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Phil. Trans. R. Soc. Lond. B 1995 **347**, 13-20
doi: 10.1098/rstb.1995.0003

The initiation and control of homologous recombination in *Escherichia coli*

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SUMMARY

The chromosome of *Escherichia coli* recombines at low frequency when it is an intact circle but recombines at high frequency when it is broken, for example by X-rays, or when a linear DNA fragment is introduced into the cell during conjugation or transduction. The high recombinogenicity of double-strand (ds) DNA ends is attributable to RecBCD enzyme, which acts on ds DNA ends and is essential for recombination and ds DNA break repair. RecBCD enzyme initiates DNA unwinding at ds DNA ends, and its nuclease activity is controlled by Chi sites (5′ G-C-T-G-G-T-G-G 3′) in such a way that the enzyme produces a potent single-stranded DNA substrate for homologous pairing by RecA and single-stranded DNA binding proteins. We discuss a unifying model for recombination and ds DNA break repair, based upon the enzymic activities of these and other proteins and upon the behaviour of *E. coli* mutants altered in these proteins.

1. INTRODUCTION

Homologous recombination plays a dual role for living organisms. First, in diploid or partly diploid organisms it produces new allelic combinations and thereby increases the variability required for evolution. Second, faithful repair of double-strand (ds) breaks in DNA provides the genomic stability required for continuity from one cell or one generation to the next. Recombination and ds DNA break repair appear to proceed by a common mechanism (Resnick 1976; Szostak *et al.* 1983) that is controlled, like many other cellular processes, at the initial stages. This article discusses the initiation and control of homologous recombination in *Escherichia coli*.

The genetic map of *E. coli* is circular, and its DNA is also circular at least some of the time. Recombination between repeated segments of the circular genome, for example the genes encoding ribosomal RNA, is rare, occurring at a rate of about 10^{-4} per cell division (Petes & Hill 1988). In contrast, broken (i.e. linear) DNA often recombines at a rate exceeding 1 per cell division (Krasin & Hutchinson 1977). The low frequency of recombination of an intact, circular genome provides stability and survival of *E. coli* as a species, whereas the high frequency of recombination of a broken genome provides rapid repair and survival of individual *E. coli* cells. Apparently, ds DNA breaks trigger recombination just when appropriate for the cell. We focus our discussion on the molecular mechanism of this control.

2. SOURCES OF DOUBLE-STRANDED DNA ENDS

Double-stranded (ds) DNA ends arise in several ways in *E. coli*. Direct DNA damage, such as that from X-irradiation, may make ds breaks. Single-strand (ss) DNA breaks (nicks or gaps) produced by other agents may be converted to ds breaks during attempted repair of a ss break or replication past it. Linear DNA is introduced into the cell during transduction, transformation (with linear DNA), or conjugation; in the last case ss DNA injected from the donor is converted into ds DNA in the recipient (Ippen-Ihler & Minkley 1986). In each case recombination (or repair) occurs at high frequency when ds DNA ends are present. The high reactivity of ds DNA ends is attributable to the strong preference for DNA ends by RecBCD enzyme, which is required for recombination and ds DNA break repair in *E. coli* (Emmerson 1968).

3. ACTIVITIES OF RecBCD ENZYME

RecBCD enzyme has multiple enzymic activities, which can be broadly separated into unwinding of linear ds DNA and DNA hydrolysis (see Taylor 1988, for review and references). Both activities require the concurrent hydrolysis of ATP, which in turn is dependent on DNA. The enzyme contains three large polypeptides, encoded by the *recB*, *recC*, and *recD* genes (M_r 134 kDa, 129 kDa, and 67 kDa, respectively). The isolated RecB polypeptide has strong DNA-dependent ATPase activity, but no other activity is present at high level with any combination except that of all three polypeptides (Masterson *et al.* 1992). Reconstitution with the three isolated polypeptides produces about 5% of the native enzyme's specific activity (Masterson *et al.* 1992); whether reconstitution is inefficient or another factor is missing is unclear. The RecB and RecD polypeptides bind ATP (Julin & Lehman 1987) and may provide the 'motor' to move RecBCD enzyme along DNA.

