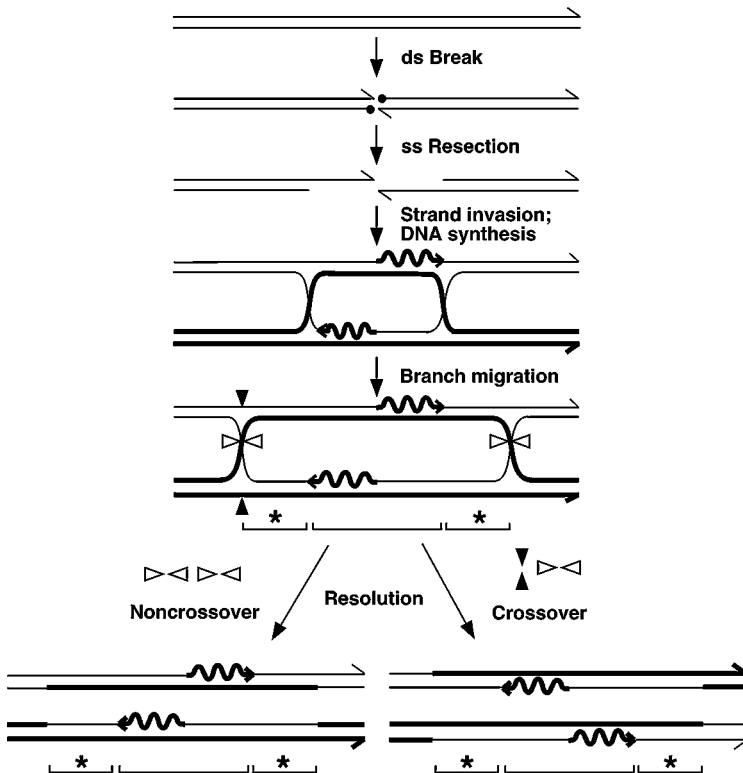
It has long been recognized that homologous genetic recombination has at least two roles—reassortment of alleles and repair of broken DNA (e.g., see 16, 54, 82). A currently popular view holds that the repair of double-strand (ds) breaks in DNA is the primary role of homologous recombination and that the generation of diversity is a byproduct of repair (e.g., see 25). This emphasis on repair of ds breaks has focused attention on recombination occurring near the ends of broken DNA, typically within  $\sim 1$  kb. Although recombination does frequently occur within such a limited region, recombination can also occur far ( $> 30$  kb) from DNA ends or the presumptive initiating lesion.

This review discusses two examples of recombination initiated by ds breaks and extensively studied at the molecular level. In the first example, meiotic recombination in the budding yeast *Saccharomyces cerevisiae*, the ends of the broken DNA are processed and invade an intact homologous duplex: resolution of the joint molecule and therefore the recombinational exchanges often occur within  $\sim 1$  kb of the initial ds break. In the second example, recombination by the RecBCD pathway of the bacterium *Escherichia coli*, the ends of the broken DNA serve as entry sites for an enzyme that can travel tens of kb before initiating joint molecule formation; recombination therefore typically occurs far from the initial ds break.

There are additional ways in which recombination can occur far from a ds break or another initiating lesion. (a) The hybrid DNA of a joint molecule formed near a break can be extended by branch migration; resolution of the joint molecule and repair of base mismatches within it can produce recombinational exchanges separated from the initial break by tens of kb. (b) The end of a broken DNA molecule can invade an intact duplex and prime DNA synthesis, which may proceed to the end of the chromosome. In this case the recombinational exchange point is at the site of the initial ds break, but all markers in the replicated region, potentially tens or hundreds of kb, undergo gene conversion, or nonreciprocal recombination. (c) A ds break can activate or induce the synthesis of recombination-promoting enzymes, potentially acting throughout the genome. Distant recombination events are expected to be statistically correlated in such "hot cells." After discussing the two well-studied cases of recombination near and far from ds breaks, I review additional data that appear to reflect each of these mechanisms.

## RECOMBINATION NEAR DOUBLE-STRAND BREAKS IN *S. CEREVISIAE* MEIOSIS

Meiotic recombination in *S. cerevisiae*, extensively studied at the genetic and molecular levels, provides a clear example of recombination occurring near ds breaks, although there appear to be exceptions noted later. Most meiotic recombination in *S. cerevisiae* can be accounted for by ds breaks that occur at hotspots of gene conversion (reviewed in 65). In brief, recombination at these hotspots occurs as follows (Figure 1) [for more extensive discussion and references, see (79, 83)].



**Figure 1** Double-strand break repair model of recombination, after Szostak et al. (112). For explanation, see the text. Alternative resolutions at the last step are not shown. Arrowheads indicate 3' ends; solid dots, Spo11 protein linked to 5' ends at the DNA break (56); wavy arrows, newly synthesized DNA; thin lines, DNA from one parent; thick lines, DNA from the other parent; open and closed triangles, points of resolution by strand cutting, swapping, and ligation. Brackets with and without an asterisk indicate regions of symmetric and asymmetric hybrid DNA, respectively, which can give rise to aberrant 4:4, 5:3, or 6:2 segregations.

After the induction of meiosis the chromatin structure surrounding a hotspot (e.g., that at *ARG4* or *HIS4*) is altered, as assayed by the accessibility of micrococcal nuclease to the DNA in isolated chromatin. This alteration appears to occur during premeiotic replication and may allow access to the DNA by recombination-promoting proteins induced during meiosis (14). One of these proteins, Spo11, in conjunction with several others, makes a ds break at one of several potential sites spread over a region of a few hundred bp defining the hotspot. Spo11 remains covalently bound to the 5' end of the DNA on each side of the break (56). Resection of the 5' end and the bound protein requires Mre11 and Rad50, although they may not be the catalysts. Typically, ~600 to 800 nucleotides are resected in wild-type cells, but resection continues farther (~2 kb) in mutants lacking Rad51 or Dmc1, proteins acting at the next stage (13, 109).

The Rad51 and Dmc1 proteins, like the *E. coli* RecA protein, bind to single-stranded (ss) DNA and, in the presence of a ss DNA binding protein (SSB), promote strand exchange between the protein·ss DNA filament and homologous duplex DNA (110). The purified proteins promote formation of D-loops (displacement loops), in which the ss DNA of the filament replaces its identical (or nearly identical) strand in the duplex. Strand exchange may continue beyond the region of resection and allow formation of symmetric hybrid DNA and the cross-stranded structure called a Holliday junction (Figure 1). DNA forms consistent with D-loops and Holliday junctions have been isolated from meiotic cells (55a). In addition, there are molecules with double Holliday junctions, formed by the invasion of both ends at the initial ds break into the same duplex (10, 92). The distance between these two Holliday junctions is determined by a combination of the length of resection and the length of symmetric strand exchange, also called branch migration. This distance is reported to range from ~0.1 to 1 kb (10).

Double Holliday junction molecules are presumably resolved into unbranched recombinant molecules, but the mechanism of this resolution is unclear. There may be multiple mechanisms, but two are widely considered. (A third is proposed later in this review; see Figure 6.) In the first mechanism, two strands of identical polarity in each Holliday junction are cut, and the ss ends are exchanged and ligated (100, 112). If the two junctions are resolved in the same "plane" (horizontal or vertical as diagrammed in Figure 1), DNA flanking the junctions remains in parental (non-crossover) configuration, whereas if the two junctions are resolved in opposite planes (one horizontal and one vertical), the flanking DNA is recombined, producing a crossover. In the second mechanism, the double Holliday junction is resolved without further covalent exchanges between the DNA molecules (51, 77). Conceptually, the two entwined duplexes are simply pulled apart, made possible by the unlinking action of a topoisomerase. Flanking DNA remains in parental configuration (non-crossover). Genetic markers flanking the Holliday junctions therefore undergo reciprocal recombination (crossover) or not, depending upon the mode of resolution of the junctions.

Genetic markers within the hybrid DNA region can undergo more complex recombination reactions, depending upon the fate of base mismatches within the

hybrid DNA (hDNA) spanning the two Holliday junctions. Each allelic difference between the two parents in this interval will produce a mismatch either on one duplex—in the region of ss resection and asymmetric strand exchange, or on both duplexes—in the region of symmetric strand exchange (Figure 1). In the absence of mismatch correction each duplex with a mismatch will segregate two genetic types, designated + and –, after one postmeiotic replication. Counting the eight duplexes present at this stage, an allelic difference in the asymmetric region will segregate  $5^+:3^-$  or  $3^+:5^-$ . A difference in the symmetric region will generate two postmeiotic segregations (PMS), designated an aberrant  $4^+:4^-$  (Ab  $4^+:4^-$ ) segregation. (The absence of any recombination event produces normal  $4^+:4^-$  segregation, i.e., without PMS.)

