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Biochemistry of Homologous Recombination in *Escherichia coli*

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INTRODUCTION

The process of homologous genetic recombination is essential to all organisms. It is important for the generation of genetic diversity, the maintenance of genomic integrity, and the proper segregation of chromosomes. The predictable assortment of genes by homologous recombination underlies the basic principles of genetics. Yet despite the importance of this central biological process, the biochemical details of the molecular mechanism by which segments of DNA are exchanged between chromosomal homologs are unclear.

Part of the explanation for this uncertainty lies in the biochemical uniqueness and complexity of the recombination process. In contrast to the mechanisms of many other transactions involving nucleic acids (e.g., replication, transcription, and translation), which result in net macromolecular biosynthesis, the simplest models of homologous recombination mandated little or no DNA synthesis; thus, biochemical assays for components of the recombination apparatus were not easily forthcoming. Furthermore, the isolation and characterization of bona fide intermediates of the recombination process proved impractical because of their complexity, lability, and low abundance, eliminating that potentially informative avenue of investigation. Finally, genetic analysis uncovered the complexity of the recombination processes, demonstrating the involvement of a multitude of genes. For example, in both the prokaryote *Escherichia coli* and the eukaryote *Saccharomyces cerevisiae*, dozens of genes were identified as either essential or important to genetic recombination. In most cases, the genes identified were not involved in other DNA metabolic processes but, instead, were shown to be uniquely important to recombination or recombinational repair. The genetic disclosure of redundant functions served to underscore the intricacy of the recombination process and to hinder identification of the role of each gene product in the recombination mechanism.

Nevertheless, in recent years, the combination of genetic, molecular, and biochemical analyses has revealed a new level of mechanistic detail in steps that underlie the exchange of genetic information between homologous DNA segments. Because of both the relative simplicity and the systematic application of both genetic and biochemical analyses to this process, biochemical understanding of the molecular mechanism of homologous recombination has made the greatest strides with *E. coli*. Mutational analysis has defined at least 25 genes involved in the process (Table 1). The first gene identified, *recA*, was isolated as a mutation that abolished conjugal recombination in *E. coli* but did not disrupt the conjugal process (93). The *recA* gene was shown to be essential in nearly every type of homologous recombination event, and subsequent biochemical analysis uncovered a unique and centrally critical enzymatic activity: the RecA protein could homologously pair DNA molecules, both single and double stranded, and promote the reciprocal exchange of DNA strands (71, 110, 413, 557). Similar analysis, both genetic and biochemical, led to the discovery of more components of the recombination machinery (Table 1). In addition to DNA strand exchange activity, the current list of biochemical activities needed for efficient genetic exchange includes DNA helicases, DNases, ATPases, topoisomerases, DNA-binding proteins, ATP-binding proteins, DNA polymerase, and DNA ligase. Also included in this list is a *cis*-acting element known as Chi (χ). The χ site, 5'-GCTGGTGG-3', was originally discovered as a recombination hot spot in bacteriophage λ (320, 419), but it is important to recombination events in *E. coli* as well (143).

This review focuses on the molecular mechanism of genetic recombination in *E. coli*, with a particular emphasis on biochemical aspects. The biochemical properties of proteins known to be important to the recombination process are summarized, and we attempt to integrate the known facts into

TABLE 1. Proteins and sites involved in genetic recombination in *E. coli*

Protein and site	Activity
RecA	DNA strand exchange, DNA renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease
RecBCD (exonuclease V)	DNA helicase, ATP-dependent dsDNA and ssDNA exonuclease, ATP-stimulated ssDNA endonuclease, χ hot spot recognition
RecBC	DNA helicase
RecE (exonuclease VIII) (*sbcA*[a])	dsDNA exonuclease, 5' → 3' specific
RecF	ssDNA and dsDNA binding, ATP binding
RecG	Branch migration of Holliday junctions, DNA helicase
RecJ	ssDNA exonuclease, 5' → 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 four-way junction binding; interaction with RuvB protein
RuvB	Branch migration of Holliday junctions, DNA helicase, interaction with RuvA protein
RuvC	Holliday junction cleavage, four-way junction binding
SbcB (exonuclease I) (*xonA*)	ssDNA exonuclease, 3' → 5' specific; deoxyribophosphodiesterase
SbcCD	ATP-dependent dsDNA exonuclease
SSB	ssDNA binding
DNA topoisomerase I (*topA*)	ω protein, type I topoisomerase
DNA gyrase (*gyrA* and *gyrB*)	DNA gyrase, type II topoisomerase
DNA ligase (*lig*)	DNA ligase
DNA polymerase I (*polA*)	DNA polymerase, 5' → 3' exonuclease, 3' → 5' exonuclease
Helicase II (*uvrD, uvrE, recL, mutU*)	DNA helicase
Helicase IV (*helD*)	DNA helicase
Chi (χ)	Recombination hot spot (5'-GCTGGTGG-3'), regulator of RecBCD enzyme nuclease activity

[a] *sbcA* mutations are regulatory mutations that activate *recE* function.