Phil. Trans. R. Soc. Lond. B (1995) **347**, 13–20
Printed in Great Britain

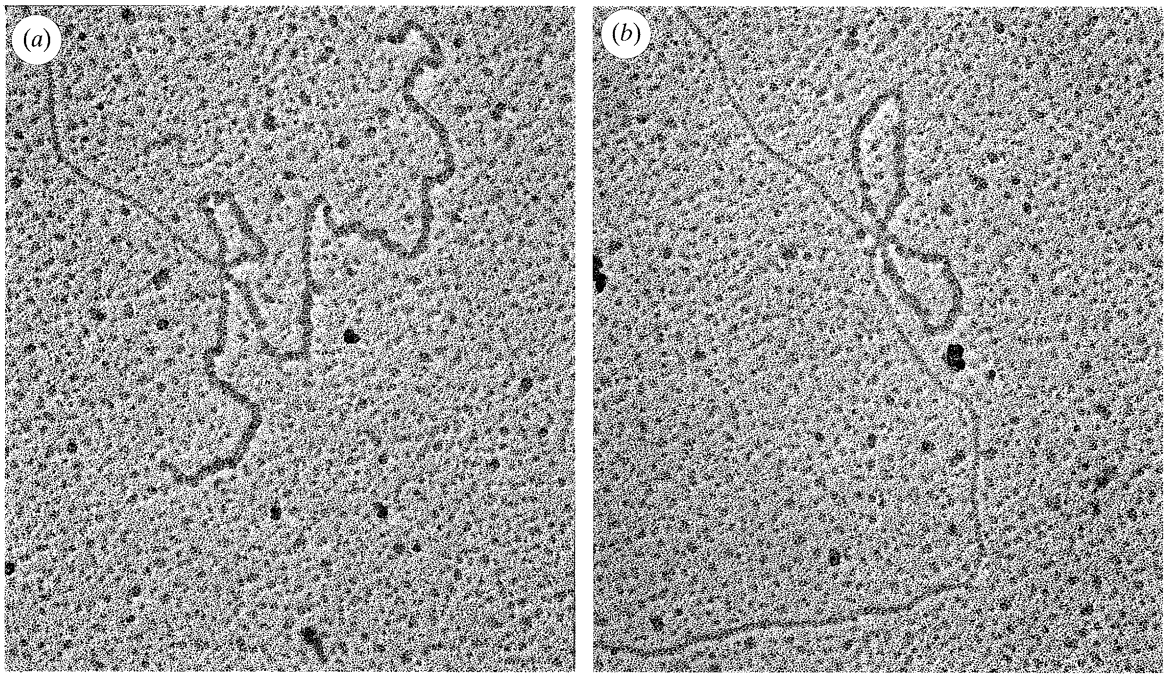

Figure 1. Loop-tail (*a*) and twin-loop (*b*) structures formed by RecBCD enzyme unwinding DNA. Thin lines are ds DNA, and thick lines are ss DNA coated with SSB. From Smith *et al.* 1984.

RecBCD enzyme has no detectable activity on circular ds DNA, but it has a high affinity for ds DNA ends: the dissociation constant is estimated to be 0.1 nM (A. F. Taylor & G. R. Smith, unpublished data). This concentration is about 1/10 that of a single ds DNA end per *E. coli* cell; consequently, the enzyme should act at its maximal rate whenever a ds DNA end occurs in the cell. This feature assures that linear ds DNA will be quickly repaired or recombined in *E. coli*.

In the absence of ATP RecBCD enzyme forms a well-defined complex with a ds DNA end (Ganesan & Smith 1992). The enzyme protects from DNase I digestion the terminal 16–17 nucleotides (nuc) of the strand with a 3′ end in the complex, and 20–21 nuc of the 5′ ended strand. UV-irradiation of the complex crosslinks the RecB polypeptide to the 3′ end and the RecC and RecD polypeptides to the 5′ end. These asymmetries are presumably responsible for the asymmetries of the unwinding and nuclease reactions, but the details remain unknown.

Upon addition of ATP to the complex, RecBCD enzyme rapidly moves along the DNA and unwinds it, with the production of a growing ss DNA loop and two ss DNA tails (figure 1*a*). These 'loop-tail' structures and the rates of their growth and movement were elucidated by electron microscopy (Taylor & Smith 1980; Telander-Muskavitch & Linn 1982). The strand with a 5′ end in the initiation complex forms the longer of the two tails, while the 3′ ended strand forms the loop and the shorter tail (Braedt & Smith 1989). Unwinding occurs at about 350 base pairs (b.p.) per second, and the loop grows at about 100 nuc per second, as measured by electron microscopy of molecules acted upon by RecBCD enzyme for 20–60 s (Taylor & Smith 1980); a similar estimate of the rate of unwinding was obtained by a continuous fluorescence-based spectroscopic assay (Roman & Kowalczykowski 1989). The loop-tail structures are frequently preserved by single-stranded DNA binding (SSB) protein when it is present in the reaction mixture; alternatively, the two tails may anneal to form a twin-loop structure (figure 1*b*). The loops of this structure grow and move along the DNA at the same rate as the loop of the loop-tail structure. RecBCD enzyme's unwinding of DNA produces ss DNA, an essential substrate for RecA protein, which pairs homologous DNA (see Rao & Radding in this volume).

During its unwinding of DNA RecBCD enzyme cuts the DNA at a frequency that depends upon the Mg^{2+} and ATP concentrations (among other factors) (reviewed by Taylor 1988). With excess Mg^{2+} (relative to ATP) the nuclease is maximally active, and oligonucleotides are quickly produced from linear ds DNA. With excess ATP, which chelates the Mg^{2+}, the nuclease activity is markedly reduced, and long ss DNA is produced by the unwinding reaction. RecBCD enzyme also hydrolyses linear ss DNA to oligonucleotides. The degradation of DNA to oligonucleotides seems antithetical to the production of intact recombinant molecules, but the interaction of RecBCD enzyme with special nucleotide sequences, Chi sites, has helped resolve this paradox.

4. CHI SITES: HOTSPOTS OF HOMOLOGOUS RECOMBINATION

During their studies of phage λ recombination in *E. coli* Stahl and his collaborators found that certain λ mutations, which they called χ, enhance homologous recombination but only near themselves (see Thaler & Stahl (1988) and Smith (1988, 1994) for reviews and references); in other words the χ mutations create a site, called Chi, that locally stimulates recombination.

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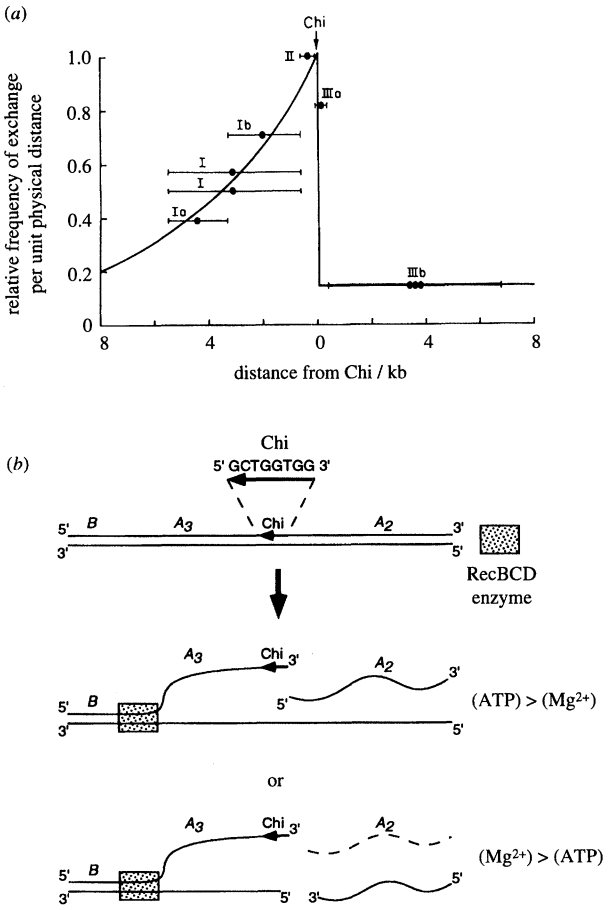