Gene conversion arises from correction of mismatches within the hDNA. Depending upon the direction of this correction, an incipient  $5^+:3^-$  (or  $3^+:5^-$ ) can be converted to a  $6^+:2^-$  (or  $2^+:6^-$ ) or restored to a normal  $4^+:4^-$ . Similarly, an incipient Ab  $4^+:4^-$  with its two mismatches will produce a  $5^+:3^-$  or  $3^+:5^-$  if there is one correction, or a  $6^+:2^-$ ,  $2^+:6^-$ , or normal  $4^+:4^-$  if there are two corrections.

By definition, markers near a hotspot convert at higher than average frequency. Double-strand DNA breaks occur near or coincident with the hotspot in several well-studied cases (65, 79). The finding of double Holliday junctions encompassing the hotspot and the frequent association of crossovers with conversion at these hotspots strongly support the ds break repair model of Szostak et al. (112), an elaboration of an earlier ds break repair model of Resnick (82). Szostak et al. (112) postulated that both strands on each side of the ds break are degraded and that resynthesis of the lost strands using an intact homolog as a template is a major source of gene conversion. At the hotspots investigated, the 3' ends on each side of the gap appear to be separated by 0 to 2 bp (27, 66, 129, 130), indicating that conversion rarely, if ever, arises from ds gapping. Furthermore, the loss of mismatch correction enzymes increases the frequency of PMS at the expense of 6:2 segregations (128). These results strongly support the model of Holliday (53) in which gene conversion results from correction of mismatches in hDNA.

Within the context of the model of Szostak et al. (112) (Figure 1) the length of hDNA extending from the initial ds break determines the distance over which markers convert at high frequency (the spread or gradient of the hotspot) and the distance over which markers are corrected in the same event (co-convert). Many genetic studies in *S. cerevisiae* confirm this prediction: meiotic gene conversion generally occurs within  $\sim 1$  kb of the initiating ds break (e.g., 28, 90). Also as predicted, crossovers are statistically associated with conversion at hotspots, but the positions of the crossovers have not, to my knowledge, been precisely determined. The paucity of Ab  $4^+:4^-$  tetrads in *S. cerevisiae* suggests, within the context of the model in Figure 1, that Holliday junctions do not frequently migrate from their point of formation at the end of the ss resection. The observed distance between double Holliday junctions, whose resolution presumably gives rise to the associated crossovers, also suggests that crossovers generally occur within  $\sim 1$  kb of the initiating ds break. Thus, the genetic and physical evidence is consistent with

*S. cerevisiae* meiotic recombination frequently occurring near a ds DNA break, but some exceptions are discussed later.

## RECOMBINATION FAR FROM DOUBLE-STRAND BREAKS BY THE RecBCD PATHWAY OF *E. COLI*

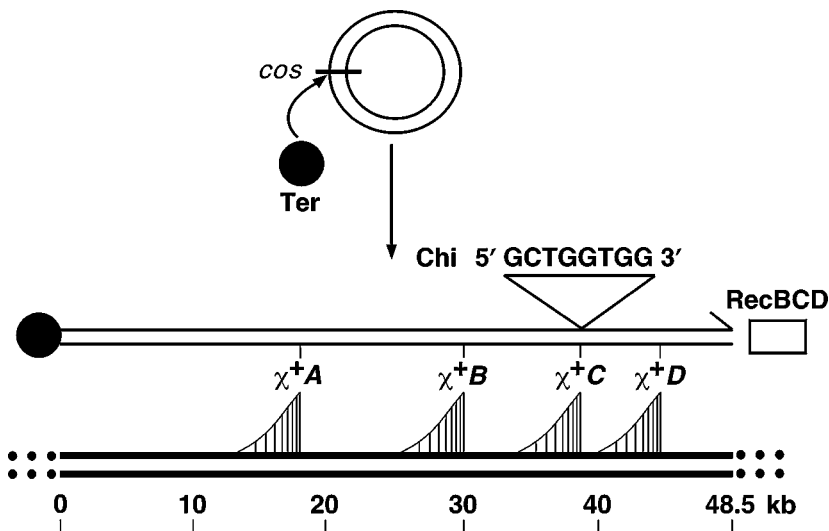
Like meiotic recombination in *S. cerevisiae*, recombination by the major pathway in *E. coli* is also initiated by ds breaks and occurs at high frequency at special sites (hotspots). But in sharp contrast, although the activity of these hotspots is dependent on the ds breaks, the hotspots are separable from the ds breaks: recombination at a hotspot can occur >30 kb from the activating ds break. This pathway (RecBCD) with its hotspot Chi provides an especially well-studied example of recombination far from a ds break.

### Genetic and Biochemical Observations

Studies primarily by F. W. Stahl and his colleagues established the following picture of the RecBCD pathway recombining phage  $\lambda$  in *E. coli* cells (Figure 2) (reviewed in 76, 95, 98, 121). These studies used  $\lambda$  Red<sup>-</sup> Gam<sup>-</sup> mutant phage to block recombination by the  $\lambda$ -encoded Red pathway and to avoid inhibition, by the  $\lambda$ -encoded Gam protein, of the *E. coli* RecBCD enzyme, an essential component of the RecBCD pathway. After infection, such phages replicate their DNA in the circular ( $\theta$ ) mode. Conversion of the circles into linear forms for packaging into mature phage particles occurs by a ds cut at a special site *cos* by the  $\lambda$ -encoded Ter protein complex, which remains bound to the genetically defined left end of the DNA. The unbound right end is available to RecBCD enzyme, which binds tightly to ds ends and subsequently travels unidirectionally along the DNA until it meets the asymmetric hotspot sequence Chi (5' GCTGGTGG 3') from the right (as written here; present in the "top" strand of  $\lambda$  as conventionally written). Assisted by other proteins including RecA and SSB proteins, RecBCD enzyme then promotes recombination at Chi and, with decreasing probability, to its left.

The remoteness of recombination from the ds break at *cos* stems from two sources: the distance from *cos* over which RecBCD enzyme travels before encountering an active Chi site and the distance from Chi over which the recombination events occur. RecBCD enzyme can travel tens of kb before encountering Chi. The activity of a Chi site ( $\chi^+A$ ) 30 to 35 kb from the activating ds break at *cos* is nearly equivalent to that of a Chi site closer to *cos* ( $\chi^+D$  at 3.5 kb or  $\chi^+C$  at 10.0 kb) (105). Recombination stimulated by Chi is detectable as far as 5 to 10 kb to the left of Chi (e.g., 22, 101). Thus, recombination can occur at least 35 kb from the ds break that provoked the event.

Recombination of the *E. coli* chromosome, which occurs by the RecBCD pathway in wild-type cells, may occur even farther from a ds break. In Hfr conjugation there can be up to ~100 kb of Chi-free F-factor DNA at the leading end of the



**Figure 2** Overview of Chi-stimulated RecBCD pathway recombination far from a DNA break at the *cos* site in phage  $\lambda$ . The  $\lambda$  Ter protein complex (filled circle) cuts circular  $\lambda$  DNA at *cos* and remains bound to the left end, allowing entry of RecBCD enzyme (open box) at the right end of the 48.5 kb linear  $\lambda$  DNA (thin lines). Upon encountering the Chi sequence 5' GCTGGTGG 3' at any one of the sites  $\chi^+A$ ,  $\chi^+B$ ,  $\chi^+C$ , or  $\chi^+D$ , the traveling RecBCD enzyme, with other proteins, promotes recombination to the left of Chi with decreasing probability (shaded graphs). The lower parental DNA (thick lines) may be either linear or circular, as indicated by the dotted lines at its ends.

transferred DNA. Presumably, RecBCD enzyme traverses this distance from the origin of transfer (*oriT*) before encountering Chi-containing DNA homologous to the recipient chromosome (96).