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consistent, if occasionally hypothetical, schemes of biochemical action. An overview of viable models for recombination in *E. coli* follows, with a description of the likely function of recombination proteins in these schemes. This overview is followed by a detailed description of the biological and biochemical properties of the individual components of the recombination machinery, which provides justification for the proposals presented in the Introduction.

MODELS

Genetic Models

The prototypic model for homologous recombination was proposed by Holliday in 1964 (234). The model is schematized in Fig. 1A and possesses three salient features. The first is that exchange of DNA strands is mediated by a strand break in both of the DNA homologs; the second is that symmetric exchange of DNA strands produces a region of heteroduplex DNA; and the third is that the resultant Holliday junction is resolved in either of two specific orientations to yield recombinant progeny. The outcome of these steps is the generation of symmetric recombinant DNA molecules that are either "spliced" or "patched." The Holliday model explained many facets of recombination biology including the phenomenon of gene conversion with or without crossing over of flanking markers and the absence of an essential role for DNA replication.

Many important variations of the Holliday model have been advanced, each attempting to explain particular details of recombinant production (see references 179, 591, and 638 for details). Although these models retain all of the essential features of the Holliday model, they differ in very significant details. For example, the Meselson-Radding (Aviemore) model (Fig. 1B) includes initiation at single-stranded DNA (ssDNA) breaks coupled with replication, DNA strand invasion, and degradation of the displaced stand. This model predicts formation of asymmetric regions of heteroduplex DNA, with genetic information being copied from the invading parental DNA molecule (427). The double-strand break repair model (Fig. 1C) proposes initiation and degradation at double-stranded DNA (dsDNA) breaks; however, in this model, the DNA molecule being invaded (i.e., the recipient) provides the genetic information needed to repair the double-strand break (502, 615). These models, together with their genetic implications, are more fully detailed elsewhere (179, 591, 638) and will not be discussed further here. Instead, this review will focus on the biochemical steps that make up the recombination process in *E. coli* and the integration of those steps into a plausible overall biochemical mechanism.

Genetic studies demonstrated the need for RecA protein, RecBCD enzyme, single-stranded DNA-binding protein (SSB protein), DNA polymerase I, DNA gyrase, and DNA ligase (93, 166, 167), as well as either the RuvA, RuvB, and RuvC proteins or the RecG protein (350), in recombination by wild-type *E. coli*. However, both mutant and suppressor analyses in various genetic backgrounds uncovered a role for many other gene products (Table 1). The need for these proteins is determined both by the genetic background of the strain and by the type of recombination event being monitored (e.g., conjugal versus plasmid versus bacteriophage recombination; inter- versus intrachromosomal recombination). In most cases, mutations result in the loss of recombination proficiency, but some mutations (e.g., *recD*, *sbcA*, *sbcB*, and *sbcC* mutations and certain *recA* and *uvrD* alleles) result in a hyperrecombination phenotype. These diverse requirements led to the concept of multiple genetic pathway for recombination (i.e., RecBCD,