Figure 2. (*a*) Localized stimulation of recombination by Chi in phage λ crosses. I, Ia, etc. are genetic intervals bounded by markers located the indicated distance from a Chi site in λ. Solid circles indicate the midpoints of each interval and the frequency of recombinants per physical length of that interval, normalized to interval II = 1. From Cheng & Smith 1989. (*b*) Action of purified RecBCD enzyme at Chi. With (ATP) > (Mg^{2+}), RecBCD enzyme unwinds ds DNA, nicks the upper strand 4–6 nuc to the right of Chi, and continues unwinding (Taylor *et al.* 1985). With (Mg^{2+}) > (ATP), RecBCD enzyme degrades the upper strand up to, but not beyond, Chi (Dixon & Kowalczykowski 1993); the lower strand is cut near Chi and is unwound beyond Chi (A.F. Taylor & G.R. Smith, unpublished). Both conditions produce a ss DNA fragment with a 3′ end near Chi and extending to its left. A_2, A_3, and *B* are genetic markers discussed in the text.

Chi stimulates RecBCD enzyme-dependent recombination but not other types, such as that promoted by λ's own recombination functions. Chi stimulates recombination, including the formation of heteroduplex DNA (hDNA), to its left but not to its right (as defined by the conventional λ genetic map) (figure 2*a*). Inversion of a Chi site in λ greatly reduces its activity. These two asymmetries – leftward directionality and orientation dependence – are accounted for in the following way. RecBCD enzyme gains access to λ DNA primarily when the circular replicative form is cut by the λ terminase complex at the cohesive end site *cos* in preparation for packaging of the linear DNA into the virion; terminase remains bound to the left cohesive end, and RecBCD enzyme enters the right end. As it travels along the DNA, RecBCD enzyme recognizes the asymmetric Chi sequence in one orientation but not the other.

Nucleotide sequence analyses of λ wild-type and χ mutants revealed Chi to be 5′ G-C-T-G-G-T-G-G 3′ (or its complement or the duplex). This sequence is recognized as Chi only when RecBCD enzyme approaches it from the right (as written here). Although wild-type λ lacks this sequence, the *E. coli* chromosome has about 1000 Chi sites, or about 1 per 5 kilobase pairs (kb). Chi is active in *E. coli* RecBCD enzyme-dependent recombination following conjugation and transduction; as far as tested, the properties of Chi in λ recombination apply to *E. coli* recombination. The *E. coli* Chi sites appear to be non-randomly oriented on the chromosome: about 80% of those in the 136 kb sequenced region around the origin of replication *oriC* are oriented such that the 5′ end of the Chi sequence above is pointed toward *oriC* over the shorter arc of the chromosome (Burland *et al.* 1993). It is unclear whether this bias reflects a role of Chi in the repair of broken replication forks (Kuzminov *et al.* 1994; see below) or the occurrence of frequently used codons within Chi (Triman *et al.* 1982) plus a bias for transcription away from *oriC* (Brewer 1988). The abundance of Chi sites on the *E. coli* chromosome makes it likely that RecBCD enzyme entering a ds DNA break anywhere on the chromosome will find within a few tens of kb a Chi site properly oriented to interact with it and effectively repair or recombine the chromosome.

5. INTERACTION OF RecBCD ENZYME AND CHI SITES

The first evidence that RecBCD enzyme interacts with Chi was the hotspot activity of Chi in RecBCD enzyme-dependent recombination but not in other pathways of recombination, as noted above. Certain non-null alleles of *recB* and *recC* that reduce or abolish Chi activity but not recombination further implicated RecBCD enzyme as the factor that recognizes Chi. Direct evidence came from the finding of Chi-dependent DNA strand cleavage (Chi nicking) by purified RecBCD enzyme; mutations of Chi or of *recB* or *recC* coordinately reduce or abolish both Chi activity in cells and Chi nicking in cell-free extracts. This correlation indicates that Chi nicking or another activity not mutationally separated from it is required for Chi's stimulation of recombination.

Chi nicking was initially studied using a reaction condition with (ATP) > (Mg^{2+}), which minimizes the Chi-independent nuclease activity (Ponticelli *et al.* 1985). Under these conditions RecBCD enzyme cuts one DNA strand, that containing 5′ G-C-T-G-G-T-G-G 3′, 4–6 nuc to the right of this sequence (figure 2*b*) (Taylor *et al.* 1985). The complementary strand is not detectably cut. Up to 40% of the substrate molecules are cut at Chi by a single passage of the enzyme (Taylor & Smith 1992), and high yields of the DNA fragments extending to the right and left of the nick are obtained. The nicked fragments are released as ss DNA, indicating that RecBCD enzyme unwinds the DNA before and after cutting at Chi. The ss DNA

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fragment extending from Chi to the left bears a 3′ OH end and is hypothesized to be a substrate for RecA and SSB protein-promoted pairing with a homologous duplex and to be a primer for repair DNA synthesis (see §7 for further discussion).