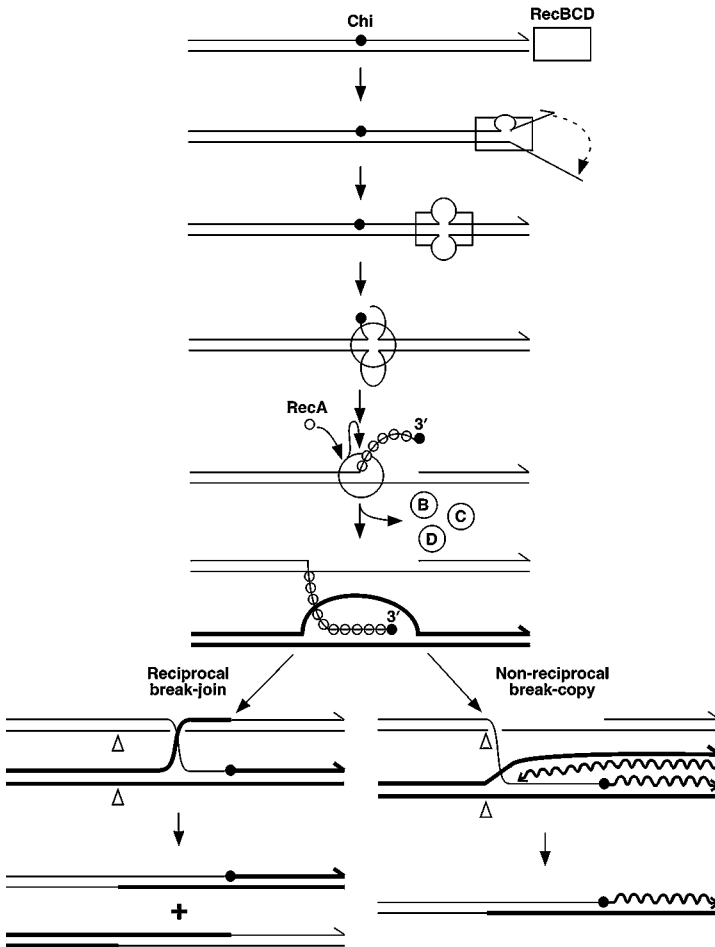
The evidence for the preceding picture rests on both genetic studies of recombination in  $\lambda$ -infected *E. coli* cells and biochemical studies of purified RecBCD enzyme, RecA and SSB proteins, and DNA. The salient genetic observations are that RecBCD pathway recombination is stimulated at and to the left of Chi (e.g., 22, 101, 105), the activity of Chi depends upon its orientation with respect to *cos* (37, 57), and a *cos* site activates Chi only if it can be cut (58, 59). These observations indicate that some entity travels from a ds break at *cos* to Chi before stimulating recombination. Since Chi is specific to the RecBCD pathway (42, 104) and its only known unique component is RecBCD enzyme (24), this enzyme was implicated as the traveling entity. Certain mutations in the *recB*, *recC*, and *recD* genes, encoding the three subunits of RecBCD enzyme, reduce or abolish Chi activity without abolishing recombination (18, 67, 91). These observations suggested a direct interaction between RecBCD enzyme and Chi.

The salient biochemical observations are the following. RecBCD enzyme binds tightly and specifically to ds DNA ends [ $K_D \approx 0.1 \text{ nM}$  or  $\sim 1/10$  the concentration of one DNA end per cell (115, 117)]. Starting from a ds end, it unwinds DNA rapidly (300–500 bp/sec) and for long distances ( $>20\text{--}30 \text{ kb}$ ) (85, 114). Upon encountering Chi in the proper orientation (that predicted by the genetic studies), it generates, by a mechanism discussed below, ss DNA extending to the left of Chi and with a 3' OH end at or near Chi (31, 80, 113). Mutations in Chi or in *recBCD* coordinately reduce Chi activity in cells and generation of this end by purified components (1, 8, 20, 21, 33, 80). After encountering Chi, RecBCD enzyme continues to travel along the DNA, elongating the ss DNA with Chi at its 3' end (31, 80). RecBCD enzyme loads RecA protein onto this ss DNA, which forms a joint molecule with homologous supercoiled DNA (7, 84). Although it is not yet clear how the RecBCD-, RecA-promoted joint molecule is converted to a recombinant molecule, the genetic and biochemical observations are consistent with the joint molecule giving rise to the observed recombinants to the left of Chi, far from the ds break at *cos*. [The DNA strand polarity of ss “patches” in  $\lambda$  recombinants measured genetically has been reported to be opposite to that predicted by cutting of the “top” strand of  $\lambda$  discussed below (50, 86, 87), but the polarity of ss “splices” and of hDNA measured physically has been reported to be that predicted (52, 93). The “patches” may reflect double splices (93), or the determination of their polarity may have been influenced by mismatch correction.] The mechanism by which Chi regulates the formation of joint molecules is discussed next.

## Multiple Effects of the Chi-RecBCD Enzyme Interaction

Biochemical studies have shown that Chi regulates at least three activities of RecBCD enzyme: (a) the generation of 3'-ended ss DNA extending to the left of Chi, (b) the loading of RecA protein onto this ss DNA, and (c) the ability to act at a subsequently encountered Chi site. After describing the biochemical observations, I discuss related genetic observations bearing on the question of Chi's role in *E. coli* recombination.

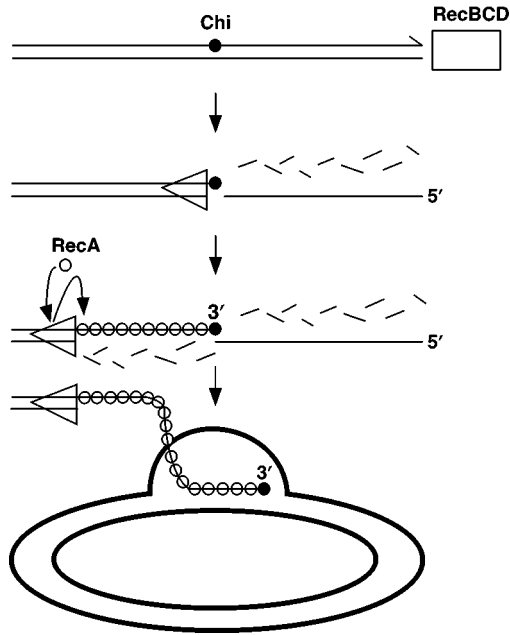
The mechanism by which RecBCD enzyme makes a DNA end at or near Chi depends upon the reaction conditions, most notably the ratio of the ATP and  $\text{Mg}^{++}$  concentrations. With  $(\text{ATP}) > (\text{Mg}^{++})$  RecBCD enzyme unwinds the DNA from the ds end to Chi, nicks the “top” strand a few nucleotides to the 3' (“upstream”) side of Chi, and continues unwinding DNA to the end (Figure 3, top five panels) (80, 113, 116). The products of this reaction, two ss DNA fragments and one full-length ss DNA, are observed with or without RecA and SSB proteins. In contrast, with  $(\text{Mg}^{++}) > (\text{ATP})$  RecBCD enzyme degrades the upper strand up to Chi, at which degradation of this strand is attenuated, the “bottom” strand is cut, and degradation of the bottom strand is augmented; continued travel and degradation produces two ss DNA fragments, the top strand to the left of Chi and the bottom strand to the right of Chi (Figure 4, top three panels) (6, 31, 118). These products are seen only in the presence of RecA and SSB proteins; in their absence all of



**Figure 3** “Nick at Chi” model of recombination, after Smith et al. (97, 99) as modified by Smith (96). For explanation, see the text. The open box indicates RecBCD enzyme; solid dot, Chi; large open circle, RecBCD enzyme after its change at Chi; small open circles, RecA protein; circled B, C, and D, the disassembled subunits of RecBCD enzyme after its dissociation from DNA. Other symbols are as in Figure 1. Alternative resolutions at the bottom are not shown.

the DNA is degraded to oligonucleotides. Both reaction conditions produce the ss DNA extending to the left of Chi (the 3' “Chi tail”) that forms a joint molecule, a likely precursor to recombinants. Thus, both reactions are compatible with the genetic observations cited above.

After encountering Chi, RecBCD enzyme loads RecA protein specifically onto the 3' Chi tail. It was first noted that RecBCD enzyme efficiently forms joint



**Figure 4** Reaction of RecBCD enzyme and formation of joint molecules under conditions of  $(Mg^{++}) > (ATP)$ , after Anderson & Kowalczykowski (7). Broken lines indicate DNA strands degraded by RecBCD enzyme. The open triangle indicates RecBCD enzyme after its change at Chi. Other symbols are as in Figures 1 and 3.

molecules between homologous supercoiled DNA and linear, Chi-containing DNA only if RecA and SSB proteins are present during RecBCD enzyme's unwinding of the linear DNA; addition of RecA protein after the initial unwinding reaction is less effective (7). A direct assay for loading of RecA protein onto ss DNA by RecBCD enzyme—protection of the ss DNA from digestion by exonuclease I—showed that efficient loading requires Chi, occurs exclusively on the 3' Chi tail, and requires the presence of RecA protein during the unwinding phase of the reaction. RecBCD enzyme may load each of the RecA monomers to form a continuous RecA · ssDNA filament, or it may load only a minority, which then nucleate the spontaneous  $5' \rightarrow 3'$  polymerization of RecA protein (81). Mutations in *recBCD* coordinately block loading of RecA protein and recombination (3, 5). Thus, self-assembly of RecA protein onto ss DNA, which can occur with purified components (e.g., 81), is not adequate for RecBCD pathway recombination in *E. coli* cells.