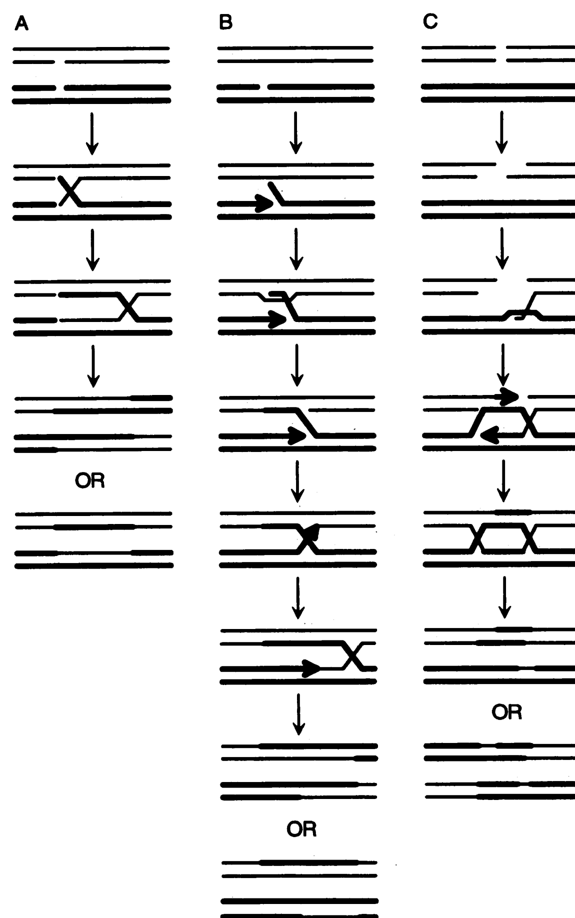

FIG. 1. Models for genetic recombination. (A) Holliday model; (B) Meselson-Radding model; (C) double-strand break model. Note that in panel B the invading molecule is the bottom DNA molecule whereas in panel C the invading molecule is the top DNA molecule. Arrowheads indicate DNA synthesis; other details are discussed in the text.

RecE, and RecF pathways [see references 92b, 393, and 576 for a detailed discussion]). However, because of significant overlap in genetic requirements, it is clear that none of these three pathways is totally independent.

The RecA protein is required for all pathways and recombination events except for RecE pathway-dependent circular and linear plasmid recombination (92a, 95, 128, 181). The RecBCD pathway (see the section on RecBCD enzyme, below), which is the major pathway for recombination in wild-type cells, requires the RecA, RecBCD, and SSB proteins, DNA polymerase I, DNA gyrase, DNA ligase, and either the RuvA, RuvB, and RuvC proteins or the RecG protein. The RecF pathway (see the section on RecF protein, below), which was defined for recombination events occurring in *recB* or *recC* strains in the presence of *sbcB* and *sbcC(D)* suppressor mutations (see the sections on SbcB and SbcCD proteins, below), generally depends on the RecA, RecF, RecJ, RecN, RecO, RecQ, RecR, RuvA, RuvB, RuvC, and RecG proteins (360, 698). The RecE pathway (see the section on RecE and RecT proteins, below), which is defined for recombination occurring in *recB* or *recC* strains in the presence of the *sbcA* suppressor mutation, has genetic requirements that are quite variable: conjugal recombination may require all of the proteins involved in the RecF pathway, but linear dimer plasmid recombination requires only the RecE and RecT proteins (289).

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Redundancy of function further obscures any generalizations regarding the need for a particular gene product in recombination. Three examples of this are particularly notable. The essential need for one of the Ruv proteins is not detected unless the redundant activity provided by the RecG protein is also mutated (350). The RecD, RecJ, and RecN proteins may provide overlapping functions (354). Similarly, the need for the RecO, RecR, or RecF protein is diminished in certain host-dependent bacteriophage λ crosses as a result of the existence of the λ-encoded *orf* gene that supplies the functions normally provided by these host proteins (544).

Although pathways were a useful construct for initially categorizing the many gene products important to a given recombination process, the unique genetic requirements for a given recombination process are, in fact, defined by the types of DNA molecules involved in recombination events being measured. Thus, the different genetic requirements for conjugal recombination and for plasmid recombination are easily appreciated in terms of the very different forms of DNA involved. The RecBCD pathway is generally active when recombination events involve DNA molecules which, at some time, exist as linear duplex species (e.g., during conjugation, transduction, or transformation with linear DNA), whereas recombination by the RecF pathway generally occurs when recombination events involve DNA molecules that are predominantly covalently closed.

The preceding statement is a generalization which simplifies the extensive literature on homologous recombination in *E. coli*; it is, nonetheless, an oversimplification. Both the overlap and the redundancy in the genetic requirements for recombination processes highlight the fact that different DNA molecules can be acted upon by a number of enzymes to yield recombinant DNA molecules by a variety of biochemical means. The virtues and limitations of the traditional pathway definitions are discussed in detail elsewhere (92b, 179, 360, 393, 576). Therefore, rather than discussing recombination reactions in terms of the classically defined pathways, we discuss them in terms of the likely series of biochemical events that make up the reaction, as suggested by the known biochemical activities of the proteins involved.

Biochemical Models

Despite the success of the various models in explaining the genetic outcome of recombination, little progress was made in identifying the biochemical steps of and enzymes involved in the exchange process until 1978, when it was demonstrated that the purified RecA protein is a DNA-dependent ATPase (467, 508) capable of promoting the exchange of DNA strands between homologous DNA molecules (71, 110, 413, 557). Such an activity is central to all models of homologous recombination.

