0014-4754/94/030204-12$1.50 + 0.20 © Birkhäuser Verlag Basel, 1994

In vitro reconstitution of homologous recombination reactions

S. C. Kowalczykowski

Division of Biological Sciences, Sections of Microbiology and of Molecular and Cellular Biology, University of California, Davis, Davis (California 95616, USA)

Abstract. The proteins essential to homologous recombination in *E. coli* have been purified and their individual activities have been identified, permitting biochemical reconstitution of steps that comprise the cellular recombination process. This review focuses on the biochemical events responsible for the initiation and homologous pairing steps of genetic recombination. The properties of an in vitro recombination reaction that requires the concerted action of recA, recBCD, and SSB proteins and that is stimulated by the recombination hotspot, Chi(χ), are described. The recBCD enzyme serves as the initiator of this reaction; its DNA helicase activity produces single-stranded DNA that is used by the recA protein to promote homologous pairing and DNA strand invasion of supercoiled (recipient) DNA. The SSB protein acts to trap the single-stranded DNA produced by recBCD enzyme and to facilitate pairing by the recA protein. The χ regulatory sequence acts in *cis* by attenuating the nuclease, but not the helicase, activity of recBCD enzyme. This attenuation assures the preservation of ssDNA produced by the DNA helicase activity and is responsible for the simulation in vitro and, presumably, in vivo. The attenuation of nuclease activity by χ results in the loss or functional inactivation of the recD subunit.
Key words. Genetic recombination; recA protein; recBCD enzyme; single-stranded DNA binding protein; recombination hotspot; DNA helicase; nuclease; homologous pairing; and DNA strand exchange.

Introduction

Genetic recombination is a fundamental biological process. All organisms rely on recombination both for maintenance of chromosomal integrity through recombinational repair and for generation of genetic diversity by random assortment of genes. Yet despite the importance of recombination, the steps that comprise this process have only recently become accessible to biochemical inquiry. This chapter focuses on the biochemical mechanism of homologous recombination in *E. coli*, with a particular emphasis on the initial steps of the major pathway. Citations to the original literature are intentionally limited and readers are encouraged to consult major review articles on recombination for comprehensive citation lists (refs 6, 22, 31, 34, 39, 43, 51, 69, 72).

Biochemical pathway for recombination in wild-type *E. coli*

All models of homologous recombination can be partitioned into at least four mechanistically distinct steps: initiation, exchange of DNA strands, DNA heteroduplex extension, and resolution (fig. 1). Initiation involves the creation of a single-stranded DNA- (ssDNA) or double-stranded DNA- (dsDNA) breaks, suitable for use by recombination enzymes. The exchange of DNA strands is a deceptively simple term for a complex process that requires recognition of DNA sequences between two potentially distant regions of DNA, disruption of existing base pairs, and formation of heteroduplex DNA. DNA heteroduplex extension can in principle occur spontaneously, but the existence of at least three proteins capable of promoting branch migration suggests otherwise. Finally, the resultant unique DNA structure, referred to as a Holliday junction, must be resolved by specific nucleolytic cleavage.

Homologous recombination in *E. coli* requires the participation of a large number of proteins (table); the specific requirements for any given recombination event is dependent on the type of DNA molecules (e.g., linear versus circular) that are participating in the reaction and on the genetic background of the strain. In wild-type strains, recombination between linear DNA molecules and the chromosome (during processes such as conjugation, transduction, and transformation by broken DNA) occurs primarily by the recBCD-pathway. Genetic studies defined a need for recA, recBCD, SSB, DNA polymerase I, DNA ligase, DNA gyrase[7,20,21] and either the ruvA, ruvB, and ruvC proteins or recG protein[33] in this process. These proteins have been purified and some of their biochemical activities have been uncovered (table).

Based on current knowledge, each of these proteins is placed in figure 1 at the step where it is thought to function; this illustration provides an overview of the likely biochemical events. The recBCD enzyme is envisioned to be the initiator of the recombination process, since it binds to a DNA end and begins unwinding, producing ssDNA (i). This heterotrimeric protein complex is a multifunctional enzyme that possesses both

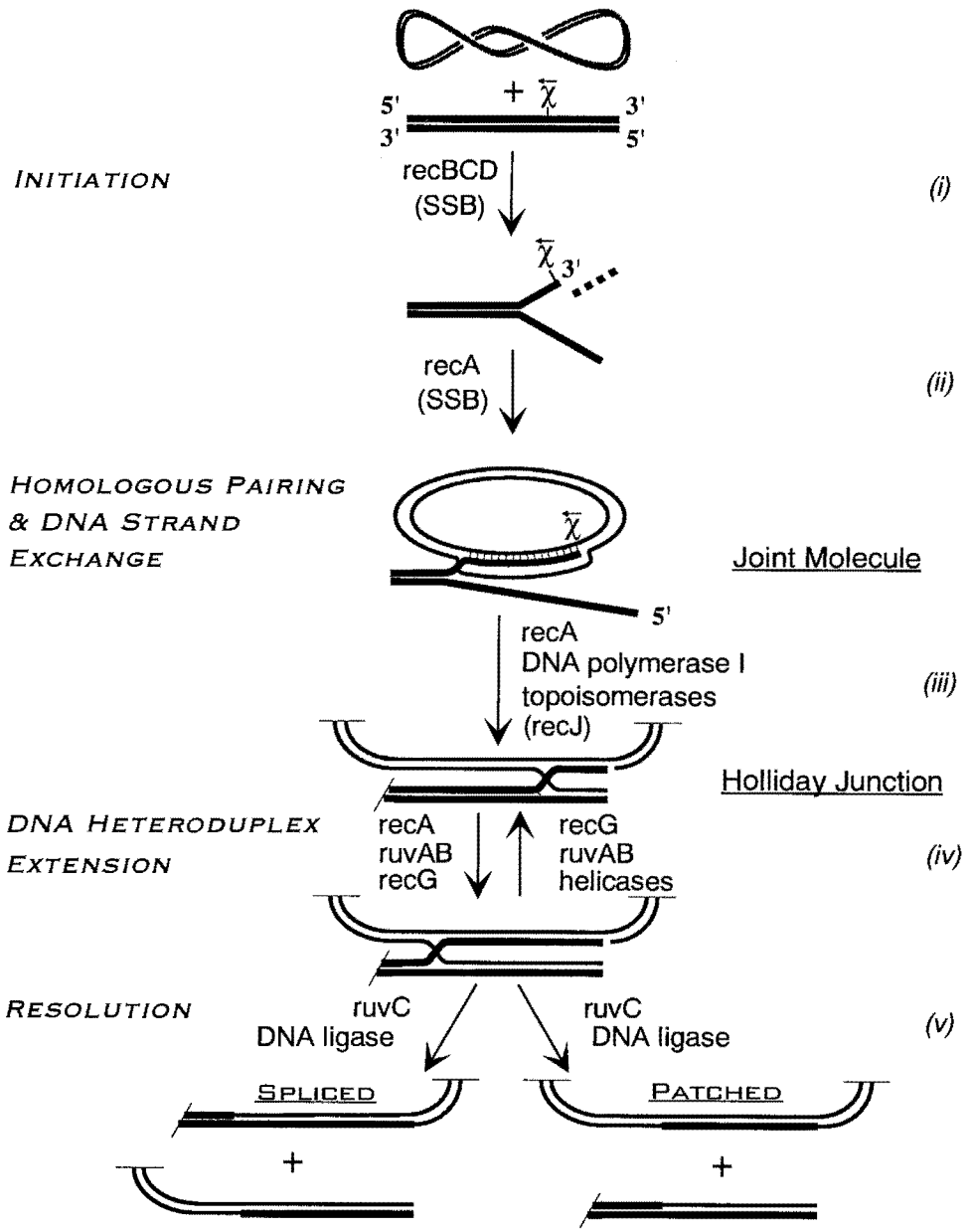

Figure 1. Biochemical model for the major pathway of homologous recombination in *E. coli*. Details are discussed in the text. (Modified from ref. 31).