The nature of the Chi-dependent change in RecBCD enzyme that permits it to load RecA protein is unclear. The *recB1080* (D1080A) mutation, which abolishes the nuclease activities (132), blocks RecA loading (3, 5), suggesting that some aspect of the Chi-nuclease interaction must precede RecA loading. In accord with a model hypothesizing the release of RecD at Chi, discussed below, RecBC enzyme

loads RecA onto the DNA strand with a 3' end at the entry point, independent of Chi [i.e., "constitutively" (23)], and genetic elimination of RecD from the RecB (D1080A) mutant enzyme renders it proficient for RecA loading and recombination (3). Other evidence discussed below does not, however, support the release of RecD at Chi. Perhaps a conformational change in RecB or RecD at Chi exposes a surface on RecBCD enzyme that allows RecA loading (3, 23, 131).

After encountering Chi, RecBCD enzyme not only gains RecA loading activity, it also gains or loses other activities, either on the same DNA molecule (in *cis*) or on a separate DNA molecule (in *trans*). As noted previously, in reactions with  $(Mg^{++}) > (ATP)$  top strand degradation after Chi is attenuated and bottom strand degradation is augmented (6). This alteration of RecBCD enzyme's nuclease activity in *cis* is not apparent in *trans*, presumably because upon leaving the DNA substrate the enzyme quickly reverts to its former state under this reaction condition [ $(Mg^{++}) > (ATP)$ ] (30). With  $(ATP) > (Mg^{++})$  Chi-dependent changes both in *cis* and in *trans* are apparent: after nicking a DNA substrate at a Chi site, RecBCD enzyme does not detectably nick at a subsequently encountered Chi site in *cis*, although it does unwind the DNA beyond the first Chi and cleaves a distal hairpin end (116). Subsequently added DNA is neither unwound nor hydrolyzed in *trans* (116, 119).

The Chi-dependent inactivation of RecBCD enzyme in *trans* persists for at least 2 h and results from disassembly of the three enzyme subunits (119). Examination of the Chi-inactivated enzyme by centrifugation or native gel electrophoresis revealed that all three subunits were separate (119). The disassembly of the enzyme appears not to occur at Chi, however: unwinding and hairpin cleavage activities are maintained after Chi, but the isolated subunits have low or undetectable unwinding activity, and subunit combinations other than the holoenzyme have low or undetectable nuclease activity (19, 71). Experiments using polystyrene beads attached to RecBCD enzyme and monitored by light microscopy during unwinding also failed to detect release at Chi of the one subunit tested (RecD) (32). The Chi-dependent disassembly may occur upon dissociation of the enzyme from the DNA substrate, perhaps at the end of the substrates used.

In summary, Chi induces complex changes in RecBCD enzyme, some of which occur at Chi and some subsequently. The nature of these changes depends upon the reaction conditions. These alternative results and other observations have given rise to contrasting views of how Chi and RecBCD enzyme promote recombination in *E. coli* cells. In the following section I evaluate the genetic and biochemical support for these views.

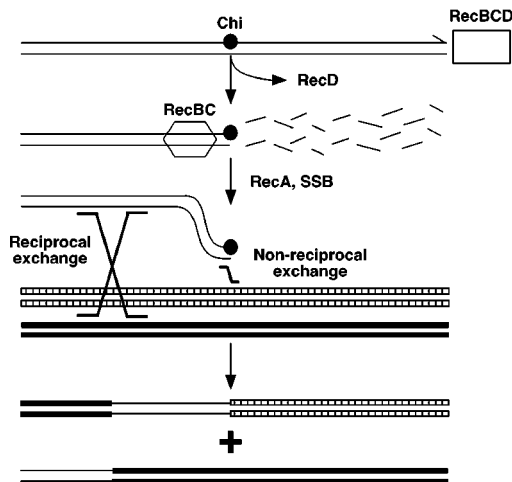
## Contrasting Views of the Role of Chi in *E. coli* Recombination

Two views of how RecBCD enzyme and Chi promote recombination can be called the "nick at Chi" model and the "stop degradation at Chi" model.

In the "nick at Chi" model, RecBCD enzyme enters a ds DNA end, unwinds the DNA up to Chi, nicks one strand at Chi, and continues unwinding, thereby

producing a recombinogenic 3' ss DNA Chi tail (Figure 3) (97, 99). This model parallels the action of RecBCD enzyme under conditions with  $(ATP) > (Mg^{++})$ . Based on the findings of Anderson & Kowalczykowski (7), an important addition to this model is the loading of RecA protein specifically onto the Chi tail. Consequently, the Chi tail, unlike the 3' end at which RecBCD enzyme entered the DNA, is recombinogenic; recombination is more frequent at Chi than at *cos* (e.g., 101). Another addition is the ability of RecBCD enzyme to cut at only one Chi site and its disassembly upon leaving the DNA (116, 119). Consequently, one RecBCD enzyme promotes one recombinational exchange on each end of linear DNA, the minimum required to effect recombination or ds break repair of *E. coli*'s circular chromosome (96).

In the "stop degradation at Chi" model, RecBCD enzyme is a destructive nuclease until it reaches Chi, at which it is changed into a "recombinase" (Figure 5) (61, 76, 107, 120). This change is hypothesized to be the release of the RecD subunit, and the Chi-altered enzyme is hypothesized to be equivalent to RecBC enzyme, i.e., the holoenzyme lacking RecD. In this model, RecA and SSB proteins promote at Chi a nonreciprocal exchange by either a break-join or a break-copy reaction and to the left of Chi a reciprocal exchange with a third homolog by an unspecified mechanism.



**Figure 5** "Stop degradation at Chi" model of recombination, after Thaler et al. (120) as modified by Stahl et al. (107), Kuzminov et al. (61), and Myers & Stahl (76). RecBCD enzyme (*open box*) degrades DNA up to Chi (*solid dot*), at which point RecD is ejected and degradation ceases. RecBC enzyme (*open hexagon*) continues to travel along the DNA. RecA and SSB proteins promote a non-reciprocal exchange at Chi with a second parental DNA (*hatched lines*) and a reciprocal exchange to the left of Chi with a third parental DNA (*thick lines*).

The hypothesis that RecD is ejected at Chi (120) was based in part on the observation that *recD* null mutants are nuclease-deficient but recombination-proficient, although they lack Chi activity (2, 18). In other words, RecBC enzyme is viewed as constitutively Chi-activated. In some ways this model parallels the action of RecBCD enzyme under conditions with  $(Mg^{++}) > (ATP)$  (Figure 4): RecBCD enzyme degrades the top DNA strand up to Chi, attenuates that degradation, and loads RecA protein onto the Chi tail. The Chi-independent (constitutive) loading of RecA protein by RecBC enzyme supports the RecD-ejection aspect of this model. Retention of nuclease activity on the bottom strand and on a distal hairpin and retention of the RecD subunit after Chi, however, are not so easily compatible with this model (4, 32, 118).

A major difference in these two models is the fate of the DNA between *cos* and Chi (to the right of Chi). In the first model this DNA survives, but in the second it is degraded. The following genetic evidence bears on the distinction between these models.

Reciprocity of recombination, the generation of two complementary recombinants in one event, requires that the DNA bearing the markers in question not be destroyed. Thus, the “nick at Chi” model readily accommodates reciprocity, whereas the “stop degradation at Chi” model does not. The evidence for reciprocity of Chi-stimulated recombination, all from  $\lambda$  crosses, has oscillated over the years: some evidence has supported reciprocity (58, 89, 102, 106), other evidence nonreciprocity (63, 105), and other evidence both (33a, 103, 107). Determining reciprocity in phage infections is complicated by the inability to count all of the input and output DNA molecules, as can be done in meiotic tetrad analysis, and by the possibility of phage DNA undergoing more than one recombination event in an infected cell. The latter appears to contribute frequently to Chi-stimulated recombination, since triparental recombinants are detected and the apparent reciprocity depends on the multiplicity of infection (103, 107). When observed, non-reciprocity could be accounted for either by degradation up to Chi (Figure 4) or by a break-copy event in which the 3' OH end at Chi primes replication templated by a homolog (Figure 3, right) (96). It seems difficult to make a firm conclusion about the degradation of DNA between *cos* and Chi based on these observations.