The pairing of this Chi-dependent fragment with a homologous supercoiled DNA has been demonstrated in a coupled system containing purified RecBCD enzyme, RecA protein and SSB protein (Dixon & Kowalczykowski 1991). Formation of the joint molecule product requires that all three proteins be present simultaneously, suggesting a coordinated action among the proteins. The joint molecule contains the supercoiled DNA and the ss DNA fragment extending from Chi to the left but apparently not the other parts of the linear Chi-containing substrate.

The reaction condition used by Dixon & Kowalczykowski (1991) to produce these joint molecules employs $(Mg^{2+}) > (ATP)$, which maximizes the activity of RecA protein. Under this condition RecBCD nuclease activity is strong, though it is inhibited by SSB protein (see Taylor 1988), and the enzyme degrades some of the DNA to oligonucleotides. Degradation is strongest on the strand with a 3′ end at which RecBCD enzyme initiated its action but is greatly diminished on that strand after encounter with a properly oriented Chi site (Dixon & Kowalczykowski 1993) (figure 2*b*). Thus, under these conditions Chi attenuates on one DNA strand the nuclease activity of RecBCD enzyme, rather than inducing the nicking activity of the enzyme, as under the conditions $((ATP) > (Mg^{2+}))$ discussed above. Under both conditions, however, RecBCD enzyme produces from the strand containing 5′ G-C-T-G-G-T-G-G 3′ a Chi-dependent fragment extending to the left of this site.

Under conditions with $(Mg^{2+}) > (ATP)$ the RecBCD enzyme reaction at Chi is complex (A. F. Taylor & G. R. Smith, unpublished results). In addition to degradation of the 3′ ended ('upper') strand up to Chi, we observe high frequency Chi-dependent cutting of the complementary ('lower') strand (figure 2*b*). Cuts on the 'upper' strand are distributed over a 10 nuc interval encompassing the Chi octamer, rather than being concentrated 4–6 nuc to the right of Chi as under conditions with $(ATP) > (Mg^{2+})$. Cuts on the 'lower' strand are distributed over a 15 nuc interval slightly offset to the left of the interval on the 'upper' strand. This reaction therefore produces three DNA fragments, one with a 5′ end near Chi ('lower' left, figure 2*b*) and two with 3′ ends near Chi ('upper' left and 'lower' right). The last two fragments are potential substrates for RecA protein-promoted homologous pairing.

A major question is which reaction condition, $(ATP) > (Mg^{2+})$ or $(Mg^{2+}) > (ATP)$, more nearly reflects the condition inside *E. coli* cells. Estimates of the free (ATP) and free (Mg^{2+}) are approximately 3 mM (Matthews 1972; Alatossava *et al.* 1985); thus, the entity in excess is uncertain. We have used mutant Chi sites, mutant *E. coli* RecBCD enzyme, and RecBCD enzymes from other bacterial species in both intracellular and extracellular reactions to address this question. There is an excellent correlation of Chi hotspot activity inside cells and Chi nicking outside cells using conditions with $(ATP) > (Mg^{2+})$ (Ponticelli *et al.* 1985; Cheng & Smith 1987; McKittrick & Smith 1989); similar studies using conditions with $(Mg^{2+}) > (ATP)$ have not, to our knowledge, been done but may help resolve this question. Regardless of the reaction condition, a 3′ OH ss DNA fragment extending from Chi to its left is produced by RecBCD enzyme. In §7 we discuss how this fragment may produce recombinants and prime DNA synthesis for ds DNA break repair.

In addition to the DNA being changed (cut) at Chi, RecBCD enzyme is also changed when it cuts at Chi. With $(ATP) > (Mg^{2+})$ the enzyme loses the ability to cut at a subsequently encountered Chi site, even though the enzyme continues to travel past this site (Taylor & Smith 1992). With $(Mg^{2+}) > (ATP)$ the enzyme's nuclease activity, at least on the 'upper' strand discussed above, is attenuated (Dixon & Kowalczykowski 1993), so that a DNA end would not be expected to be produced at a subsequently encountered Chi site; this prediction has not, to our knowledge, been tested. The physical basis for the change of RecBCD enzyme upon cutting at Chi has not been established. Taking note of the phenotypes of *recD* mutants and Chi sites, Thaler *et al.* (1988) hypothesized that the RecD subunit is lost from RecBCD enzyme upon its encounter with Chi. The similar effect of (Mg^{2+}) on RecBCD enzyme after its encounter with Chi and on RecBC protein (i.e. lacking RecD polypeptide) has been interpreted as support for this hypothesis (Dixon, Churchill & Kowalczykowski 1994). Alternatively, the change may be the loss or alteration of a small RNA that is associated with purified RecBCD enzyme and which appears to be required for DNA unwinding and Chi cutting by RecBCD enzyme (S. K. Amundsen & G. R. Smith, unpublished data). Regardless of the physical basis for the change, cutting at only a single Chi site provides an enzymic basis for RecBCD enzyme promoting only a single recombinational or repair event near a ds DNA end (see §7).

6. FURTHER ENZYMIC STEPS FOR RECOMBINATION AND REPAIR

Production of ss DNA at and distal to a Chi site by RecBCD enzyme only initiates recombination or repair; further enzymic steps are required to convert this intermediate into completed recombinants or repaired molecules. These steps include homologous pairing, strand exchange, branch migration, and resolution, and can be promoted by the RecA, RuvAB, RecG, and RuvC proteins of *E. coli* (see below and articles by Rao & Radding and by West in this volume).