DNA helicase and nuclease activities[52,61]. The recBCD enzyme is also needed for the recombination hotspot activity[32,55] that is associated with the DNA sequence (5′-GCTGGTGG-3′)[53] known as Chi (χ) (see Smith in this issue). The χ sequence is asymmetric and is recognized by recBCD enzyme only when the enzyme is traveling through the dsDNA from the 3′-side of χ[42,62]. Interaction with χ results in an attenuation of the nuclease, but not the helicase, activity of recBCD enzyme, permitting the enzyme to continue unwinding without further DNA degradation[15,16]. A consequence of this χ-dependent attenuation is that ssDNA with a 3′-end in the vicinity of χ is created (the product of step (i)). This ssDNA is the substrate for invasion of the homologous dsDNA promoted by recA and SSB proteins (ii) (see Stasiak and Egelman, and Heyer in this issue; ref. 15). Both the χ-dependent generation of the 3′ ssDNA invasive end and the χ-dependent attenuation of the recBCD enzyme nuclease activity are sufficient to explain the recombination hotspot activity of χ. After formation of the homologously paired joint molecule (product of step (ii)), a Holliday junction can form, presumably through the action of either DNA polymerase I or DNA gyrase (iii). The resultant cross strand structure can undergo either thermal or protein-mediated branch migration but, given the slow speed of the

Proteins and DNA sites involved in genetic recombination in *Escherichia coli*[a]

Protein	Activity
recA	DNA strand exchange; DNA renaturation; DNA-dependent ATPase; DNA- and ATP-dependent coprotease
recBCD (exonuclease V)	DNA helicase; ATP-dependent ssDNA and dsDNA exonuclease and ATP-stimulated ssDNA endonuclease; χ-hotspot recognition
recBC	DNA helicase
recE (exonuclease VIII) (*sbcA*)	dsDNA exonuclease, $5' \rightarrow 3'$ specific
recF	ssDNA and dsDNA binding; ATP binding
recG	branch migration of Holliday junctions; DNA helicase
recJ	ssDNA exonuclease, $5' \rightarrow 3'$ specific
recN	unknown, ATP binding consensus sequence
recO	interaction with recR and (possibly) recF proteins
recQ	DNA helicase
recR	interaction with recO and (possibly) recF proteins
recT	DNA renaturation
ruvA	Holliday-, cruciform-, and 4-way junction binding; interaction with ruvB protein
ruvB	branch migration of Holliday junctions; DNA helicase; interaction with ruvA protein
ruvC	Holliday junction cleavage; 4-way junction binding
sbcB (exonuclease I) (*xonA*)	ssDNA exonuclease, $3' \rightarrow 5'$ specific; deoxyribophosphodiesterase
sbcC	unknown, ATP binding consensus sequence
sbcD	unknown
SSB	ssDNA binding
DNA topoisomerase I (*topA*)	ω protein, type I topoisomerase
DNA gyrase (*gryA & B*)	DNA gryase, type II topoisomerase
DNA ligase (*lig*)	DNA ligase
DNA polymerase (*polA*)	DNA polymerase; $5' \rightarrow 3'$ exonuclease; $3' \rightarrow 5'$ exonuclease
helicase II (*uvrD, uvrE, recL, mutU*)	DNA helicase
helicase IV (*helD*)	DNA helicase
Chi(χ)	recombination hotspot: 5'-GCTGGTGG-3'; regulator of recBCD enzyme nuclease activity

[a]from ref. 31.

random process and its inability to proceed past DNA mismatches[41a], it is almost certain that the branch migration activity of either recA, ruvAB or recG protein is used (iv) (see West in this issue). Finally, resolution of the Holliday junction and completion of the process is accomplished by the ruvC protein (v) (see West in this issue). The result is recombinant progeny that represent either 'spliced' or 'patched' parental DNA (products of step (v)). Each of these biochemical steps together with some of the experimental details that support the model illustrated in figure 1 are elaborated below.

RecBCD enzyme, a DNA helicase with nuclease activity

The recBCD enzyme is encoded by the *recB*, *recC*, and *recD* genes (see refs 52, 61). The resulting heterotrimeric protein complex, which is also known as exonuclease V, constitutes an enzyme possessing both helicase and nuclease activities. The DNA helicase activity can unwind dsDNA with flush or nearly flush (having ssDNA overhangs of less than about 25 nucleotides) dsDNA ends in an ATP-dependent reaction[63]. The nuclease activities are quite diverse, and include ATP-dependent dsDNA exonuclease, ATP-dependent ssDNA endonuclease, and ATP-stimulated ssDNA exonuclease activities. This seemingly unlikely combination of activities is accomodated by the following mechanism (fig. 2)[48,60,64–66]. The enzyme initiates unwinding by binding to the ends of dsDNA; simultaneous with unwinding, it introduces endonucleolytic nicks randomly into the unwound ssDNA. The frequency of nicking is unrelated to the distance traveled, but is highly dependent both on the rate of protein translocation and on solution conditions. This results in the generation of ssDNA fragments that can range in size from short oligonucleotides which are acid soluble (i.e., less than about 15 nucleotides in length) to pieces thousands of nucleotides in length.

DNA helicase activity plays a prominent role in models for recBCD enzyme function in genetic recombination. The nick initiation model of Smith and colleagues proposed that recBCD enzyme uses its helicase activity to travel to a χ sequence where it nicks the DNA[54]. Subsequent unwinding past χ produces ssDNA for use by recA protein. Models of Thaler et al. proposed that the recBCD enzyme translocates through dsDNA until it encounters a crossover structure; there, depending on whether it encountered a χ sequence along the way, it either destroys or resolves the Holliday junctions[57,67,68]. However, it seemed paradoxical from the outset that a potent nuclease (recBCD enzyme), which is responsible for much of the degradation of foreign DNA in *E. coli*, is also required for homologous recombination. This apparent incongruity was resolved when it was discov-

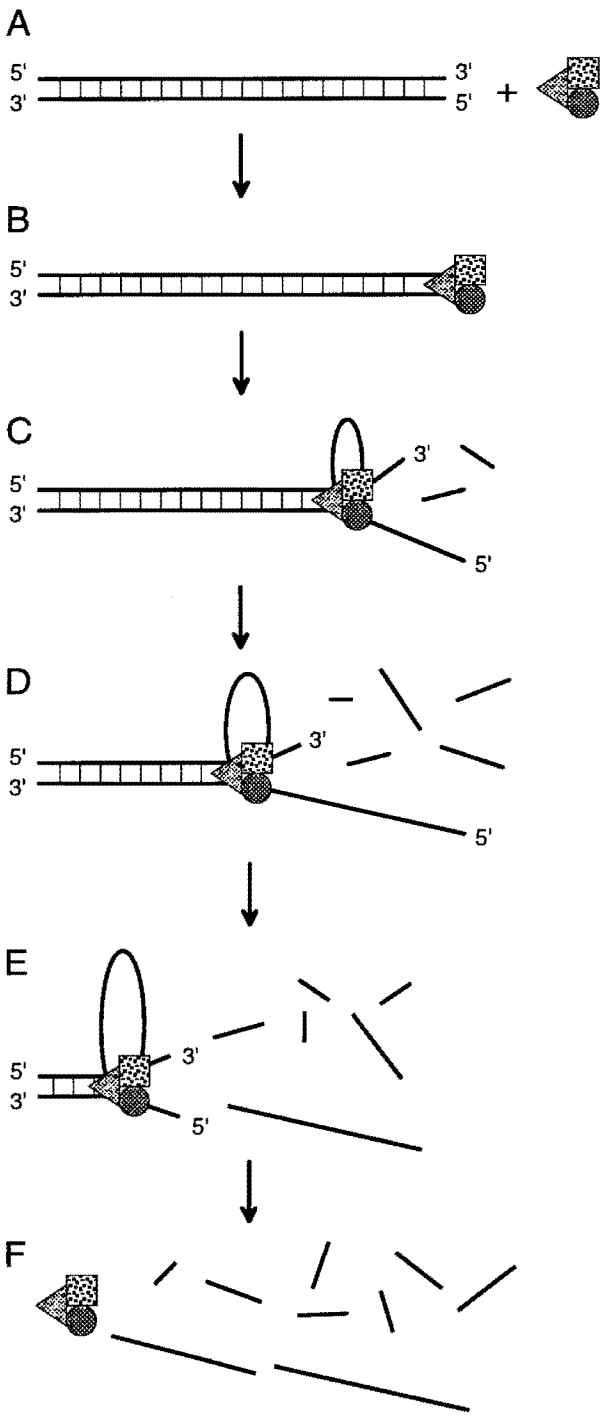

Figure. 2. Biochemical mechanism of action for recBCD enzyme.

ered that the χ sequence is a regulatory element which controls nuclease activity of recBCD enzyme; upon recognizing χ, the nuclease activity of recBCD enzyme is attenuated but its helicase activity remains[15,16]. Hence, recBCD enzyme can be viewed as a DNA helicase that possesses a regulatable nuclease activity.