There is evidence that Chi “protects” intracellular DNA, much as it protects the top DNA strand to its left in reactions with  $(Mg^{++}) > (ATP)$ , but this intracellular protection does not appear to be the simple inactivation of the RecBCD nuclease activity, as hypothesized by the “stop degradation at Chi” model. The first evidence reported was the Chi-dependent accumulation of high-molecular-weight DNA (HMW) forms of a plasmid in *E. coli* (26). In these experiments an adventitious origin of replication was activated by induction of a replication protein at high temperature. Presumably, activation of this origin initiates unidirectional rolling circle replication and entry of RecBCD enzyme into the linear tail. If the plasmid contains a properly oriented Chi site, HMW accumulates; otherwise, only circular plasmid DNA is observed. This is expected if Chi inactivates RecBCD nuclease. The accumulation of HMW, however, requires RecA protein (26), indicating that

Chi does not simply inactivate RecBCD nuclease. RecA protein could protect the DNA and allow HMW accumulation in either of two ways. (a) RecA protein loaded onto the DNA could protect it from degradation by other intracellular nucleases, but RecA protein only modestly protects dsDNA against RecBCD enzyme or  $\lambda$  exonuclease (127). (b) RecA protein could promote recombination of the Chi tail with a circular plasmid form; the resultant “dumbbell,” a linear molecule with a circle at each end, would be resistant to intracellular exonucleases, including RecBCD enzyme.

Kuzminov and colleagues (61, 62) reported more direct evidence for protection of intracellular linear DNA by Chi. In these experiments a plasmid containing *cos* was linearized after induction of Ter at high temperature. A few percent of the initial DNA survives but only if it contains a properly oriented Chi site. As in the preceding experiments, survival of this DNA requires RecA and SSB proteins. Thus, the RecBCD nuclease does not appear to be frequently inactivated at Chi, for in that case Chi-containing DNA should survive at high level and whether or not the RecA and SSB proteins are present. Furthermore, the partial length linear molecules predicted by the “stop degradation at Chi” model were not observed. The protection of DNA “upstream” of Chi, even at low level, suggested a *trans* effect of Chi; indeed, Chi on one linearized plasmid partially protects a second, Chi-free linearized plasmid (61). The protection observed in these and the preceding experiments may reflect only the *trans* effect of Chi or recombination (as noted above) or both.

Additional experiments have shown intracellular *trans* effects of Chi, presumably by an alteration of RecBCD enzyme. (a)  $\lambda$  crosses were conducted in *E. coli* cells bearing a *cos* plasmid previously linearized by Ter; if the plasmid contains Chi, the  $\lambda$  crosses manifest reduced Chi activity (75). In replication-blocked crosses in these cells recombination is focused at the right end of  $\lambda$ , as it is in *recD* mutants, suggesting that Chi on the linearized plasmid converted RecBCD enzyme into RecBC enzyme. However, all known homologous recombination independent of RecBCD enzyme, e.g., that by the RecF pathway, also focuses recombination at the right end of nonreplicating  $\lambda$  (121). (b) *E. coli* cells were treated with bleomycin, which presumably breaks the chromosome and allows RecBCD enzyme access to the many Chi sites on it; in subsequent  $\lambda$  crosses Chi activity is strongly reduced for 1 to 2 h after bleomycin-treatment (60). In addition, phage T4 gene 2 mutant phage can multiply in these cells, just as these phage can in *recB*, *recC*, or *recD* null mutants, reflecting the lack of RecBCD nuclease activity (2, 18, 60, 78).

These results suggest that RecBCD enzyme is altered by Chi such that its action on another DNA molecule (in *trans*) is changed. The alteration was inferred to be the loss of the RecD subunit (60, 75). The long-term inactivation of RecBCD enzyme, however, is reminiscent of the Chi-dependent disassembly of all three subunits with (ATP) > (Mg<sup>++</sup>) (119). In this case the retention of Chi-independent recombination proficiency may reflect DNA break-dependent induction of, for example, the RecF pathway, which is not stimulated by Chi (104). The restoration

of RecBCD enzyme activity after  $\sim 1$  to 2 cell generations may reflect *de novo* synthesis of RecBCD enzyme.

In summary, recombination is stimulated far from the DNA break at which RecBCD enzyme enters the  $\lambda$  or *E. coli* chromosome. Precisely how RecBCD enzyme processes the broken DNA into recombinants within *E. coli* remains to be determined. The observations cited here support some aspects, but not others, of two models (Figures 3, 5). Further studies of *recBCD* mutants and of recombination intermediates from *E. coli* cells may help elucidate this complex process.

## ADDITIONAL EVIDENCE FOR RECOMBINATION FAR FROM DNA BREAKS IN PHAGE AND FUNGI

In this section I discuss additional examples of recombination that occur far from DNA breaks or that can be interpreted as doing so. In some cases no DNA break has been demonstrated, but the initiating lesion may be a DNA break; in these examples, the recombination event spans a long distance on the chromosomes, often  $>10$  kb.

### Long Hybrid DNA and Meiotic Gene Conversion Tracts

Within the context of models such as those in Figures 1 and 3, hybrid DNA (hDNA), that with one strand from each parent, is a precursor to crossing over and gene conversion. The length of hDNA from an initiating DNA break determines the distance from the break over which recombination can occur. Thus, long hDNA can give rise to recombination far from a DNA break.

In phage  $\lambda$ , hDNA can extend nearly the length of the chromosome, 48.5 kb (40). Long hDNA can be detected physically when DNA replication is blocked and the  $\lambda$  Red recombination pathway and the *E. coli* RecA protein are active. From mixed infections by density-labeled phage, DNA in progeny phage containing closely linked markers ( $O^+$ ,  $P^+$ ) from each parent has a wide range of densities, indicating that either parent can contribute anywhere from  $\sim 10\%$  to  $90\%$  of the nucleotides (126). Much of the DNA has hybrid sections that can be nearly the length of the chromosome (40). Long hDNA can also be detected genetically when replication is permitted and the progeny from a six-factor cross are plated on *mutL* bacteria deficient in mismatch repair. Of phages with hDNA spanning a central marker (*cl*), 50% have hDNA  $>10$  kb long, and 25%  $> 17$  kb long (55). Thus, long hDNA is common in  $\lambda$  recombination.

Although the event initiating hDNA formation was not investigated in these studies, other studies indicate that the  $\lambda$  Red exonuclease initiates ss DNA digestion from a ds break at *cos*; the resultant 3' ss DNA end invades an intact duplex to form hDNA (121). This hDNA may be extended by branch migration the length of the  $\lambda$  chromosome. Correction of mismatches within the hDNA can produce a

recombinational exchange tens of kb from *cos*, the site of the presumed initiating DNA break.

Perhaps surprisingly, the hDNA in  $\lambda$  crosses can encompass deletion heterologies of 0.7 or 1.3 kb (64); larger deletions were not tested. hDNA encompassing a 0.6-kb deletion heterology is also formed by the Chi-stimulated RecBCD pathway (52). Although deletion heterologies of this size block spontaneous branch migration, purified RecA protein can promote formation of hDNA across large deletion heterologies at low frequency (12). Intracellular activities such as RuvAB (125) may aid the formation of long hDNA containing large deletion heterologies. In fungi, large deletions or insertions undergo gene conversion at frequencies comparable to those of single bp mutations (38, 88, 133). Furthermore, frequent PMS of 1.5-kb insertions and occasional PMS of 5.6-kb insertions are observed in *S. cerevisiae* strains deficient in Rad1 or Rad10, proteins involved in nucleotide excision repair (55b). Thus, hDNA formation in phage and fungi does not appear to be dramatically impeded by deletion heterologies of  $\sim 1$  kb or more.

Long hDNA initiated by DNA breaks provides a plausible mechanism for long co-conversion tracts produced during *S. cerevisiae* meiosis. A study employing 12 restriction site mutations scattered over a 16-kb interval of chromosome III showed that meiotic gene conversion tracts were usually continuous and often  $< 4$  kb long, but of 64 tracts analyzed 15% were at least 4 to 7 kb long and 5% were  $> 12$  kb long (111). Another study employed unsequenced mutations in four genes spanning 14 kb of chromosome VI (29). Many conversion tracts included only one marker, but of those that included an internal marker (*sup6*) 7.8% included three markers and 2.3% included all four. The minimum lengths of these tracts were, respectively, 3 to 9 kb and 8 to 14 kb, the uncertainty being due to the unknown positions of the markers within the genes. Furthermore, 6% of the tetrads manifesting PMS of *sup6* manifested PMS of three markers, indicating hDNA at least 6 to 8 kb long. In both studies the absence of markers outside the clusters analyzed precludes putting an upper limit on the length of hDNA, but these studies suggest that hDNA can occasionally be  $> 10$  kb long in *S. cerevisiae* meiosis.

## Meiotic Recombination Far from Prominent DNA Breaks

Although there is substantial evidence that *S. cerevisiae* meiotic recombination frequently occurs near ds DNA breaks (see above), there is suggestive evidence that some occurs far from breaks in *S. cerevisiae* and in the fission yeast *Schizosaccharomyces pombe*.