7. A UNIFYING MODEL FOR RECOMBINATION AND DS DNA BREAK REPAIR IN *E. COLI*

Based upon other people's work and ideas, as well as our own, we previously proposed a model (figure 3) for recombination by the RecBCD pathway of *E. coli*

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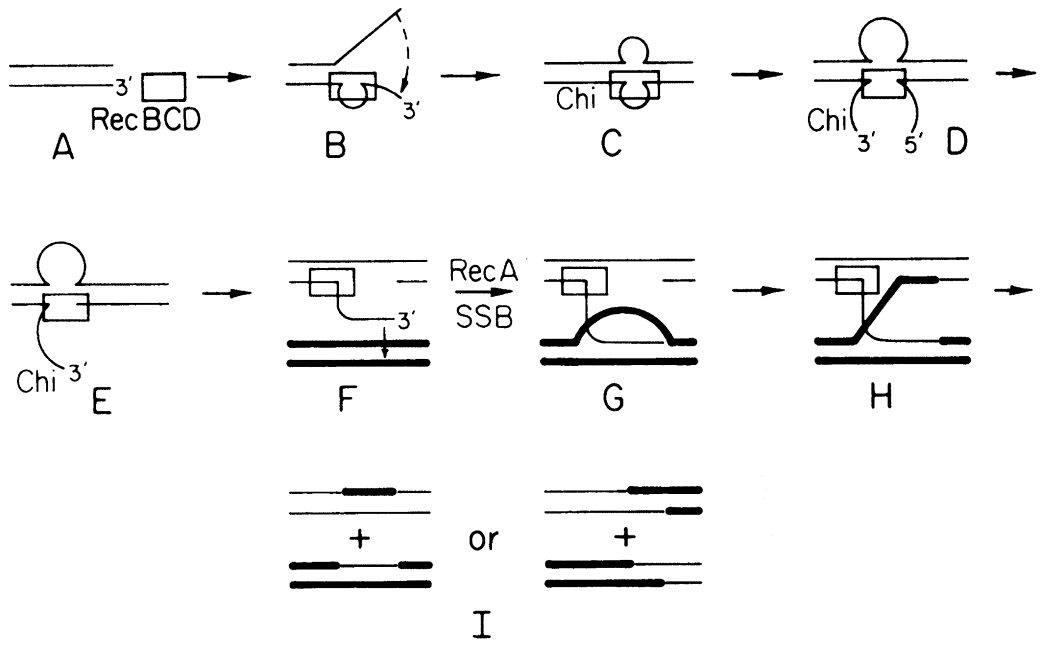

Figure 3. Model for recombination promoted by RecBCD enzyme (open box), Chi, RecA protein, and SSB protein. Thin and thick lines represent parental ds DNA. Resolving enzymes, such as RuvABC and RecG proteins, acting at the last two steps are not shown. For explanation see text. Modified from Smith *et al.* (1984).

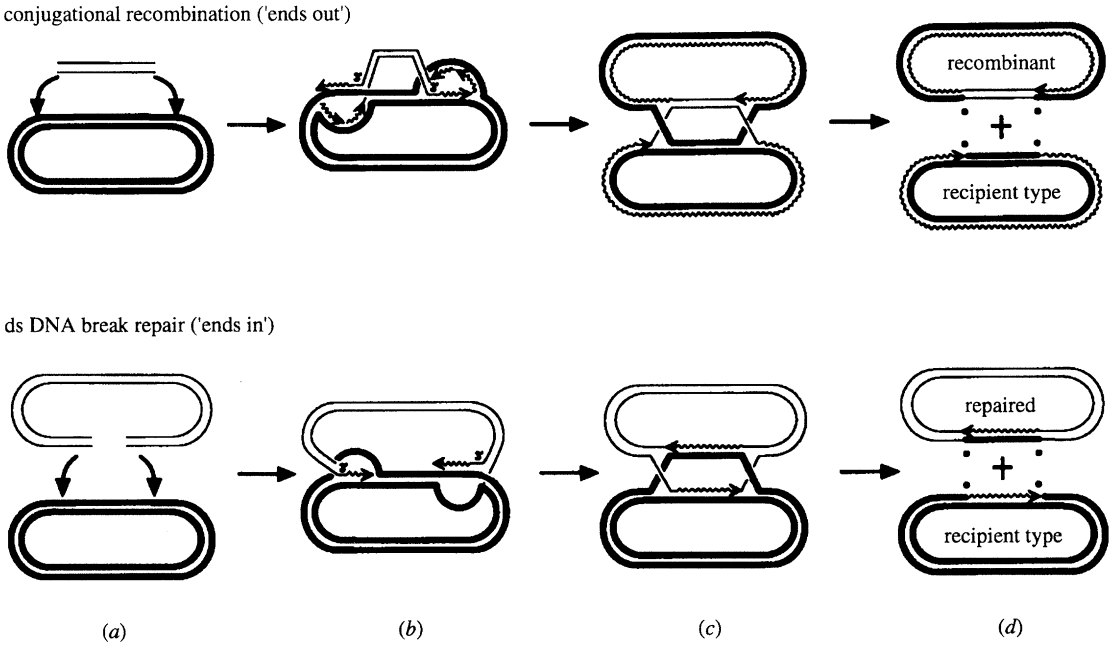

Figure 4. Model for recombination and ds DNA break repair by RecBCD enzyme and Chi, involving non-reciprocal resolution by DNA synthesis. Thin and thick lines are parental ds DNA, and wavy lines are newly synthesized DNA. Solid dots are probable sites of heteroduplex DNA. For explanation, see text and figure 3. From Smith (1991).