Electron microscopic analysis determined that, under the conditions used, the enzyme unwound at a rate of 300 bp/sec, producing characteristic looped structures which grew at about 100 bp/sec. Kinetic analysis of the unwinding process was facilitated by a continuous, spectrofluorometric assay for DNA helicase activity[49]. The helicase activity of recBCD enzyme was found to depend on reaction conditions that typically influence all protein-nucleic acid interactions. Optimal helicase activity at 37°C is about 1000–1300 bp/sec/recBCD enzyme molecule. This rate, which has an optimum at about 80–100 mM NaCl, decreases with increasing NaCl concentrations (decreasing about 40% at 200 mM) and with decreasing temperatures. Increasing the ATP concentration results in a hyperbolic increase in the rate of DNA unwinding with a K_m of about 130 μM. The enzyme also has a relatively high apparent affinity for DNA ends, yielding a K_m of $\approx$1 nM (0.6–2.1 nM).

The unwinding of dsDNA is highly processive[47,60]. Genetic studies demonstrated the recBCD enzyme could recognize χ sites located at the distal end of bacteriophage λ relative to the *cos* entry site[56]. Biochemically, this translates into a requirement for recBCD enzyme to translocate a distance of about 30 Kb. Electron microscopy showed that recBCD enzyme could travel processively for at least 20 Kb[60,66] and subsequent quantitative analysis showed that the enzyme will unwind an average of 30 Kb before dissociating from the DNA, with about 15% of the enzymes unwinding 50 Kb[47]. As observed for the rate of DNA unwinding, the processivity of unwinding decreases with increasing NaCl (by 50% at 280 mM) and with decreasing ATP (apparent $K_N \approx 40$ μM). During the course of this processive unwinding, 2–3 molecules of ATP are hydrolyzed for each base pair unwound, suggesting that two subunits (probably the recB and recD subunits) are involved in the processive helicase activity of the holoenzyme[48], which is reduced about 15-fold when the recD subunit is absent[28]. The enzymatic parameters for apparent substrate affinity are well within the estimates for the physiological concentration of each of these substrates, which lends support to the potential importance of this activity in vivo.

The availability of these kinetic parameters for recBCD enzyme helicase activity greatly facilitated in vitro experiments designed to address the role of DNA unwinding in recombination processes. The first reactions tested a simple but important question: could the helicase activity of recBCD enzyme be used to produce ssDNA that could be used by recA protein in DNA pairing reactions? The answer, from two different reactions (fig. 3), was yes[46,50]. The first reaction used ssDNA and dsDNA under conditions that failed to support recA protein-promoted DNA strand invasion; when recBCD enzyme was added, it produced ssDNA that was utilized by recA protein to produce heteroduplex DNA via its DNA renaturation activity. The second reaction used linear dsDNA and

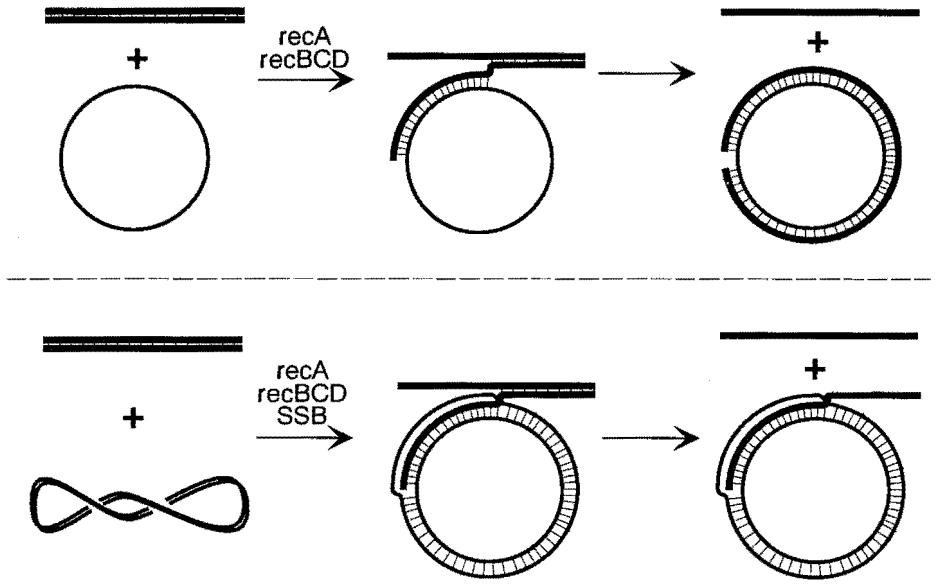

Figure. 3. DNA substrates used for reconstitution of in vitro homologous pairing reactions.

supercoiled DNA; recBCD enzyme produced ssDNA that was utilized by recA protein to produce homologously paired joint molecules via its DNA strand invasion activity.

The second reaction, in particular, displayed some significant properties[46]. Besides requiring recBCD enzyme unwinding, it was optimal at concentrations that approached one functional recBCD enzyme molecule per DNA end, demonstrating that the rate of homologous pairing was maximal when the rate of DNA unwinding was maximal[49]. Second, the maximal rate of joint molecule formation was obtained in the presence of an SSB protein concentration that saturated about 50% of the ssDNA produced by enzymatic unwinding, consistent with behavior of SSB protein in other recA protein-promoted reactions[10]. Third, the ability of recA protein to form joint molecules from the ssDNA produced by recBCD enzyme was equivalent to its use of heat denatured DNA, indicating that ssDNA produced by either method was equivalent with respect to recA protein function[46]. Fourth, the optimal concentration of recA protein represented the amount needed to nearly saturate the ssDNA produced, consistent with the properties of other homologous pairing reactions (see Stasiak and Egelman in this issue). Fifth, the behavior of mutant recA proteins in these homologous pairing reactions corresponded to their genetic phenotype (see ref. 29). Finally, the actions of the recA, recBCD, and SSB proteins were coordinated, in that optimal joint molecule formation was obtained only when all three proteins were present simultaneously[46]. Although no evidence exists for protein-protein interactions, these proteins 'cooperate' to promote this in vitro reaction. RecBCD enzyme unwinds the dsDNA and SSB serves as the primary trapping protein. Trapping by SSB protein, in turn, allows recA protein to bind; however, recA protein must be present during the unwinding phase because it must bind to the ssDNA coincidently with SSB protein so that it can rapidly displace the SSB protein.

These studies established the basic properties of joint molecule formation by recA, recBCD, and SSB proteins in vitro and afforded an experimental route to the next important topic: Does the presence of a χ sequence have any effect on joint molecule formation in this in vitro recombination reaction?

Chi, a regulator of recBCD enzyme nuclease and recombination activities

The χ recombination hotspot stimulates recombination in its vicinity in a polar manner (see Smith in this issue). Recombination is 5 to 10 times more frequent near χ and is dependent on *recB* and *recC*[17,32,55]. Simulation is primarily to the 5′ side of the χ site and extends as far away as 10 kb[5,55]. In vivo, it is assumed that all recBCD enzyme-dependent recombination requires interaction with χ or χ-like sequences. In vitro, recognition of χ produces a ssDNA fragment that terminates near χ; cleavage occurs in the DNA strand containing the χ sequence, 4 to 6 nucleotides to the 3′ side of χ[42,62].