At the *S. cerevisiae* *HIS4* locus there is a prominent DNA break site that accounts for some but not all meiotic recombination at this locus (28, 36, 79). The frequency of gene conversion and the amount of meiotic DNA breakage are reduced by mutations eliminating transcription factors or their binding sites and increased by mutations introducing telomeric DNA sequences. There is a good linear relation between the conversion frequency and the amount of breakage, but this line indicates 10% conversion at zero breakage (36). In this strain background

the “wild-type” conversion frequency is  $\sim 30\%$ , and that of the most active hotspot is  $\sim 70\%$ . Thus, about one third of the “wild-type” conversion appears independent of the prominent DNA breaks at *HIS4*. This recombination may result from prominent breaks at distant sites or from low-level breaks throughout the *HIS4* region.

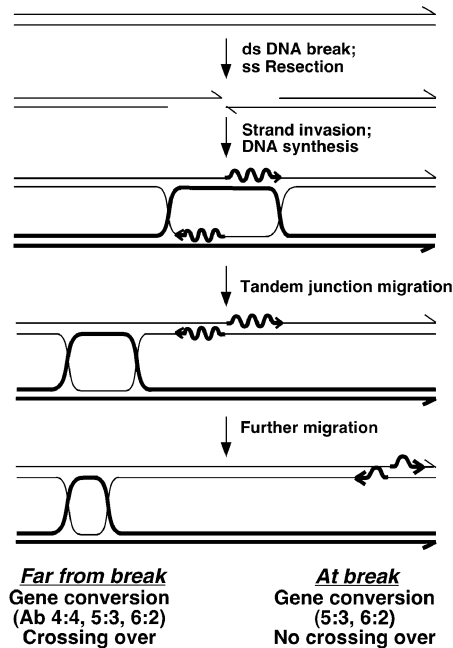
There also appears to be a discrepancy between the amount of meiotic DNA breakage and the amount of recombination in another region of chromosome III of *S. cerevisiae* (9). Across this 340-kb chromosome there are two “hot” regions of  $\sim 70$  kb and  $\sim 100$  kb in which many sites of prominent DNA breakage occur. At each site within these regions the frequency of observed breaks ranges from 0.2% to 8.8%; the cumulative frequencies of breaks in these regions are 32% and 44%. Between these two regions is a “cold” region of  $\sim 90$  kb in which there are only three sites of observed breakage; these occur within a 3-kb interval and have a cumulative frequency of breaks of only 1.7%. Although the amount of DNA breakage in the “hot” and “cold” regions differs by a factor of  $\sim 20$ , the amount of recombination differs by a factor of only 2 to 3:  $\sim 35$  cM and  $\sim 55$  cM in the two “hot” regions and 15 to 20 cM in the “cold” region (9, 15). The available data thus suggest that recombination frequently occurs in the “cold” region tens of kb from the observed breaks. Recombination in this region could stem from widely distributed low-level, undetected breaks or from the distant, observed breaks.

A similar discrepancy between meiotic recombination and DNA breaks appears in *S. pombe*. Meiotic DNA breaks occur in *S. pombe* and are dependent on the products of eight *rec* genes, which are also required for meiotic recombination (17; R. Schreckhise, J. Young, & G. Smith, unpublished data). Thus, these breaks appear to be required for meiotic recombination, but the wide spacing of the breaks does not seem to coincide with the more uniform distribution of recombination. A 501-kb region of chromosome I has been examined extensively (J. Young, G. Hovel-Miner, C. Rubio & G. Smith, unpublished data). There are six sites of prominent DNA breakage spaced  $\sim 30$  to 100 kb apart; at each site  $\sim 2\%$  to  $\sim 12\%$  of the DNA is broken, or  $\sim 25\%$  cumulative. Recombination between eight single-*bp* transition markers in this region has been studied; the most distant markers, *lys3-37* and *pro1-1*, are separated by 476 kb physically and 45 cM genetically. The ratio of  $\sim 25\%$  breaks: 45 cM is similar to that observed in the “hot” region of *S. cerevisiae* chromosome III mentioned above (9). The intensity of recombination is 0.9 cM/10 kb for the entire *lys3-37-pro1-1* interval. For 12 subintervals this value ranges from 0.7 to 1.6 cM/10 kb, whether the subinterval studied contains no prominent DNA break site or one to five such sites. Recombination thus appears to be more uniformly distributed than the prominent break sites, implying that recombination must occur far from these prominent sites. As for the 90-kb “cold” region of *S. cerevisiae* chromosome III, there may be widely distributed, low-level breaks responsible for some *S. pombe* meiotic recombination. The available evidence suggests, however, that recombination frequently occurs tens of kb from DNA breaks.

How might recombination occur so far from DNA breaks? One possibility is that a DNA break relieves torsion within a domain of the chromosome and

that recombination can occur, by an unspecified mechanism, anywhere within the relaxed domain. Another possibility is the entry of a “recombination machine,” exemplified by RecBCD enzyme (Figures 2–5), at a DNA break and its travel to a distant point before promoting recombination.

A third possibility for recombination far from a ds DNA break is outlined in Figure 6: a double Holliday junction is formed at the site of a ds DNA break, as in the model in Figure 1 (112). The junctions migrate in tandem to one or the other side for a substantial distance before being resolved with or without a crossover. Heteroduplex DNA at the site of the initial break is confined to one chromatid and is not flanked by an adjacent crossover. This outcome is seen in ~10% of events at the *S. cerevisiae* ARG4 locus and has been attributed to the “topoisomerase pullout” resolution of double Holliday junctions mentioned earlier (41). The scheme in Figure 6 provides an alternative that has the same genetic consequences close to the initial break but, in addition, can account for crossovers far from the DNA break.

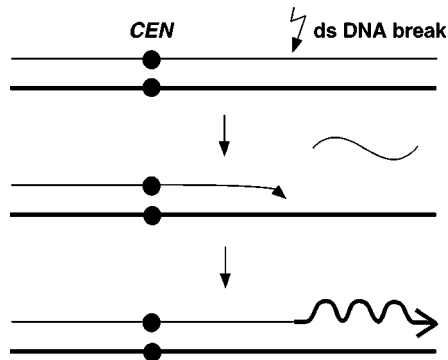


**Figure 6** Proposal for recombination far from a DNA break due to long-distance branch migration of a double Holliday junction, established from a ds break as in Figure 1. Symbols are as in Figure 1. At the site of the initial break, PMS (5:3 segregation) or gene conversion (6:2) can occur, but crossing over cannot (*bottom right*). At the site of resolution of the double Holliday junction, PMS (aberrant 4:4 or 5:3) or gene conversion (6:2) with or without crossing over can occur (*bottom left*; see Figure 1).

## Long Mitotic Conversion Tracts: Priming of DNA Replication by a Broken DNA End

In *S. cerevisiae*, a DNA ds break introduced by the HO endonuclease normally promotes localized mitotic gene conversion of the adjacent *MAT* locus, i.e., mating-type switching (49). But in abnormal circumstances the broken DNA can be processed in alternative ways. For example, if the cells lack Rad51 protein, the conversion tracts can extend to the end of the chromosome, a distance of 120 kb (68). Diploid cells heterozygous for four markers spanning 300 kb of chromosome III were transiently induced for *HO* expression; viable cells, recovered at high frequency, were tested for homozygosity of the markers. In ~60% of the cells there was homozygosity for *MAT* and the two centromere-distal markers used; a marker on the other arm of the chromosome remained heterozygous. In the remaining cells the two arms of the broken chromosome were lost. Slightly modifying a previous model for break-copy recombination of phage  $\lambda$  (94) and T4 (39, 74), the authors proposed that the broken end of the centromere-containing arm frequently invades the homolog and primes DNA replication to the telomere (Figure 7). For this to give homozygosity of the *MAT* locus, immediately centromere-proximal to the break, the broken end must be degraded at least 1 kb (to remove the *MAT* locus) and be processed into a 3' ss DNA end (to produce a primer for replication).

A subsequent study (70) indicated that degradation frequently proceeds >13 kb from the HO-induced ds break. A marker, *URA3*, was inserted at sites 3, 13, 48, or 78 kb from the break toward the centromere. At the first two sites *URA3* was rarely,



**Figure 7** Break-induced replication can give rise to long gene conversion tracts. A ds DNA break (*jagged arrow*) generates an invasive 3' end (*arrowhead*), which primes DNA synthesis. The replication fork (not shown) progresses to the end of the invaded chromosome (see Figure 3, bottom right). The broken fragment (*sinuous line*) distal to the centromere (*CEN*, *solid dot*) is lost. The thin line indicates duplex DNA from one homolog, and thick from the other. The wavy line indicates newly replicated duplex DNA.

if ever, retained, but at the latter two sites it was retained in ~50% or ~80% of the cells that repaired the broken chromosome. Located between these two pairs of sites, at 44 kb from the break, is a site important for the retention of a centromere-proximal *URA3* marker. This site is close to an origin of replication *ARS310* but is functionally separable from it. Thus, the inferred gene conversion tracts can extend ~50 to 100 kb to each side of the break. This event also occurs in Rad<sup>+</sup> cells, but at lower frequency (~2%). [See below for previous, similar observations in Rad<sup>+</sup> cells and the same proposed mechanism (123).]