(Smith *et al.* 1984). In this model RecBCD enzyme binds to a ds DNA end, produced by any of several means (see §2). The enzyme unwinds DNA with the production of growing ss DNA loops (steps B and C) and cuts one strand at a properly oriented Chi site (D), to produce with continued unwinding a ss DNA 'tail' with Chi near the free 3′ OH end (E and F). RecA and SSB proteins pair this ss DNA with a homologous duplex to form a D-loop (G). Continued DNA unwinding by RecBCD enzyme and DNA strand transfer by RecA and SSB proteins extends the heteroduplex DNA in the D-loop.

At this point we envisage two alternatives for resolution of the joint molecule into recombinants. In the first alternative the D-loop is cut to allow RecA and SSB proteins to convert the D-loop into a Holliday junction (figure 3, step H). Branch migration and resolution of the Holliday junction by RuvAB and RuvC proteins or by RecG protein plus others produce reciprocal recombinants, either 'patches' (I, left) or 'splices ' (I, right) depending upon which pair of strands is cleaved in the resolution.

In the second alternative (figure 4) the invading ss DNA with its 3′ OH in the D-loop primes DNA

replication, which may be converted into a replication fork (Smith 1991). This process is especially attractive for recombination and repair of circular chromosomes, such as *E. coli*'s, in which a linear (broken or fragmented) DNA molecule interacts with an intact circular molecule. Action of RecBCD enzyme at properly oriented Chi sites near each end of the linear fragment (step *a*), followed by D-loop formation by RecA and SSB proteins (*b*), produces two replication primers pointed in opposite directions. Replication from these two points produces two intact circles joined by two Holliday junctions (*c*). Resolution of the Holliday junctions in the same directions (both 'horizontal' or both 'vertical') produces two intact monomeric circles (*d*); resolution in the opposite directions produces a dimeric circle (not shown), which can be resolved into monomeric circles by the XerCD site-specific recombination enzyme (see Sherratt *et al.* in this volume). In either case the occurrence of intact circles stops recombination, as circular DNA is refractory to RecBCD enzyme.

The model in figure 4 has several attractive features for *E. coli* recombination and ds DNA break repair. Note that repair of a broken chromosome (bottom panels) is topologically equivalent to recombination of a linear fragment introduced into *E. coli* during conjugation or transduction (top panels).

1. The same mechanism can account for both processes, as proposed by Resnick (Resnick 1976) and amplified by Szostak *et al.* (1983). In *E. coli* several enzymes are required for both processes: RecA protein, SSB protein, RecBCD enzyme, DNA polymerase I, and DNA ligase (see Smith 1988 for references). In addition a second genome is required for repair or recombination of a chromosome fragment (Krasin & Hutchinson 1977).

2. The loss of Chi nicking activity by RecBCD enzyme upon cutting at one Chi site (Taylor & Smith 1992) ensures that a RecBCD enzyme at each end will promote a single exchange (i.e., two exchanges on the linear fragment). As the donor DNA in conjugation or transduction is a linear chromosomal fragment, even numbers of exchanges are required to produce a circular (viable) chromosome. In conjugation and transduction the majority of the events (about 80 % and > 95 %, respectively) appear to involve exactly two exchanges (reviewed by Smith 1991). Breakage of the linear fragment into two, three, ... *n* pieces would result in four, six, ... 2*n* exchanges, or always an even number, as required for viability. During DNA repair this feature of RecBCD enzyme would limit the process to the minimum number of exchanges needed for repair.

3. The generation of circular DNA molecules would limit recombination and repair to a single cycle of events. In contrast, reciprocal resolution without replication (figure 3) would generate a succession of linear fragments and cycles of recombination, perhaps until the linear fragment were destroyed by exonucleolytic degradation. The occurrence of recombinants with just two exchanges in the majority of the cases argues against repeated recombination being a principal outcome, but it may occur occasionally.

The model in figure 4 can account for both reciprocal and non-reciprocal exchange of markers during recombination, depending upon their location. Consider a set of markers A_1, A_2, A_3 and B, with B to the left of a leftward-directed Chi (figure 2*b*). If A_1 is outside the linear fragment, recombination between it and B will, of course, be non-reciprocal. If A_2 is on the linear fragment but to the right of Chi, recombination will be non-reciprocal if the linear fragment is degraded by RecBCD enzyme up to Chi but may be reciprocal if RecBCD enzyme nicks DNA at Chi (see §5). If A_3 is between Chi and B, recombination may be either reciprocal or non-reciprocal depending upon whether heteroduplex DNA covers both B and A_3 or just A_3 and depending upon the correction of mismatches in the heteroduplex DNA. The available evidence from Chi-stimulated recombination of phage λ in *E. coli* suggests that both types of recombination may occur (see Smith 1994 for references); this evidence is inconclusive, however, primarily because of the inability to monitor all of the substrate and product molecules. Further studies are needed to resolve this issue.

8. TEST OF THE TWO CHI MODEL: TRANSFORMATION WITH LINEAR DNA

The model in figure 4 predicts that recombination of a linear DNA fragment with the *E. coli* chromosome requires two Chi sites on the fragment, each oriented to activate RecBCD enzyme approaching it from the nearer end. We have begun such a test with a linear fragment bearing about 3.0 kb of a Chi-free part of the *E. coli his* operon flanked by about 1.5 and 0.8 kb of Chi-free pBR322 vector (P. Dabert & G. R. Smith, unpublished). The test χ^+ DNA has a properly oriented Chi site at each *his*–pBR322 junction, whereas the control χ^0 DNA has no Chi sites. Addition of these DNA's to $CaCl_2$-treated *E. coli* produced, in 15 independent transformations, a total of 155 *his* integrated transformants with χ^+ DNA but 0 with an equal amount of χ^0 DNA. (Neither DNA transformed a *recA* mutant, but both transformed a *recD E. coli* mutant with equal efficiencies. *recD* mutants are recombination-proficient but have no detectable ATP-dependent nuclease, Chi-cutting or Chi-hotspot activity; these mutants may recombine by a pathway different from that of wild-type *E. coli* (Smith 1988).) The strong Chi-dependence of transformation of wild-type *E. coli* with linear DNA supports the model in figure 4, but additional tests, such as transformation using DNA's with one Chi site or with inverted Chi sites, are needed.