The nick initiation model for Chi action predicts that during the process of DNA unwinding, a nick will be introduced at χ[54]. For the DNA substrates typically used in vitro (4–7 Kb), the nick at χ should result in three discrete ssDNA fragments due to the high processivity of unwinding (disregarding the heterogeneously sized fragments produced by random nucleolytic nicking) (fig. 4). Two result from cleavage of the χ-containing strand (top

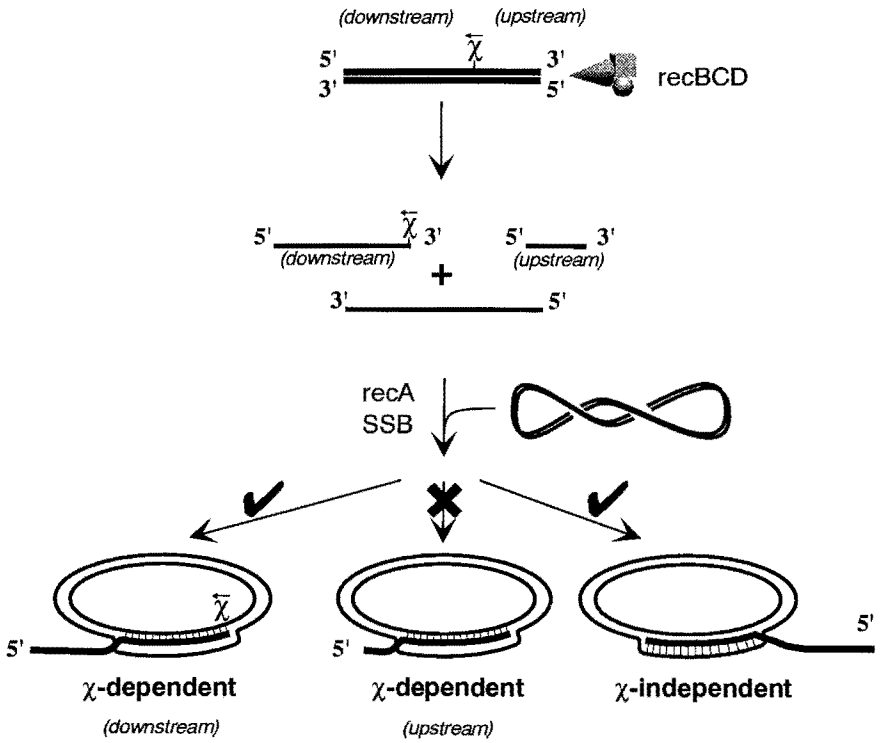

Figure 4. Expected pattern of joint molecule formation resulting from DNA unwinding, with a nick at χ, mediated by recBCD enzyme. The joint molecules observed experimentally are indicated with a ✓ and the one not observed is marked by an ×.

strand in figure 4), and the sizes of these fragments will be χ-specific (i.e., they will depend on the location of χ). The third discrete-sized ssDNA should be the full-length strand that originates from the bottom strand in figure 2. This pattern is expected for recBCD enzyme entering from the right side of dsDNA; recBCD enzyme which enters from the left side of the DNA (50% of the events) will fail to recognize the improperly oriented χ sequence.

In vitro reactions using recA, recBCD, and SSB proteins and the DNA substrates depicted in figure 4 readily detect formation of a single χ-specific joint molecule as the predominant species[15]. This result experimentally established one of the principle tenets of the nick-initiation model. The formation of joint molecules derived from full length ssDNA generated by the expected complete unwinding of dsDNA is also detected. Significantly, however, the χ-specific ssDNA fragment derived from the upstream side of χ is not observed under the reaction conditions employed, neither as a participant in a joint molecule nor as a ssDNA fragment[15,16]. This, and other observations, lead to the conclusions that the DNA 3′ to χ is degraded and, furthermore, the interaction with χ causes an attenuation of the nuclease activity of recBCD enzyme. This attenuation lasts as long as the enzyme remains associated with the DNA molecule that contains χ. The nuclease-attenuated enzyme continues to unwind the DNA (for at least 10 Kb), ultimately dissociating when it reaches the end of the DNA duplex. Thereafter, the nuclease activity is reactivated, and the cycle of unwinding, χ-recognition, and nuclease attenuation is repeated indefinitely[15].

These experiments also uncovered another unrecognized feature of recBCD enzyme nuclease activity: degradation during DNA unwinding is highly asymmetric[16]. The strand 3′ at the entry site is degraded much more extensively than the strand 5′ at the entry site. Furthermore, upon encountering a χ site, this asymmetric degradation is significantly attenuated. These observations offer a very simple explanation for the failure to detect a ssDNA fragment from the upstream side of χ – it is simply degraded. However, upon attenuation of nuclease activity, the DNA downstream of χ is saved from degradation. Thus, χ is a regulatory sequence.

The differential degradation is sensitive to solution conditions, with one of the primary determinants being the free Mg^{+2} concentration[18] (determined experimentally by the ratio of the concentrations of Mg^{+2} relative to ATP). The nuclease activity of recBCD enzyme displays maximal nucleolytic activity at free Mg^{+2} concentrations above about 0.1–0.5 mM and decreases continuously to near zero at a free Mg^{+2} concentration of about 5 μM[18]. This observation readily explains the detection of fragments derived only from the downstream side of χ at higher Mg^{+2} concentrations, whereas detection of both upstream and downstream fragments

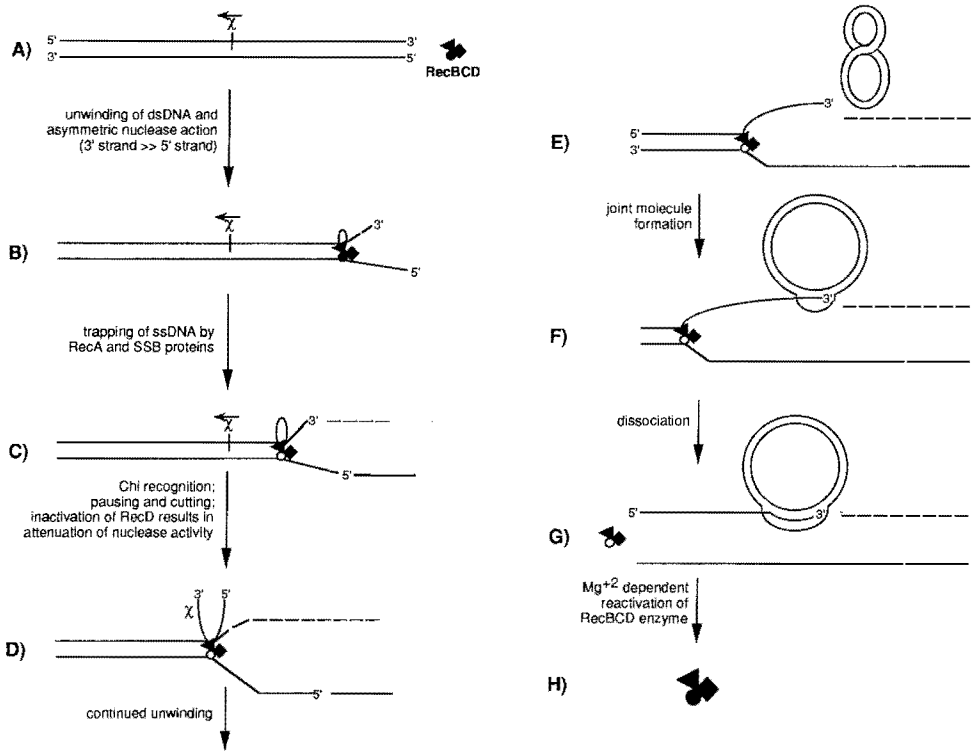

Figure 5. Nuclease attenuation model for initiation of homologous recombination by recBCD enzyme. Details are discussed in the text. (Modified from ref. 15).

occurs at lower free Mg^{+2} concentrations[13]. If both the frequency of cleavage and the distance to the χ site are low, then an upstream χ-specific fragment is recovered[42]. But regardless of the solution conditions used, interaction with χ resulits in a modification of recBCD enzyme that attenuates its nuclease activity. The attenuation is greater than 500-fold at higher Mg^{+2} concentrations and as low as 5–10-fold at the lowest concentrations examined[13]; conceivably at very low free Mg^{+2} concentrations, degradation would be minimal and attenuation could go undetected.