In vitro, RecA protein can promote the formation of homologously paired joint molecules between suitable DNA substrate pairs (see the section on RecA protein, below). If there is no topological constraint to prevent the entwining of DNA strands in the region of heteroduplex DNA formation (e.g., if there is a free DNA end in this region), then plectonemically wound joint molecules result (i.e., the individual strands are intertwined as in normal duplex DNA); if there is a topological constraint (e.g., both are covalently closed circles), paranemically wound joint molecules form (i.e., although base paired, the strands are not topologically intertwined). The one absolute requirement for all homologous pairing reactions promoted by RecA protein is the need for ssDNA. For plectonemically paired DNA complexes, one of the two homologs must be single stranded at a region that is homologous to the other dsDNA partner; for paranemically paired complexes, the ssDNA is essential, but it need not be opposite homology in the dsDNA. This necessity places an important constraint on all biochemically realistic models for homologous recombination in *E. coli* and presumably in other prokaryotes as well, since RecA-like proteins have been identified in all prokaryotes examined (306a, 432, 509). A similar constraint may also apply to eukaryotes, given the recent discovery of structural homologs in *S. cerevisiae* (40, 468, 562a), fission yeast (562), chicken (34), and mouse and human (562) cells (for reviews, see references 225a, 306a, and 466a). Thus, for homologous pairing to occur between two DNA molecules which are normally double stranded, some processing of one of the DNA molecules to produce a region of ssDNA is essential (see Fig. 2).

The essential requirement for ssDNA in RecA protein-dependent pairing processes readily rationalizes the need for both helicases and nucleases in genetic recombination. Helicases produce ssDNA by unwinding dsDNA; nucleases can produce ssDNA by strand-specific degradation of dsDNA. The discovery that the RecBCD enzyme is a DNA helicase which possesses both nuclease and χ-recognition activities provided a biochemical means for the requisite processing of dsDNA into ssDNA during conjugal recombination in wild-type *E. coli* (578, 585, 626). However, the RecBCD enzyme is not the only DNA helicase implicated in the recombination process. The RecQ protein is also a helicase (651), whose need in conjugal recombination is manifest in strains that are defective in either the *recB* or *recC* gene (and contain the additional *recBC*-suppressing mutations *sbcBC*). An alternative means for processing dsDNA to yield ssDNA is through nucleolytic activity, and the RecE protein is, in fact, a strand-specific dsDNA exonuclease that generates 3′ ssDNA tails on dsDNA (255, 316a).

Helicases and nucleases are also needed for late steps in the recombination process, that is, after DNA heteroduplex formation by RecA protein. Branch migration is expected in all recombination models, and resolution of the paired DNA molecules is essential. The realization that the RuvAB and RecG proteins are specialized DNA helicases that possess branch migration activities which can augment the branch migration activity of RecA protein places these enzymes in this later phase of the recombination reaction (251, 360, 361, 644, 698). Finally, the discovery that the RuvC protein can specifically recognize and cleave Holliday junctions completes the recombination pathway by defining the nuclease that acts in the final step in the reaction pathway (149). The RuvC protein, in concert with RuvA and RuvB proteins, and, perhaps alternatively, the RecG protein play a major role in resolution of these recombination intermediates (318a, 360, 626a, 691a). Thus, taken together, these findings now form the basis of a conceptual framework for likely biochemical pathways for genetic recombination in *E. coli*, which are detailed below.

DNA strand invasion mechanisms. The original Holliday model envisioned ssDNA breaks as the initiators of the DNA exchange events (234). The dsDNA break repair model modified this view and, instead, proposed a dsDNA break followed by exonucleolytic degradation as the initiator of recombination events (502, 615). In *E. coli*, recombination during conjugation or transduction or between λ phage is believed to initiate at dsDNA breaks (638), since the DNA involved in each of these physiological processes is at least transiently linear. In conjugation and transduction, the homologous partner (recipient) is normally the covalently closed-circular chromosome. Thus, these processes involve a reaction between linear dsDNA and