These marker arrangements were also tested for meiotic gene conversion prompted by the DNA break at the HO cut site (69). A *rad50* mutation blocked formation of the normal meiotic breaks, and expression of *HO* by the meiosis-specific promoter of *SPO13* led to a meiotic DNA break at the HO cut site. A *spo13* deletion mutation allowed recovery of viable spores in dyads. Among these dyads at least 29% had converted one or both markers (*URA3* and *THR4*) flanking *MAT* and the HO cut site. Thus, these events, like the mitotic events, can be accounted for by degradation >10 kb from the DNA break, followed by replication to the end of the chromosome, a distance of >100 kb.

## Distant, Correlated Mitotic Recombination Events

Analysis of spontaneous mitotic recombination in diploid cells is not as simple as that of meiotic recombination, since the presumed initiating lesion is not known, recovery of all the participating chromosomes can be difficult, and recombination can occur in the pre- or postreplication phase of the cell division cycle or both. Nevertheless, the occurrence of widely separated mitotic recombination events suggests that recombination can occur far from an initiating lesion.

The *HOT1* mitotic recombination hotspot of *S. cerevisiae* promotes the formation of long, continuous conversion tracts (123), as seen by the following evidence. *HOT1* is part of the rDNA gene cluster and includes the enhancer-promoter complex (122) and overlaps a replication pause site (124). Presumably, ss or ds DNA breaks at one or both of these sites trigger mitotic recombination at high frequency. One study employed five heterozygous markers on chromosome III and selected cells homozygous for a marker (Ura<sup>-</sup>) 48 kb centromere-distal to a 570-bp transplanted *HOT1* fragment (123). Of these cells, >90% were homozygous for all markers between the *URA3* marker and *HOT1*, and more than half of these were also homozygous for a marker 27 kb centromere-proximal to *HOT1*. Only rarely, <5% of the time, were these events loss of the entire *URA3* chromosome. *HOT1* in *cis* to *URA3* stimulated the formation of Ura<sup>-</sup> cells much more than *HOT1* in *trans*, indicating that the events were rarely, if ever, due to crossing over. The simplest interpretation is that the homozygosity results from conversion tracts including *HOT1* and extending >48 kb to one side and more than half the time >27 kb to the other side, or ≥75 kb total. The inferred conversion tract covering *HOT1* need not have an end at *HOT1*; the ends can be >27 kb from *HOT1*. The authors proposed that the centromere-proximal end of the chromosome broken at *HOT1* is degraded by an exonuclease (sometimes a substantial distance) and that this

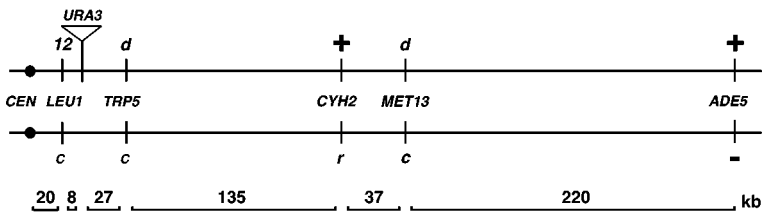
end primes DNA replication (templated by the homolog) to the centromere-distal end of the chromosome, thereby giving rise to long conversion tracts covering *HOT1*.

In addition, there is a substantial class of *HOT1*-stimulated events that are homozygous *Ura*<sup>-</sup> but remain heterozygous for two other markers between *URA3* and *HOT1*; these appear to have conversion tracts both of whose ends are >20 kb from *HOT1* but not covering *HOT1*. They evidently did not arise by the proposed break-copy mechanism; they may have arisen by an entity moving from *HOT1* and promoting recombination at a distance, perhaps by a mechanism such as that in Figure 3 or 6.

In the following examples of spontaneous mitotic recombination, no initiating lesion is known, but events covering a long distance or widely separated from each other can be interpreted within the framework of the preceding examples.

A long series of studies examined mitotic recombination in diploid cells carrying up to six markers spanning ~425 kb on one arm of chromosome VII of *S. cerevisiae* (Figure 8) (11, 34, 43–47). Heteroalleles at *LEU1*, *TRP5*, or *MET13* allowed the selection of prototrophic intragenic recombinants (“convertants”). Heterozygosity at *ADE5* allowed the detection of red-white sectoring colonies, since the cells were homozygous for the unlinked *ade2* mutation. (*ADE5 ade2* colonies are red, and *ade5 ade2* colonies are white.) Sectoring colonies arise from a cell in which recombination happened immediately before or shortly after plating; examination of the progeny within this colony allows inferences about all of the participating chromosomes and the reciprocal or nonreciprocal nature of the recombination event(s).

As noted below, the recombination event(s) often span long distances (>100 kb). These were initially interpreted as a reflection of long hDNA (43, 44), and some events may indeed reflect this. But three other interpretations are: (a) break-copy (Figures 3, 7), (b) a moving entity (Figures 3, 6), and (c) multiple, independent events that occur in a subpopulation of cells especially proficient for recombination (“hot cells”). All four factors may contribute, perhaps some concurrently.



**Figure 8** Multiply marked chromosome VII of *S. cerevisiae* used to demonstrate widely separated mitotic recombination events. The *LEU1*, *TRP5*, *CYH2*, *MET13*, and *ADE5* genes were marked on each homolog with alleles *c*, *d*, *r*, *12*, <sup>+</sup> and <sup>-</sup> as indicated. The *URA3* insertion was present in some strains but not others. The distances in kb between loci are indicated. A single line indicates duplex DNA, and a filled circle the centromere.

An early observation was the high coincidence of doubly prototrophic cells reflecting recombination at widely separated loci (44). For example, a doubly heteroallelic strain (Figure 8) produces  $\text{Leu}^+$  cells at a rate of  $3.4 \times 10^{-6}$  per cell division and  $\text{Trp}^+$  at  $2.6 \times 10^{-6}$ .  $\text{Leu}^+ \text{Trp}^+$  are produced at  $1.1 \times 10^{-8}$ , or 1200 times higher than the rate expected from independent events. Similarly,  $\text{Leu}^+ \text{Met}^+$  are produced at a rate 200 times higher than expected. Thus, conversions at loci 35 kb or 207 kb apart are statistically correlated. These could arise from hDNA spanning these long distances, followed by mismatch correction. They are not easily accounted for by the break-copy model (Figure 7): a break near *leu1*, for example, followed by hDNA formation and mismatch correction might produce  $\text{Leu}^+$ , but the subsequent copying would produce a cell homozygous for either *trp5-c* or *trp5-d*. Two separate events in “hot cells” is an alternative discussed in the next section.

To test whether the events at *LEU1* and *TRP5* were connected, *URA3* was inserted between them on one homolog (45, 46). Among doubly selected  $\text{Leu}^+ \text{Trp}^+$  cells 39% became homozygous  $\text{Ura}^-$ , and 12% became homozygous *URA3/URA3*. If the *URA3* insertion was flanked by a direct repeat of 6.1 kb, thereby allowing intrachromosomal events such as a deletion, 70% became homozygous  $\text{Ura}^-$ , and 2% homozygous *URA3/URA3*. (The *URA3* markers are lost in <0.2% of unselected cells, indicating that their conversion is strongly associated with events at *LEU1* or *TRP5*.) These high frequencies of conversion of the marker between the selected  $\text{Leu}^+ \text{Trp}^+$  events suggest that frequently a single event spans the 35-kb region. If these events result from hDNA spanning the 35 kb, then hDNA must frequently encompass 5.5- or 11.6-kb insertion heterologies, since these insertions do not significantly alter the rates of  $\text{Leu}^+$ ,  $\text{Trp}^+$ , or coincident  $\text{Leu}^+ \text{Trp}^+$  recombinant formation (45, 46). Among singly selected  $\text{Leu}^+$  cells, *URA3* converted in 9%, or in 30% if *URA3* was flanked by direct repeats. Thus, singly selected events appear frequently to extend >8 kb.