These results are accommodated by the nuclease attenuation model for initiation of genetic recombination depicted in figure 5[13–16]. The recBCD enzyme binds to the ends of dsDNA and begins unwinding and degrading the DNA. Unwinding and the introduction of endonucleolytic breaks is coincident and results in the production of ssDNA fragments. Degradation of dsDNA is asymmetric, with the DNA strand 3′ at the entry site being nicked more frequently than the 5′ strand. This process of unwinding and random endonucleolytic nicking of primarily (but not excusively) the 3′-terminal strand adequately describes the biochemical characteristics of the ATP-dependent dsDNA exonuclease activity of recBCD enzyme. This course of action continues until a χ sequence is encountered. Recognition of the χ sequence results in a pause of the recBCD enzyme. This pause, in turn, assures a high probability of an endonucleolytic cleavage to occur in the vicinity (4 to 6 nucleotides) of χ. Recognition of χ causes an alteration of the biochemical activity of recBCD. The nuclease activity is reduced by as much as 500-fold so that now the enzyme is primarily a helicase rather than a helicase with nuclease activity[13,15,16]. Continued unwinding of the dsDNA, without further degradation, results in the formation of an intact ssDNA fragment originating from the 3′-side of χ and continuing downstream to the point of recBCD enzyme dissociation. This distance is at least 10 Kb and, for the unaltered enzyme, averages 30 Kb[47]. The act of continued unwinding by the nuclease-attenuated enzyme results in production of the ssDNA strand that is the preferred substrate for recA protein-promoted DNA strand invasion. Attenuation of nuclease activity persists as long as the χ-altered recBCD enzyme remains associated with the DNA molecule that effected the attenuation. However, upon exiting or dissociating from the DNA, the recBCD enzyme typically reverts to the state that is seen prior to interaction with χ, i.e., the nuclease activity is restored[13,15,16] (however, see below and ref. 14). This reactivated enzyme is indistinguishable from the enzyme prior to its interaction with χ and is fully capable of subsequent rounds of DNA unwinding, nucleolytic degradation, χ-recognition, and nuclease attenuation. Thus, under these conditions, the process is completely catalytic and the protection afforded by χ is a *cis*-effect.

This model is consistent with all of the known in vitro and in vivo properties of recBCD enzyme and χ sites. It explains the polarity of χ hotspot activity with the direct demonstration that at χ, but not before, the nuclease activity of recBCD enzyme is substantially reduced. Consequently, the DNA that is downstream of χ is protected relative to the DNA that is upstream of χ. Thus, χ exerts its effect on genetic recombination not because of a biochemical activiation at χ but, rather, because of a biochemical inactivation; this inactivation of nuclease activity is presumably responsible for the cellular stimulation of recombination because it results in preservation of a DNA intermediate that is an essential ingredient of the initiation phase of homologous pairing. Hence χ is a regulatory sequence for genetic recombination as was initially proposed to explain the behavior of *recD*‡ mutuations[67,68]; however, rather than acting at a resolution step, the in vitro data argue for an initiation function[13,15,16].

The interaction between recBCD enzyme and χ observed in vitro is paralleled in vivo. In vivo, the presence of a χ sequence upstream from a second χ causes the stimulation of recombination resulting from the downstream χ to be reduced[57,76]. In agreement, in vitro, a χ sequence downstream from another χ site is not recognized when the first χ is recognized[63a]. Both of these observations are consistent with the attenuation model: once the nuclease activity is attenuated, both non-specific and χ-specific cleavages are reduced dramatically. The protection of DNA downstream, but not upstream, of χ from degradation by recBCD enzyme was also deduced from in vivo work. Recombination of bacteriophage λ to produce χ-containing recombinant progeny requires the participation of three individual λ phage: two parents that contain χ and one that need not[57]. This study indicated that the DNA upstream of χ is degraded by recBCD in one of the χ^+ parents, but that DNA downstream of χ is not[57]. A similar conclusion was derived from studies of plasmids replicating by a rolling-circle mechanism. The presence of χ in the proper orientation with respect to the free dsDNA end results in the formation of high molecular weight linear multimers, but when the orientation of χ is reversed, degradation by recBCD enzyme ensues[12]. Thus both the in vivo and in vitro data suggest that DNA is degraded by recBCD enzyme up to χ, but, upon interaction with χ, this degradation is diminished. These observations also offer an explanation for why DNA upstream of χ is not highly recombinogenic both in vivo and in vitro: it is degraded. Furthermore, consistent with the in vitro studies, χ increases the amount of heteroduplex DNA in vivo, in a process that requires both *recA* and *recB* function[22a]. These observations are readily explained by the nuclease attenuation model (fig. 5).

The strong functional relationship between χ recombination hotspot activity and χ-recognition by recBCD enzyme is established by extensive mutational analysis (see ref. 52). The conformity between χ-dependent stimulation of recombination in vivo and the detection of χ-specific fragments (i.e., nicking activity) in vitro is virtually exact (see Smith in this issue). The nick at χ was envisioned to generate a 3′-terminal ssDNA fragment that participated in recA protein-dependent DNA strand invasion[54], a prediction that was demonstrated in vitro[15]. However, the attenuation model suggests that the close correlation between these phenomena has, at its root, a more fundamental origin. The nick at χ and the persistence of the downstream χ-specific ssDNA result from a pause at χ, with subsequent attenuation of nuclease activity[15,16]. The production of χ-specific fragments in vitro is therefore a manifestation of the attenuation event. Thus, this view sees in vivo hotspot activity, in vitro χ-specific cutting, and nuclease attenuation as consequences of χ-recognition producing the biochemically altered recBCD enzyme[14–16].

The properties of a purified mutant recBCD enzyme are consistent with the expectations of the nuclease attenuation model 1[18,19]. The *recB2109* mutation is phenotypically defective in homologous recombination[1]. In vitro, the purified $recB^{2109}CD$ enzyme retains all of the activities of the wild-type enzyme, save one[18,19]. Within a factor of 2–3, the $recB^{2109}CD$ enzyme has the same DNA helicase, dsDNA exonuclease, ssDNA exonuclease, and ssDNA endonuclease activities at physiological levels of ATP (although it does have an ≈6-fold lower affinity for ATP than the wild-type enzyme). The mutant enzyme demonstrates no ability to recognize χ as manifested by the inability to produce χ-specific ssDNA fragments[1,18]. As a consequence, this mutant enzyme continues to degrade the ssDNA produced by DNA unwinding. Thus, $recB^{2109}CD$ enzyme can be viewed as a constitutively active nuclease that fails to promote recombination since χ is unable to regulate its nuclease activity, either because $recB^{2109}CD$ enzyme cannot recognize χ or because the mutant enzyme cannot attenuate its nuclease activity[18,19].

The unusual phenomenon of attenuation of nuclease activity invites an explanation as to its biochemical basis. One potential explanation has at its origin the unique attributes of *rec*‡ mutations[2,4]. This class of mutants primarily map to the *recD* gene and is phenotypically recombination proficient[2,4]; in fact, these mutants are hyper-recombinogenic in some assays despite their insensitivity to the presence of χ[2,4,37,68]. To explain the behavior of these mutations, Thaler et al. hypothesized that interaction with χ converted the antirecombinogenic recBCD enzyme to the recombinogenic recBC enzyme, accompanied by loss of the recD subunit[68]. This recBC enzyme was proposed to productively resolve Holliday junctions. An element of this hypothesis was recently tested in vitro[14]. The recBC enzyme (reconstituted without the recD subunit) bio-

chemically mimics certain characteristics of the recBCD enzyme that has productively interacted with χ[14]. It has helicase activity but little or no nuclease activity[14,28,41] and its activities can be completely inhibited, affording protection to DNA in trans[14]. In agreement with the failure of *rec*‡ mutants to recognize χ in vivo, the recBC enzyme does not produce χ-specific fragments in vitro[14]. Despite this defect, because of the near absence of nuclease activity, the recBC enzyme efficiently promotes joint molecule formation in a coupled recA, recBC, and SSB protein-mediated reaction in vitro[13]. Thus, the biochemical evidence suggests that interaction with χ results in either the loss or functional inactivation of the recD subunit and that, as a consequence of this event, the nuclease activity of the resultant (χ-modified) enzyme is lost[14]. Nevertheless, the altered enzyme is still capable of producing ssDNA by DNA unwinding which can be used by recA protein, arguing for an initiation purpose for χ[13,15,16].