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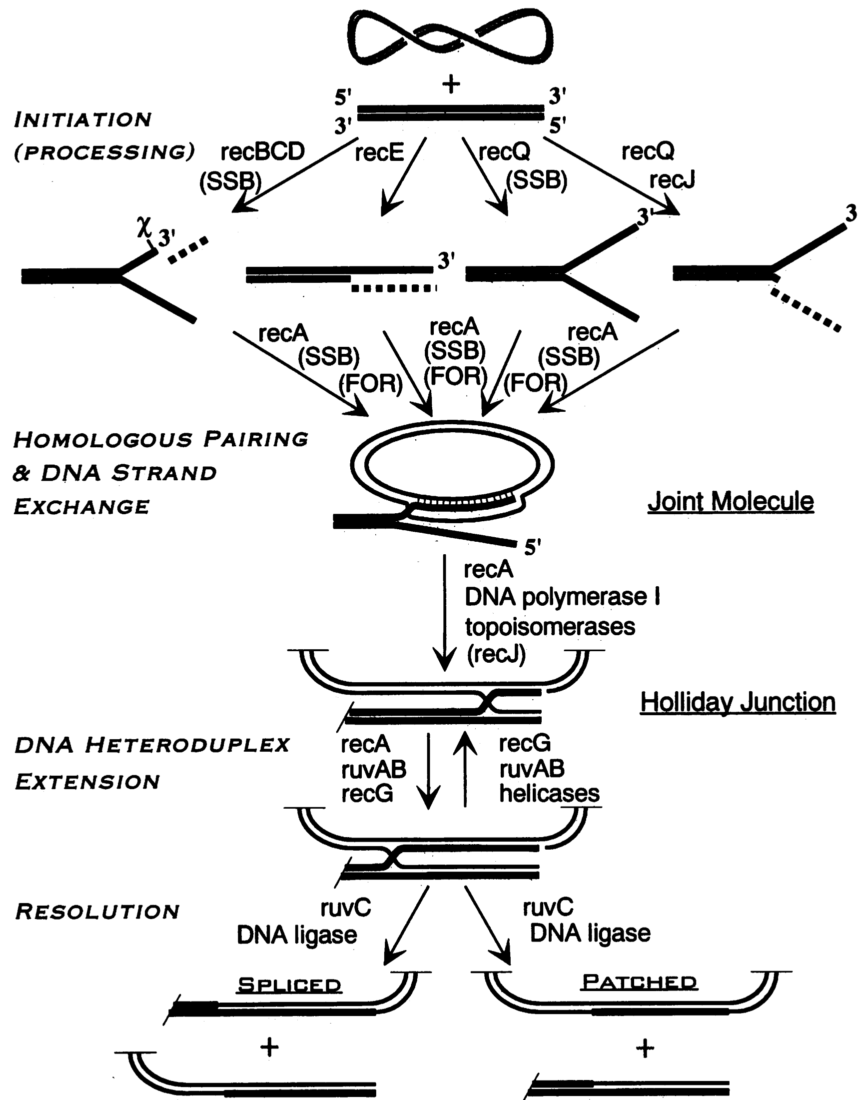

FIG. 2. Biochemical model for genetic recombination involving DNA strand invasion. Details are discussed in the text. FOR, RecFOR proteins.

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supercoiled DNA molecules (Fig. 2). The entire recombination reaction can be thought of as consisting of four steps: (i) initiation (processing), (ii) homologous pairing and DNA exchange, (iii) DNA heteroduplex extension (branch migration), and (iv) resolution.

(i) Initiation. For the reasons outlined above, the initiation phase of recombination requires processing of the dsDNA to produce ssDNA suitable for RecA protein function; this can occur by the collection of alternatives depicted in Fig. 2. In wild-type *E. coli*, the combined helicase and nuclease actions of the RecBCD enzyme convert intact dsDNA into unwound dsDNA with splayed ssDNA ends (625, 633). The RecBCD enzyme unwinds and degrades linear dsDNA asymmetrically until it encounters a χ sequence (141, 142) (see the section on RecBCD enzyme, below). The χ sequence is a regulatory element that attenuates the nuclease but not the helicase activity of RecBCD enzyme, thereby controlling the potentially destructive (to recombination) degradative capacity of this enzyme (141, 142). These events result in the generation of ssDNA terminating near χ with the 3′ invasive end that is preferred for RecA protein-dependent invasion of supercoiled (chromosomal) DNA (141, 142, 484, 585, 627) (see the section on RecA protein, below). The ssDNA produced by the RecBCD enzyme is trapped, bound, and therefore protected by SSB and RecA proteins. Other DNA helicases also could potentially process the linear dsDNA by simple unwinding without degradation. The *recQ* gene encodes a helicase (651) that is clearly important to recombination in the absence of unwinding function normally provided by RecBCD enzyme (see the section on RecQ protein, below); whether it actually serves to initiate recombination reactions is as yet unknown.