9. PERSPECTIVES

The mechanism of homologous recombination and ds DNA break repair discussed here may apply, with modifications, to other organisms. RecBCD-like enzymes and RecA-like proteins are widely distributed in bacteria (Telander-Muskavitch & Linn 1981; Miller & Kokjohn 1990), and they may use a mechanism very similar to that of *E. coli*. Although Chi sites are, as

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expected, frequently found in genomes from diverse organisms, they appear to be active as recombinational hotspots only in the enteric bacteria (McKittrick & Smith 1989 and references therein). Chi is active in phage λ crosses in *Salmonella typhimurium*. Diverse enteric bacteria, both terrestrial and marine, contain RecBCD-like enzymes with Chi cutting activity, and cloned *recBCD* genes from several of these bacteria confer Chi hotspot activity to *E. coli recBCD* deletion mutants. Non-enteric bacteria contain RecBCD-like enzymes that do not detectably recognize Chi, even when, in the case of two *Pseudomonas* spp, their genes are in *E. coli*. These bacteria may use another nucleotide sequence as a recombinational hotspot, but with this modification they may repair and recombine their DNA by the same mechanism as *E. coli*.

In eukaryotes ds DNA breaks also trigger repair and recombination (Thaler & Stahl 1988). Furthermore, genes encoding proteins with amino acid sequences homologous to that of *E. coli* RecA protein have been described, and, in the case of *Saccharomyces cerevisiae*, mutants lacking these proteins are deficient in recombination and repair (Bishop *et al.* 1992; Shinohara *et al.* 1992). It is likely, therefore, that enzymes enter ds DNA ends and form ss DNA substrates for homologous pairing with intact duplexes by the RecA-like proteins. Alternative mechanisms, such as initiation by ss nicks and gaps, have not been excluded, however. To our knowledge, there are no substantiated reports of RecBCD-like enzymes (i.e. ATP-dependent nucleases) from eukaryotes. Thus, the putative ss DNA substrates for pairing may be produced by exonucleases that digest one DNA strand (perhaps in conjunction with helicases); such a mechanism has been inferred for the λ Red and *E. coli* RecE and RecF pathways (reviewed by Smith 1988; Thaler & Stahl 1988). Fungi and perhaps other eukaryotes contain hotspots of meiotic recombination with genetic properties similar to those of Chi (Smith 1994). In *S. cerevisiae* these appear, however, to be sites of ds DNA break formation controlled by chromatin structure (Wu & Lichten 1994), rather than sites acting, as Chi does, spatially and temporally downstream of the ds DNA break. Such secondary control sites in eukaryotes have not been excluded, however. The overall mechanism of homologous recombination and repair in eukaryotes may be similar to that in *E. coli*, but the details of the mechanism may be different. The control of the initiation of recombination in eukaryotes also remains to be elucidated; knowledge of that control in *E. coli* may aid its elucidation.

We are grateful to our past and present colleagues for their contributions to our work. We thank Wayne Wahls for figure 2*b*. Research in our laboratory is supported by grants from the United States Public Health Service (NIH grants GM31693 and GM32194). P.D. is a scientist of the Institut National de la Recherche Agronomique (France) and the recipient of an EMBO fellowship (ALTF 622–1992).

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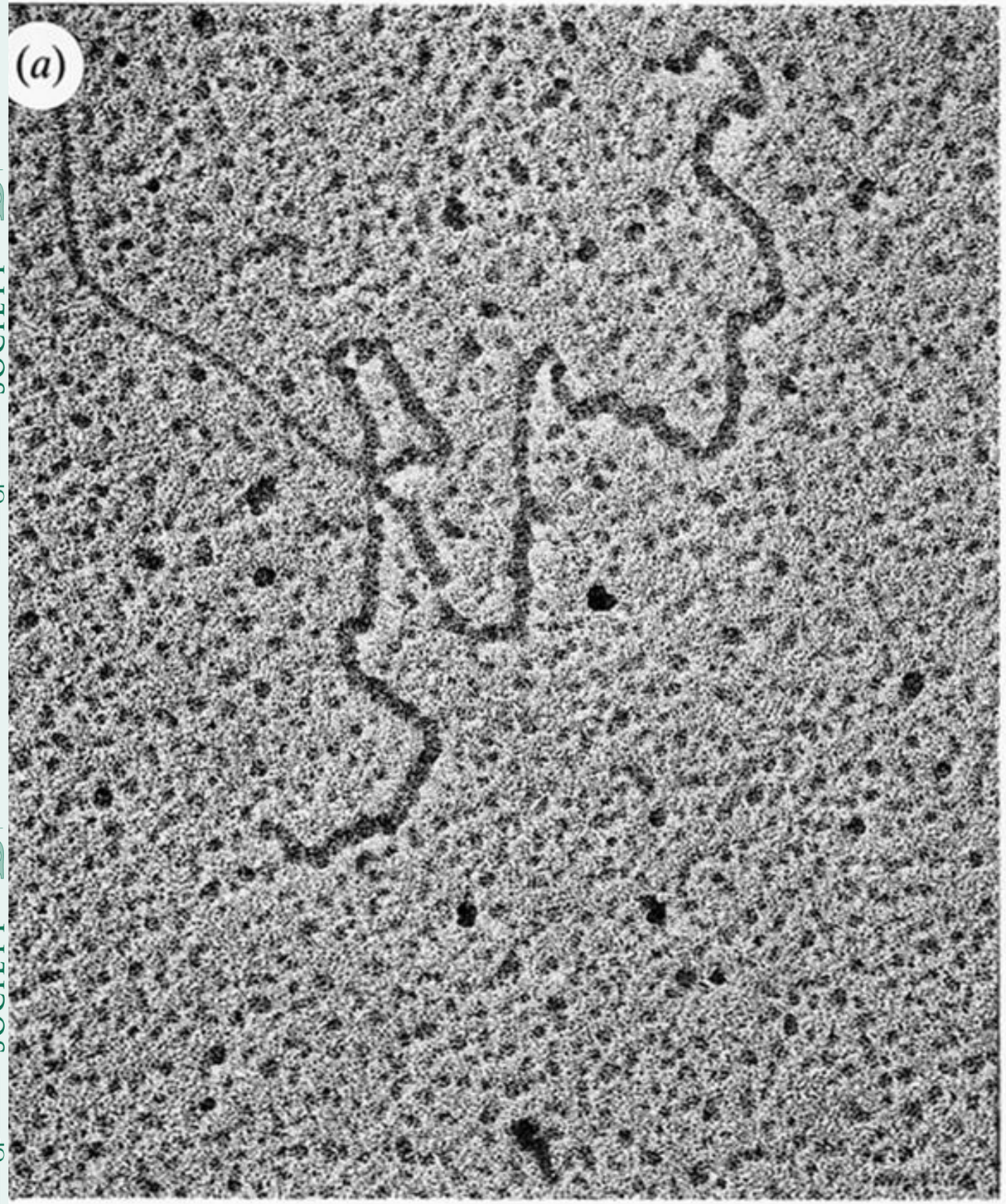