These in vitro results further illuminate the replication-blocked intracellular behavior of the recBC(D‡) enzyme. Recombination of λ phage in *recD*‡ strains occurs primarily at the ends of λ[68], consistent with the retention of helicase but not nuclease activity by the recBC enzyme (i.e., the DNA ends are not degraded). Conjugal and P1-mediated, but not λ phage, recombination in *recD*‡ strains requires *recJ* function[37,68]. The recJ protein is a $5' \rightarrow 3'$ ssDNA specific exonuclease[38] and the combined action of recBC and recJ enzymes should produce ssDNA with 3′-tails. Thus, the nuclease activity lost by the recBC(D^-) enzyme can be compensated by the non-specific nuclease activity of recJ protein. The specific need for this nuclease activity is unclear but perhaps degradation of one DNA strand prevents reannealing of the two complementary ssDNA strands, an event that would compete with recA protein-dependent DNA strand invasion. Finally, the recombination proficiency of *recD*‡ strains without any χ recognition appears to contradict the primacy of χ in *recBCD*-dependent recombination events. This apparent violation is readily explained, however, by proposing that the major function of χ is to convert the nucleolytically-active recBCD enzyme to the nucleolytically-attenuated recBC enzyme[15,16]. The nuclease-deficient, helicase-proficient recBC enzyme, therefore, represents the constitutively χ-altered recBCD enzyme[14]. Thus, the characteristics of the *recD* mutations and the recBC enzyme are in complete accord with the nuclease attenuation model.

RecA protein, promoter of DNA strand invasion

The recA protein is a remarkably complex protein. The wide scope of its activities prevents a detailed discussion here (but see Stasiak and Egelman in this issue), so only the properties relevant to its participation in the coupled in vitro reaction will be discussed. The recA protein can promote homologous pairing and DNA strand exchange between a variety of DNA substrates (see reviews[30,31a,43–45,58,59,72]). The in vitro reaction that is most relevant to the events envisioned in figure 1 is the reaction between linear ssDNA and covalently closed supercoiled DNA. Within a few minutes, recA protein can promote homologous plectonemic pairing (i.e., the paired DNA strands are intertwined as in conventional dsDNA) between this pair of substrates, provided that the ssDNA is linear and the other is negatively supercoiled[24–27]. The supercoiled DNA molecule serves as the recipient and is invaded by the linear donor ssDNA molecule. The 3′-end of the ssDNA is more invasive than the 5′-end due to the polymerization properties of recA protein[27]. Since recA protein polymerizes $5' \rightarrow 3'$ along ssDNA and initial binding is random[70], the 3′-end of linear ssDNA has a higher probability of coverage by recA protein than the opposite end. Furthermore, if SSB protein is present, this preference is exacerbated because the binding of SSB protein is more rapid and subsequent displacement of the SSB protein by recA protein occurs in the same $5' \rightarrow 3'$ direction[70].

Formation of joint molecules in the coupled recA-, recBCD-, and SSB protein-reaction requires participation of recA protein in a similar way. The ssDNA generated by dsDNA unwinding is a suitable substrate for recA protein-promoted pairing with supercoiled DNA[15,46]. The joint molecules formed are plectonemic in nature, as evidenced by their stability in the absence of bound recA protein[15,46]. The 3′-end generated by degradation up to the χ-site is the invasive end in the χ-specific pairing step[15], as was predicted by the nick-initiation model[54]. The DNA sequences at this 3′-end define the homology requirements for the pairing process in vitro: if the DNA sequence immediately downstream of χ is homologous to pBR322 DNA, then pBR322 DNA sequences are required in the supercoiled DNA recipient; if those sequences are M13 DNA, then M13 DNA sequences are required for χ-specific DNA strand invasion[15].

Holliday junction formation and resolution

The formation of homologously paired joint molecules by the combined actions of recA, recBCD, and SSB proteins has been reproduced in vitro[15,46], although, in this reaction, formation of Holliday junctions is yet to be demonstrated. But recA protein is capable of forming Holliday junctions in vitro[73,74], so it certainly remains plausible that, as shown in figure 1, the step following joint molecule formation is Holliday junction formation. Formation of the Holliday junction should simply require pairing of the DNA strand displaced from the supercoiled DNA, with the remaining DNA strand from the linear dsDNA. This may require either

extension of the invading ssDNA strand by DNA polymerase I to regerate the ssDNA lost to degradation by recBCD enzyme, or the involvement of a topoisomerase (see Gangloff, Lieber and Rothstein in this issue) to permit plectonemic pairing of the longer 5'-terminal strand depicted in figure 1, or both. Though topoisomerases have been shown to permit recA protein-promoted plectonemic pairing between topological constrained DNA substrates[3,11,75], their participation in a coupled system is yet to be demonstrated.

After formation of the Holliday junction, all models of homologous recombination propose branch migration of the crossover point to extend the region of DNA heteroduplex. There now exist three candidate proteins that are capable of effecting branch migration: recA[9], recG[35,36], and ruvAB proteins[23,40,71]. In vitro, any of these proteins can promote branch migration, though the characteristics of their reactions are somewhat different (see West in this issue). In vivo, *recG* and either *ruvA*, *B*, or *C* mutations dramatically reduce recombination levels[33], implying that if the major function of the recG and ruvAB protein is branch migration, then recA protein is not an effective substitute.

The final step in figure 1 requires resolution of the Holliday junction. Since ruvC protein is the only known activity in *E. coli* that demonstrates specificity for such unique structures[8], ruvC protein is a likely resolver of Holliday junctions (see West in this issue).

Future directions

This brief review has only touched upon some of the unique features of recombination enzymology that were uncovered by in vitro reconstitution of recombination reactions. I have attempted to highlight the needed coordination between the rapid helicase activity of recBCD enzyme and the relatively slower pairing activity of recA protein; the singular role that the χ recombination hotspot plays as a regulator of recombination processes; and the dual nature of the recBD enzyme as a helicase with nuclease activity. I have focused on biochemical events important to initiation of recombination, leaving discussion of details important to the behavior of recombination hotspots, homologous pairing and branch migration, and Holliday junction resolution to others (see Smith, Stasiak and Egelman, Heyer, Gangloff, Lieber and Rothstein, and West, respectively, in this issue). Since essentially all of the individual steps illustrated in figure 1 have been reproduced using purified components, reconstitution of even more complex reactions seems imminent. Not only will the complete reconstitution of homologous recombination in vitro represent a major milestone, but certainly new, unexpected discoveries will emanate from the biochemical and genetic studies that will be required to achieve this endpoint.

Acknowledgments. The author is grateful for the helpful comments that were provided by G. Smith, F. Stahl, A. Eggleston, J. New, B. Rehrauer, R. Tracey and by members of the Thibodeaux Laboratory, and is indebted to A. Eggleston for providing figure 2. The work in this laboratory is supported by NIH grants AI-18987 and GM-41347.