Also shown in Fig. 2 are means for initiating recombination events through the action of nucleases. The linear dsDNA can be resected by a strand-specific exonuclease to produce a ssDNA-tailed dsDNA molecule. The product of the *recE* gene is a dsDNA exonuclease that is encoded by a cryptic lambdoid phage and that is functionally similar to the λ exonuclease (255, 256) (see the section on RecE protein, below). The RecE protein processively degrades the 5′-terminal strand of dsDNA to produce a molecule with a 3′ ssDNA tail, which, as

mentioned above, is the preferred polarity for RecA protein-dependent invasion of the supercoiled recipient DNA. Finally, a nuclease can work in concert with a helicase like the RecQ protein to ensure that reannealing of the two complementary ssDNA strands does not compete with the subsequent pairing step. The RecJ protein is a recombination-specific nuclease that is a possible candidate for such a function (377) (see the section on RecJ protein, below). This protein is a nuclease that preferentially degrades ssDNA from the 5′ end. Thus, the combination of a helicase like RecQ protein and a nuclease such as RecJ protein would generate ssDNA with a 3′ end preferred by RecA protein.

(ii) Homologous pairing. The next step in Fig. 2 involves homologous pairing with and invasion of the supercoiled (chromosomal) DNA by the 3′ end of the newly produced ssDNA to form a joint molecule. The RecA protein, aided by the SSB protein (see the section on the SSB protein, below) and, perhaps, by the RecFOR proteins polymerizes on ssDNA, forming a presynaptic complex. Because initial binding is random and subsequent polymerization by the RecA protein occurs in the $5' \rightarrow 3'$ direction (500), the 3′ end of a linear ssDNA fragment is always more likely to be coated than the 5′ end, contributing to the enhanced invasiveness of 3′ ends (290–293). This functional presynaptic complex then conducts a rapid and efficient search for homology within a supercoiled DNA recipient that results in the formation of a plectonemic joint molecule. It should be noted that homologous pairing by RecA protein does not require a free end on the ssDNA. RecA protein can also pair internal regions of ssDNA within gapped dsDNA (as might arise from DNA damage) with homologous dsDNA (71, 695). If the dsDNA is covalently closed, a paranemic joint molecule is formed, but this paranemic joint can be converted to a plectonemic joint by topoisomerase I (120, 717) (see the section on DNA topoisomerases, below). Finally, joint molecules can give rise to Holliday junctions by pairing of the strand displaced from the supercoiled DNA with the other ssDNA strand derived from the originally linear dsDNA molecule. Although this has not yet been demonstrated for this pair of DNA substrates, RecA protein can form a Holliday junction in vitro when provided with DNA substrates that are not topologically constrained (e.g., linear dsDNA and gapped circular dsDNA) (695, 699). This capability, coupled with the isolation of a Holliday junction-cleaving protein (106, 107, 450), supports the contention that Holliday junctions are formed during recombination in *E. coli*, although there is little genetic or physical evidence for this supposition.

(iii) Heteroduplex extension. The third step depicted in Fig. 2 is extension of the DNA heteroduplex region, presumably by protein-promoted branch migration. Although branch migration can occur without the intervention of enzymes, thermal branch migration is slower (449, 476b), is bidirectional, and cannot bypass base pair mismatches (476a). In contrast, the branch migration activity of RecA protein is more rapid, is unidirectional (110), and can traverse regions of dsDNA heterology as large as several hundred nucleotides (37); each of these properties is essential to the cellular process. Although the branch migration activity of RecA protein may be sufficient for DNA heteroduplex extension in vivo, DNA helicases may also participate at this step (see the section on DNA helicases, below). DNA helicases can either antagonize the action of a DNA-pairing protein by disrupting nascent joint molecules or enhance its action by promoting a higher rate of DNA heteroduplex extension. In the *E. coli* and bacteriophage T4 recombination systems, the UvrD and Dda helicases, respectively, can act in both capacities, depending on when the helicase encounters the joint molecule (70, 280, 281, 441a). In *E. coli*, the RuvAB protein complex stimulates DNA heteroduplex extension in RecA protein-promoted reactions by about fivefold (644, 698) (see the section on RuvAB proteins, below). The complex can act on both RecA protein-free and RecA protein-bound Holliday junctions, and branch migration promoted by the RuvAB protein appears to have no preferred directionality, resulting in roughly equivalent amounts of original substrate and product DNA molecules. Equally surprising is the existence of a second protein, the RecG protein, with biochemical activities similar to those of the RuvAB protein (361, 362). The RecG protein is also a DNA helicase that displays specificity for Holliday junctions (see the section on RecG protein, below); however, it has a propensity for reversal of RecA protein-mediated DNA strand exchange, formally antagonizing the efforts of RecA and RuvAB proteins but nevertheless providing an alternative mechanism for resolution of recombination intermediates (699a). Thus, as suggested in Fig. 2, DNA helicases may either competitively disrupt joint molecules or, alternatively, augment the DNA heteroduplex extension activity of RecA protein.