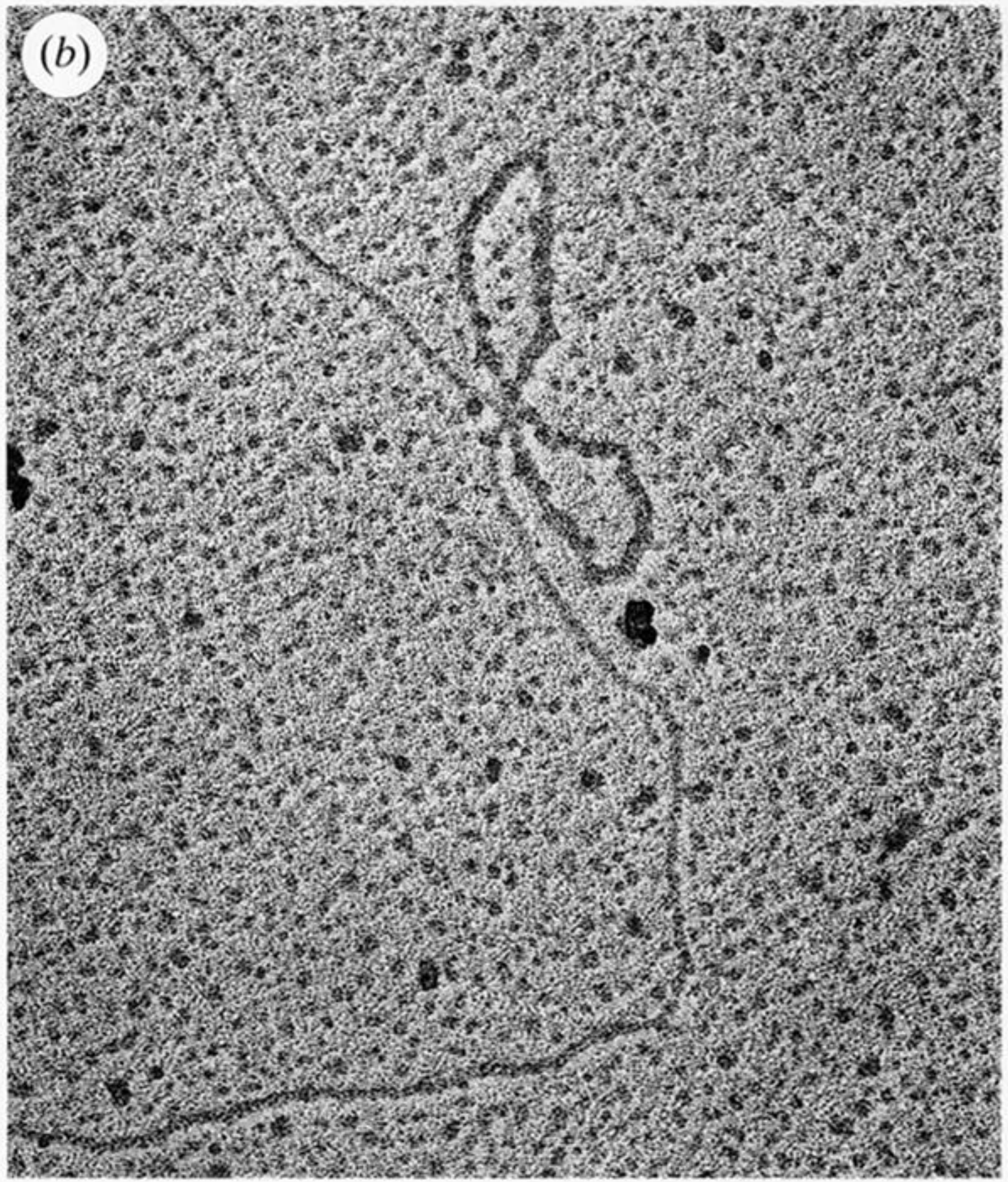

igure 1. Loop-tail (*a*) and twin-loop (*b*) structures formed by RecBCD enzyme unwinding DNA. Thin lines are ds
)NA, and thick lines are ss DNA coated with SSB. From Smith *et al.* 1984.