1 Amundsen, S. K., Neiman, A. M., Thibodeaux, S. M., and Smith, G. R., Genetic dissection of the biochemical activities of recBCD enzyme. Genetics *126* (1990) 25–40.
2 Amundsen, S. K., Taylor, A. F., Chaudhury, A. M., and Smith, G. R., *recD*: The gene for an essential third subunit of exonuclease V. Proc. natl Acad. Sci. USA *83* (1986) 5558–62.
3 Cassuto, E., Formation of covalently closed heteroduplex DNA by the combined action of gyrase and recA protein. EMBO J. *3* (1984) 2159–2164.
4 Chaudhury, A. M., and Smith, G. R., A new class of *E. coli recBC* mutants: Implications for the role of recBC enzyme in homologous recombination. Proc. natl Acad. Sci. USA *81* (1984) 7850–7854.
5 Cheng, K. C., and Smith, G. R., Distribution of Chi-stimulated recombinational exchanges and heteroduplex endpoints in phage lambda. Genetics *123* (1989) 5–17.
6 Clark, A. J., and Low, K. B., Pathways and systems of homologous recombination in *Escherichia coli*, in: The Recombination of Genetic Material, pp. 155–215. ed. K. B. Low. Academic Press, Inc., San Diego 1988.
7 Clark, A. J., and Margulies, A. D., Isolation and characterization of recombination deficient mutants of *E. coli* K-12. Proc. natl. Acad. Sci. USA *53* (1965) 451–459.
8 Connolly, B., Parsons, C. A., Benson, F. E., Dunderdale, H. J., Sharples, G. J., Lloyd, R. G., and West, S. C., Resolution of Holliday junctions *in vitro* requires the *Escherichia coli ruvC* gene product. Proc. natl Acad Sci USA *88* (1991) 6063–7.
9 Cox, M. M., and Lehman, I. R., RecA protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange. Proc. natl Acad. Sci. USA *78* (1981) 3433–3437.
10 Cox, M. M., Soltis, D. A., Livneh, Z., and Lehman, I. R., On the role of single-stranded DNA binding protein in recA protein-promoted DNA strand exchange. J. biol. Chem. *258* (1983) 2577–2585.
11 Cunningham, R. P., Wu, A. M., Shibata, T., DasGupta, C., and Radding, C. M., Homologous pairing and topological linkage for DNA molecules by combined action of *E. coli* recA protein and topisomerase I. Cell *24* (1981) 213–223.
12 Dabert, P., Ehrlich, S. D., and Gruss, A., Chi squence protects against *recBCD* degradation of DNA *in vivo*. Proc. natl Acad. Sci. USA *89* (1992) 12073–12077.
13 Dixon, D. A. Biochemical and mechanistic examination of the interaction between the recBCD enzyme and the recombination hotspot, Chi of *Escherichia coli* (Ph.D. Thesis). Northwestern University, Evanston 1993.
14 Dixon, D. A., Churchill, J. J., and Kowalczykowski, S. C., Reversible inactivation of the *Escherichia coli* recBCD enzyme by the recombination hotspot, Chi, in vitro: Evidence for functional inactivation or loss of the recD subunit. Proc. natl Acad. Sci., USA (1994) in press.
15 Dixon, D. A., and Kowalczykowski, S. C., Homologous pairing in vitro stimulated by the recombination hotspot, Chi. Cell *66* (1991) 361–71.
16 Dixon, D. A., and Kowalczykowski, S. C., The recombination hotspot, Chi, is a regulatory sequence that acts by attenuating the nuclease activity of the *Escherichia coli* recBCD enzyme. Cell *73* (1993) 87–96.
17 Dower, N. A., and Stahl, F. W., Chi activity during transduction-associated recombination. Proc. natl Acad. Sci. USA *78* (1981) 7033–7.
18 Eggleston, A. K., and Kowalczykowski, S. C., Biochemical characterization of a mutant recBCD enzyme, the recB^{2109}CD enzyme, which lacks χ-specific, but not non-specific, nuclease activity. J. molec. Biol. *231* (1993) 605–620.

19 Eggleston, A. K., and Kowalczykowski, S. C., The mutant recBCD enzyme, recB^{2109}CD enzyme, has helicase activity but does not promote efficient joint molecule formation *in vitro*. J. molec. Biol. *231* (1993) 621–633.
20 Emmerson, P. T., Recombination deficient mutants of *Escherichia coli* K-12 that map between *thyA* and *argA*. Genetics *60* (1968) 19–30.
21 Ennis, D. G., Amundsen, S. K., and Smith, G. R., Genetic functions promoting homologous recombination in *Escherichia coli*: A study of inversions in phage lambda. Genetics *115* (1987) 11–24.
22 Fishel, R., General Genetic Recombination, in: Modern Microbial Genetics, pp. 91–121. Wiley-Liss, New York 1991.
22a Holbeck, S. L., and Smith, G. R., Chi enhances heteroduplex DNA levels during recombination. Genetics *132* (1992) 879–891.
23 Iwasaki, H., Takahagi, M., Nakata, A., and Shinagawa, H., *Escherichia coli* ruvA and ruvB proteins specifically interact with Holliday junctions and promote branch migration. Genes Dev. *6* (1992) 2214–20.
24 Konforti, B. B., and Davis, R. W., 3′ homologous free ends are required for stable joint molecule formation by the recA and single-stranded binding proteins of *Escherichia coli*. Proc. natl Acad. Sci. USA *84* (1987) 690–4.
25 Konforti, B. B., and Davis, R. W., The preference for a 3′ homologous end is intrinsic to recA-promoted strand exchange. J. biol. Chem. *265* (1990) 6916–20.
26 Konforti, B. B., and Davis, R. W., DNA substrate requirements for stable joint molecule formation by the recA and single-stranded DNA-binding proteins of *Escherichia coli*. J. biol. Chem. *266* (1991) 10112–21.
27 Konforti, B. B., and Davis, R. W., ATP hydrolysis and the displaced strand are two factors that determine the polarity of recA-promoted DNA strand exchange. J. molec. Biol. *227* (1992) 38–53.
28 Korangy, F., and Julin, D. A., Kinetics and processivity of ATP hydrolysis and DNA unwinding by the recBC enzyme from *Escherichia coli*. Biochemistry *32* (1993) 4873–4880.
29 Kowalczykowski, S. C., Biochemical and biological function of *Escherichia coli* recA protein: Behavior of mutant recA proteins. Biochimie *73* (1991) 289–304.
30 Kowalczykowski, S. C., Biochemistry of genetic recombination: Energetics and mechanism of DNA strand exchange. A. Rev. Biophys. biophys. Chem. *20* (1991) 539–575.
31 Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D., and Rehrauer, W. M., Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. (1994) in press.
31a Kowalczykowski, S. C., and Eggleston, A. K., Homologous pairing and DNA strand-exchange proteins. A. Rev. Biochem. *63* (1994) in press.
32 Lam, S. T., Stahl, M. M., McMilin, K. D., and Stahl, F. W., *Rec*-mediated recombinational hot spot activity in bacteriophage lambda. II. A mutation which causes hot spot activity. Genetics *77* (1974) 425–33.
33 Lloyd, R. G., Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli* K-12 depends on *recG*. J. Bact. *173* (1991) 5414–8.
34 Lloyd, R. G., and Sharples, G. J., Genetic analysis of recombination in prokaryotes. Curr. Opin. Genet. Dev. *2* (1992) 683–90.
35 Lloyd, R. G., and Sharples, G. J., Dissociation of synthetic Holliday junctions by *E. coli* recG protein. EMBO J. *12* (1993) 17–22.
36 Lloyd, R. G., and Sharples, G. J., Processing of recombination intermediates by the recG and ruvAB proteins of *Escherichia coli*. Nucl. Acids Res. *21* (1993) 1719–1725.
37 Lovett, S. T., DeLuca, C. L., and Kolodner, R. D., The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. Genetics *120* (1988) 37–45.
38 Lovett, S. T., and Kolodner, R. D., Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli*. Proc. natl Acad. Sci. USA *86* (1989) 2627–2631.
39 Mahajan, S. K., Pathways of homologous recombination in *Escherichia coli*, in: Genetic Recombination, pp. 87–140. Eds R. Kucherlapati and G. R. Smith. American Society for Microbiology, Washington, D.C. 1988.
40 Müller, B., Tsaneva, I. R., and West, S. C., Branch migration of Holliday junctions promoted by the *Escherichiva coli* ruvA and ruvB proteins. I. Comparison of ruvAB- and ruvB-mediated reactions. J. biol. Chem. *268* (1993) 17179–17184.
41 Palas, K. M., and Kuhsner, S. R., Biochemical and physical characterization of exonuclease V from *Escherichia coli*. Comparison of the catalytic activities of the recBC and recBCD enzymes. J. biol. Chem. *265* (1990) 3447–54.
41a Panyutin, I. G., and Hsien, P., Formation of a single base mismatch impedes spontaneous DNA branch migration. J. molec. Biol. *230* (1993) 413–424.
42 Ponticelli, A. S., Schulz, D. W., Taylor, A. F., and Smith, G. R., Chi-dependent DNA strand cleavage by recBC enzyme. Cell *41* (1985) 145–151.
43 Radding, C. M., Helical RecA nucleoprotein filaments mediate homologous pairing and strand exchange. Biochim. biophys. Acta *1008* (1989) 131–145.
44 Radding, C. M., Helical interactions in homologous pairing and strand exchange driven by recA protein. J. biol. Chem. *266* (1991) 5355–8.
45 Roca, A. I., and Cox, M. M., The recA protein: Structure and function. Crit. Rev. Biochem. molec. Biol. *25* (1990) 415–56.
46 Roman, L. J., Dixon, D. A., and Kowalczykowski, S. C., RecBCD-dependent joint molecule formation promoted by the *Escherichia coli* recA and SSB proteins. Proc. natl Acad. Sci. USA *88* (1991) 3367–3371.
47 Roman, L. J., Eggleston, A. K., and Kowalczykowski, S. C., Processivity of the DNA helicase activity of *Escherichia coli* recBCD enzyme. J. biol. Chem. *267* (1992) 4207–4214.
48 Roman, L. J., and Kowalczykowski, S. C., Characterization of the adenosinetriphosphatase activity of the *Escherichia coli* recBCD enzyme: Relationship of ATP hydrolysis to the unwinding of duplex DNA. Biochemistry *28* (1989) 2873–2881.
49 Roman, L. J., and Kowalczykowski, S. C., Characterization of the helicase activity of the *Escherichia coli* recBCD enzyme using a novel helicase assay. Biochemistry *28* (1989) 2863–2873.
50 Roman, L. J., and Kowalczykowski, S. C., Formation of heteroduplex DNA promoted by the combined activities of *Escherichia coli* recA and recBCD proteins. J. biol. Chem. *264* (1989) 18340–18348.
51 Smith, G. R., Homologous recombination in procaryotes. Microbiol. Rev. *52* (1988) 1–28.
52 Smith, G. R., RecBCD Enzyme, in: Nucleic Acids and Molecular Biology, vol. 4, pp. 78–98. Eds F. Eckstein and D. M. J. Lilley. Springer-Verlag, Berlin 1990.
53 Smith, G. R., Kunes, S. M., Schultz, D. W., Taylor, A., and Triman, K. L., Structure of Chi hotspots of generalized recombination. Cell *24* (1981) 429–436.
54 Smith, G. R., Schultz, D. W., Taylor, A. F., and Triman, K., Chi sites, recBC enzyme, and generalized recombination. Stadler Symp. *13* (1981) 25–37.
55 Stahl, F. W., and Stahl, M. M., A role for recBC nuclease in the distribution of crossovers along unreplicated chromosomes of phase λ. Molec. gen. Genet. *131* (1974) 27–30.
56 Stahl, F. W., Stahl, M. M., Malone, R. E., and Crasemann, J. M., Directionality and nonreciprocality of Chi-stimulated recombination in phage lambda. Genetics *94* (1980) 235–48.
57 Stahl, F. W., Thomason, L. C., Siddiqi, I., and Stahl, M. M., Further test of a recombination model in which χ removes the recD subunit from the recBCD enzyme of *Escherichia coli*. Genetics *126* (1990) 519–533.
58 Stasiak, A., and Egelman, E. H., Visualization of recombination reactions, in: Genetic Recombination, pp. 265–308. Eds R. Kucherlapati and G. R. Smith. American Society for Microbiology, Washington, D.C. 1988.
59 Stasiak, A. Z., Rosselli, W., and Stasiak, A., RecA-DNA helical filaments in genetic recombination. Biochimie *73* (1991) 199–208.