(iv) Resolution. The final step is nucleolytic resolution of the Holliday junction. Symmetric cleavage yields recombinant progeny that either have undergone exchange of flanking markers and contain heteroduplex DNA (spliced molecules) or have simply exchanged ssDNA strands, resulting in heteroduplex DNA (patched molecules). A Holliday junction-cleaving enzyme was recently identified as the RuvC protein (106, 107). This protein recognizes and specifically cleaves Holliday junctions in the symmetric manner required to yield both types of recombinants (see the section on RuvC protein, below). Furthermore, it is possible that the RuvAB proteins help target the RuvC protein to its ultimate site of action.

The steps proposed in this illustration (Fig. 2) represent primarily the types of events occurring in conjugal and transductional recombination in wild-type *E. coli*, with the added consideration that integration of a linear fragment requires two exchanges, one at each end of the molecule. The biochemical feasibility of the steps depicted in Fig. 2 is high, given that two different coupled in vitro recombination reactions establish the fundamental assumptions invoked. The first consists of purified RecA, RecBCD, and SSB proteins in a coordinated homologous pairing reaction that is responsive to stimulatory effects of the recombination hot spot, χ; this system is capable of carrying out the first two steps (helicase-dependent unwinding and RecA protein-dependent homologous pairing) in Fig. 2 (141, 303a, 310, 510). The second system consists of RecA and RuvC proteins in a reaction that can both form and resolve Holliday junctions, as outlined in the last steps of Fig. 2 (149). It seems entirely reasonable to suggest that a system consisting of these purified components should promote a complete recombination reaction in vitro from parents to progeny (303a).

(v) Paranemic pairing. The strand invasion mechanism described in Fig. 2 requires that dsDNA be processed to reveal ssDNA that is homologous to the DNA recipient. However, it is also conceivable that homologous pairing between regions of fully duplex DNA might precede the generation of homologous ssDNA. Although the latter mechanism is plausible, the seeming absolute requirement of homologous ssDNA for DNA strand exchange by RecA protein seemed to preclude such a mechanism. Recent studies, however, demonstrate that RecA protein can, indeed, pair two fully duplex regions of homologous DNA, provided that one of the DNA molecules contains a region of ssDNA (which need not be homologous) and that the other is negatively supercoiled (81, 104, 105). The joint molecule formed between such DNA substrates is

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paranemic, and its stability requires the continued presence of bound RecA protein. Thus, in a hypothetical paranemic pairing mechanism, the initiation step which creates a region of ssDNA remains similar to that proposed in Fig. 2, but the requirement that this processing occur at a site of DNA sequence homology is relaxed. In this case, because the ssDNA region is heterologous, it does not participate directly in the pairing reaction but instead serves simply as a loading site for RecA protein binding. Once bound, RecA protein can polymerize (in a $5' \rightarrow 3'$ direction) toward a region of sequence homology.

The paranemic joint formed by RecA protein between homologous duplex regions would be similar in appearance to the joint molecule depicted in Fig. 2, except that pairing would occur in a region of duplex DNA distal to the ssDNA and, hence, dsDNA would flank both sides of the pairing site. Although relaxation of the requisite supercoiling disrupts the paranemic joint, the action of topoisomerases at the site of the joint could catenate and stabilize the paranemic joint molecule by converting it to a plectonemically wound joint molecule (69, 275). The resultant joint molecule would then contain two Holliday junctions, one at each end of the region of homologous pairing, which could be acted upon by RecA, RuvAB, or RecG protein as described above. Resolution of both Holliday junctions by RuvC protein would result in the formation of recombinant progeny. Although conceptually feasible, the coupling of paranemic joint molecule formation between dsDNA molecules to any subsequent steps has not been demonstrated in vitro.

DNA strand-annealing mechanisms. Conservative models of recombination (i.e., those which maintain the number of DNA molecules involved in the process) invoke DNA strand invasion and exchange as described above, but recombination events need not always be so preserved, and, in fact, many are not (595, 621, 638). Intramolecular recombination between directly repeated sequences in plasmids (621) and intermolecular recombination of bacteriophage λ (595, 638) often do not produce equal amounts of progeny. Such nonconservative recombination can be accommodated by DNA strand invasion mechanisms if a portion of the linear DNA is destroyed in the processing step (595) or if only one of the DNA ends produced by a dsDNA break participates in DNA strand invasion (621, 638). However, an alternative explanation proposes that recombination proceeds by a DNA strand-annealing mechanism (268, 567a, 613).