Many of the mitotic events studied appear to result from a conversion, perhaps near a spontaneous DNA break, and a nearby reciprocal exchange (“crossover”). This event is readily seen as a selected  $\text{Trp}^+$  (or  $\text{Leu}^+$ ) colony that is red-white sectored. The conversion at *TRP5* (or at *LEU1*) gives the selected prototrophy, and, with appropriate segregation of centromeres at mitosis, the associated crossover gives homozygosity for *CYH2*, *MET13*, and *ade5* on the white side of the sector. Sectoring is highly correlated with conversion: for example, 1.1% of selected  $\text{Trp}^+$ ,  $\text{Leu}^+$ , or  $\text{Met}^+$  colonies are sectored, whereas the frequency of sectors among unselected colonies is  $\sim 0.03\%$  (11, 34). Analysis of cells in the red sector and in the white sector confirms, in  $\sim 50\%$  of the cases, the recovery of markers expected for a conversion and a nearby crossover (11, 43).

In the other cases ( $\sim 50\%$ ), more complex events appear. In one study,  $\sim 25\%$  of the  $\text{Trp}^+$  red-white sectored colonies had apparently converted all markers centromere-distal to *TRP5* but the centromere-proximal marker was rarely altered (11); these can be accounted for by the break-copy mechanism (Figures 3, 7), with a break near *TRP5*. But  $\sim 7\%$  (16/227) had recombinational exchange points between *CYH2* and *MET13* or between *MET13* and *ADE5*; these points are at least 135 kb or 170 kb, respectively, from *TRP5*. In another study (43),  $\sim 10\%$  (7/71)

were of the latter type. Thus, widely separated recombination events occur fairly frequently; these events, like the coincident  $\text{Leu}^+$   $\text{Trp}^+$  events, are not accounted for by the break-copy mechanism.

More complex events also appear: in  $\sim 14\%$  of the  $\text{Trp}^+$  sectored colonies there are three to six genotypes ("mosaics"). Among these,  $\sim 15\%$  have exchanges in the *CYH2-MET13* or *MET13-ADE5* interval or both; i.e.,  $>135$  kb from the selected  $\text{Trp}^+$  event (11, 47). Similar values are obtained among  $\text{Leu}^+$  or  $\text{Met}^+$  selected colonies. Some of these complex outcomes could result from a single event, such as long hDNA resolved by a crossover and one or more mismatch corrections. Arguing for a single event spanning a long distance is the "polarization" of the mosaic genotypes: markers centromere-distal to a selected event are more frequently recombinant in the mosaics than are centromere-proximal markers (11). A similar polarization occurs among the non-mosaic sectored colonies (43).

Other mosaic colonies, such as those containing cells with five or six genotypes, must involve multiple events. Furthermore, some statistically correlated events involve two nonhomologous or three homologous chromosomes (11, 47, 73). These events are most easily accounted for by the "hot cell" notion, discussed next.

### Induction of Recombination Potential in *trans*: "Hot Cells"

DNA damaging agents stimulate recombination apparently in two ways: the DNA lesions may be sites of enhanced recombination (in *cis*), and they may induce recombination potential on undamaged DNA (in *trans*). A similar induction of DNA repair capacity is well established as exemplified by the DNA damage-inducible SOS repair regulon of *E. coli*. In the absence of exogenous DNA damaging agents, a subpopulation of cells may be induced for spontaneous recombinational potential. In these "hot cells" recombination occurs at higher frequency than in most cells in this population. Two separate events in these cells may appear as a single event covering a long distance.

Evidence for the induction of recombination potential is seen in X-irradiated *S. cerevisiae* cells (35). If a haploid *ade6-21,45* double mutant is irradiated and then mated with an unirradiated *ade6-21/ade6-45* heteroallelic diploid, the frequency of  $\text{Ade}^+$  recombinants increases as a function of X-ray dose. Most of the  $\text{Ade}^+$  recombinants appear to arise from a single recombination event between the unirradiated chromosomes of the diploid, since their frequency is about 3000 times higher than expected from the frequency of two recombination events between the doubly mutant chromosome and each singly mutant chromosome. Furthermore, radiation of the haploid increases recombination even when nuclear fusion is largely prevented by a *kar1* mutation. UV-irradiation of the haploid also increases recombination, but this effect is diminished when the UV-induced lesions are removed by exposure to visible light (photoreactivation) before mating. These observations imply that a DNA lesion induces a diffusible agent that promotes recombination, presumably anywhere in the genome.

There is also evidence for an induced state of hyper-recombination ("hyper-Rec") in a subpopulation of untreated yeast cells. With appropriate markers one

can select cells with recombination event #1, or with #2, or with both. If the events occur independently, the frequency of the double event should equal the product of the frequencies of the individual events ( $f_{1,2} = f_1 \cdot f_2$ ). If the events occur preferentially in a subpopulation of cells, they will not occur independently, and  $f_{1,2} = C \cdot f_1 \cdot f_2$ , where  $C$ , the coefficient of coincidence, is  $> 1$ . If the events occur exclusively and independently within a distinct subpopulation, the fraction of the total cells in this subpopulation is  $1/\sqrt{C}$ .

Several studies of spontaneous mitotic recombination show that  $C$  is  $> 1$  in both *S. cerevisiae* and *S. pombe*. For example, one study (73) used strains similar to those discussed previously (Figure 8) but also heteroallelic at loci on other chromosomes. The rates of recombination to produce prototrophy (e.g., *TRP5*) and to produce white colonies (*ade5/ade5*) were measured separately and simultaneously. For *TRP5* and white (both on chromosome VII),  $C = 340$ . For *LYS2*, *TYR1*, or *URA3* (on chromosomes II, II, or V, respectively) and white,  $C = 1200, 800, \text{ or } 720$ . Thus, the coincidence of events on separate chromosomes is as great as those on the same chromosome, which implies that this coincidence must be due to separate recombination events, presumably in a subset of "hot" cells. From the assumptions in the preceding paragraph, this subset is a few percent of the total cells. These observations call into question the interpretation that the prototrophic red-white sectorized colonies discussed previously arise from one event covering a long distance. The polarization of the events and the high frequency of a second event among cells with a selected event (up to 70%), however, argue that some single events cover a long distance.

In *S. pombe* also there is coincidence between spontaneous mitotic recombination events on the same or separate chromosomes; this holds for pairs of intragenic or intergenic events or one of each (48, 72). The measured coefficients of coincidence are similar for the three types of double events, ranging from  $\sim 10$  to 100.

The fraction of hot cells in the total population appears to be increased in mutants defective in DNA metabolism since  $C$  is decreased in such mutants. For example, in an *S. cerevisiae cdc9* mutant deficient in DNA ligase,  $C$  is reduced to 7–36 for the events discussed above (73). This suggests that the *cdc9* mutation increases the hot cell population from  $\sim 3\%$  to  $\sim 20\%$  of the total population. Similarly, in an *S. pombe rad2* mutant deficient in the "flap" endonuclease important for completing DNA replication,  $C$  for two intragenic events on different chromosomes is reduced to 4 from 29 in *rad2*<sup>+</sup> cells; for two intergenic events on different chromosomes  $C$  is reduced to  $\sim 10$  from  $\sim 100$  (48). Presumably, the defects in DNA metabolism increase the level of unrepaired DNA damage, which induces recombination potential throughout the genome in a substantial fraction of the population. This effect is similar to that of X rays discussed at the beginning of this section.

## CONCLUSION AND PERSPECTIVE

In numerous organisms, including phage, bacteria, and fungi, recombination can occur either near a DNA break or far from it. The relative frequencies of these two types of recombination are still unclear, as too few studies have focused on

recombination remote from DNA breaks. In no case is the molecular mechanism completely understood; in some cases the genetic and biochemical observations are not in complete accord. Further investigations, including reconstruction of a complete pathway of recombination in cell-free preparations, are needed to answer these questions.

There are multiple mechanisms by which recombination can occur far from a DNA break. More than one of these mechanisms may play a role in a given event. For example, a recombination machine may enter a ds DNA break, travel to the left, and establish a replication fork toward the right (e.g., Figure 3); this event may also form long hDNA and induce recombination-promoting enzymes. Sorting out the molecular mechanism underlying such events may require multiple genetic and physical approaches.

There is circumstantial evidence for a cellular state conferring high recombination potential ("hyperRec" state). Additional studies are needed to confirm and elucidate this state. There is also evidence for a hyper-mutable state ("hyperMut"), for example, in starved *E. coli* cells (39a). Perhaps in some circumstances the hyperRec and hyperMut states are coincident, creating a hyper-unstable state. A suggestion of such a state comes from the high rate of mutation near recombination events stimulated by a ds DNA break (108). It is conceivable that the hyperRec or hyperMut states, or both, persist for several cell generations after the occurrence of DNA damage. Such a hyper-unstable state, especially if persistent, may contribute to the development of cancer.

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