60 Taylor, A., and Smith, G. R., Unwinding and rewinding of DNA by the recBC enzyme. Cell *22* (1980) 447–457.
61 Taylor, A. F., The recBCD enzyme of *Escherichia coli*, in: Genetic Recombination, pp. 231–263. Eds R. Kucherlapati and G. R. Smith. American Society for Microbiology, Washington, D.C. 1988.
62 Taylor, A. F., Schultz, D. W., Ponticelli, A. S., and Smith, G. R., RecBC enzyme nicking at Chi sites during DNA unwinding: Location and orientation-dependence of the cutting. Cell *41* (1985) 153–163.
63 Taylor, A. F., and Smith, G. R., Substrate specificity of the DNA unwinding activity of the recBC enzyme of *E. coli*. J. molec. Biol. *185* (1985) 431–443.
63a Taylor, A. F., and Smith, G. K., RecBCD enzyme is altered upon cutting DNA at a Chi recombination hotspot. Proc. natl Acad. Sci. USA *89* (1992) 5226–5230.
64 Telander-Muskavitch, K., and Linn, S., RecBC-like enzymes: Exonuclease V deoxyribonucleases, in: The Enzymes, pp. 233–250. Ed. P. D. Boyer. Academic Press, New York 1981.
65 Telander-Muskavitch, K., and Linn, S., A unified mechanism for the nuclease and unwinding activities of the recBC enzyme of *E. coli*. J. biol. Chem. *257* (1982) 2641–2648.
66 Telander-Muskavitch, K. M., and Linn, S., Electron microscopy of *E. coli* recBC enzyme reaction intermediates, in: Mechanistic Studies Of DNA Replication and Genetic Recombination, vol. 19, pp. 901–908. Eds B. Alberts and C. F. Fox. Academic Press, New York 1980.
67 Thaler, D. S., Sampson, E, Siddiqi, I., Rosenberg, S. M., Stahl, F. W., and Stahl, M., A hypothesis: Chi-activation of recBCD enzyme involves removal of the recD subunit., in: Mechanisms and Consequences of DNA Damage Processing, pp. 413–422. Eds E. Friedberg and P. Hanawalt. Alan R. Liss, Inc., New York 1988.
68 Thaler, D. S., Sampson, E., Siddiqi, I., Rosenberg, S. M., Thomason, L. C., Stahl, F. W., and Stahl, M. M., Recombination of bacteriophage lambda in *recD* mutants of *Escherichia coli*. Genome *31* (1989) 53–67.
69 Thaler, D. S., and Stahl, F. W., DNA double-chain breaks in recombination of phage lambda and of yeast. A. Rev. Genet. *22* (1988) 169–97.
70 Thresher, R. J., Christiansen, G., and Griffith, J. D., Assembly of presynaptic filaments. Factors affecting the assembly of recA protein onto single-stranded DNA. J. molec. Biol. *201* (1988) 101–13.
71 Tsaneva, I. R., Müller, B., and West, S. C., ATP-dependent branch migration of Holliday junctions promoted by the ruvA and ruvB proteins of *E. coli*. Cell *69* (1992) 1171–80.
72 West, S. C., Enzymes and molecular mechanisms of genetic recombination. A. Rev. Biochem. *61* (1992) 603–40.
73 West, S. C., Cassuto, E., and Howard-Flanders, P., Postreplicaiton repair in *E. coli*: Strand exchange reactions of gapped DNA by recA protein. Molec. gen. Genet. *187* (1982) 209–217.
74 West, S. C., Countryman, J. K., and Howard-Flanders, P., Enzymatic formation of biparental figure-eight molecules from plasmid DNA and their resolution in *E. coli*. Cell *32* (1983) 817–829.
75 Wu, A. M., Bianchi, M., DasGupta, C., and Radding, C. M., Unwinding associated with synapsis of DNA molecules by recA protein. Proc. natl Acad. Sci. USA *80* (1983) 1256–1260.
76 Yagil, E., and Shtromas, I., *Rec*-mediated recombinational activity of two adjacent Chi elements in bacteriophage lambda. Genet. Res. *45* (1985) 1–8.