(i) Initiation. The biochemical steps proposed for such a mechanism are readily illustrated by considering intramolecular recombination between direct sequence repeats of a normally supercoiled DNA molecule (Fig. 3). A prerequisite is either a random double-strand break or single-strand break that is subsequently converted to a double-strand break. As in the case of the DNA strand invasion model (Fig. 2), the site of the DNA strand breakage must be processed to produce ssDNA tails. This can occur by several different biochemical means. The most direct involves the use of a strand-specific dsDNA exonuclease to resect the ends of the dsDNA. Exonucleolytic degradation which is sufficient to uncover regions of complementary ssDNA at both ends would generate ssDNA-tailed substrates suitable for reannealing. In *E. coli*, this degradative function could be provided by the RecE nuclease.

Alternative modes of initiation also may exist in the reannealing pathway. For example, a helicase like RecQ protein acting in concert with a $5' \rightarrow 3'$ ssDNA exonuclease like the RecJ protein can functionally mimic the activity of the RecE nuclease to produce dsDNA with 3' ssDNA tails (middle path in Fig. 3). Conceivably, other ssDNA exonucleases such as exonuclease I (which is the product of the *sbcB* gene and is a $3' \rightarrow 5'$ exonuclease) can substitute for the RecJ nuclease. In this case, ssDNA tails with the opposite 5' polarity are produced (not shown); however, genetic evidence argues against this possibility for exonuclease I (see the section on SbcB protein, below). Alternatively, the solitary action of a helicase such as the RecQ protein may suffice to produce ssDNA (right-hand path in Fig. 3). In principle, the RecBCD enzyme could also serve to produce ssDNA tails, but genetic studies involving plasmid recombination do not support this proposal (see the section on RecBCD enzyme, below). This result may suggest that the combined helicase and nuclease activities of this enzyme are too potent (i.e., either too processive or too rapid) under physiological conditions to yield any significant progeny by a presumed annealing pathway. However, the plasmid DNA molecules involved in these studies were devoid of χ sites, an element which moderates the nuclease activity of the RecBCD enzyme. Recent studies involving linear multimer formation by plasmids replicating by a rolling-circle mechanism reveal that the presence of a χ sequence greatly enhances plasmid stability and multimer formation, whereas the absence of a χ sequence results in plasmid degradation by the RecBCD enzyme (121). Supporting this scenario, recombination is enhanced in cells containing *recD* mutations (11, 376), which result in production of the RecBC enzyme (11); the RecBC enzyme, lacking the RecD subunit, lacks nuclease activity and is biochemically similar to the RecBCD enzyme that has interacted with χ (140).

(ii) Renaturation. The second step of the annealing pathway requires proteins capable of renaturing DNA. The two likely proteins involved at this step are the RecA and RecT proteins of *E. coli*. In addition to its unique DNA strand exchange activity, the RecA protein possesses a DNA renaturation activity which is stimulated by ATP. Although the DNA renaturation activity of RecA protein is strongly inhibited in vitro by SSB protein, there may be conditions when this inhibition is alleviated. If SSB protein were titrated by the production of excess ssDNA (for example, under conditions of DNA damage), the renaturation activity of RecA protein would be manifest. This possibility is quite reasonable since the unwinding of a single λ phage genome by a helicase such as RecBCD enzyme is sufficient to titrate the total intracellular pool of SSB protein (310). In vitro, the RecA protein is capable of forming heteroduplex DNA from ssDNA produced by the enzymatic unwinding of dsDNA in the absence of SSB protein (515). Another argument for a potential physiological function for the DNA renaturation activity of RecA protein is obtained from biochemical analysis of mutant RecA proteins. In all cases examined, the severity of the recombination phenotype correlates with the loss of both DNA strand exchange and DNA renaturation activities (302), demonstrating that these activities are not separable. Recently, the RecT protein was shown to renature ssDNA (see the section on RecE and RecT proteins, below) (217, 218). In addition to DNA reannealing between regions of ssDNA, the RecT protein can promote DNA strand displacement, resulting in extension of the heteroduplex DNA into regions of dsDNA (218). In vivo, plasmid recombination dependent on *recT* (and *recE*) has the unique characteristic of not requiring *recA* (95). This suggests that the RecT protein, in concert with other proteins, provides the same functionality as RecA protein and implies either that the DNA renaturation activity of RecT protein (and perhaps of RecA protein) is sufficient for recombination or that RecT (together with other proteins) has an as yet undiscovered DNA strand invasion activity. A final candidate for DNA renaturation activity in vivo might be the SSB protein, since this

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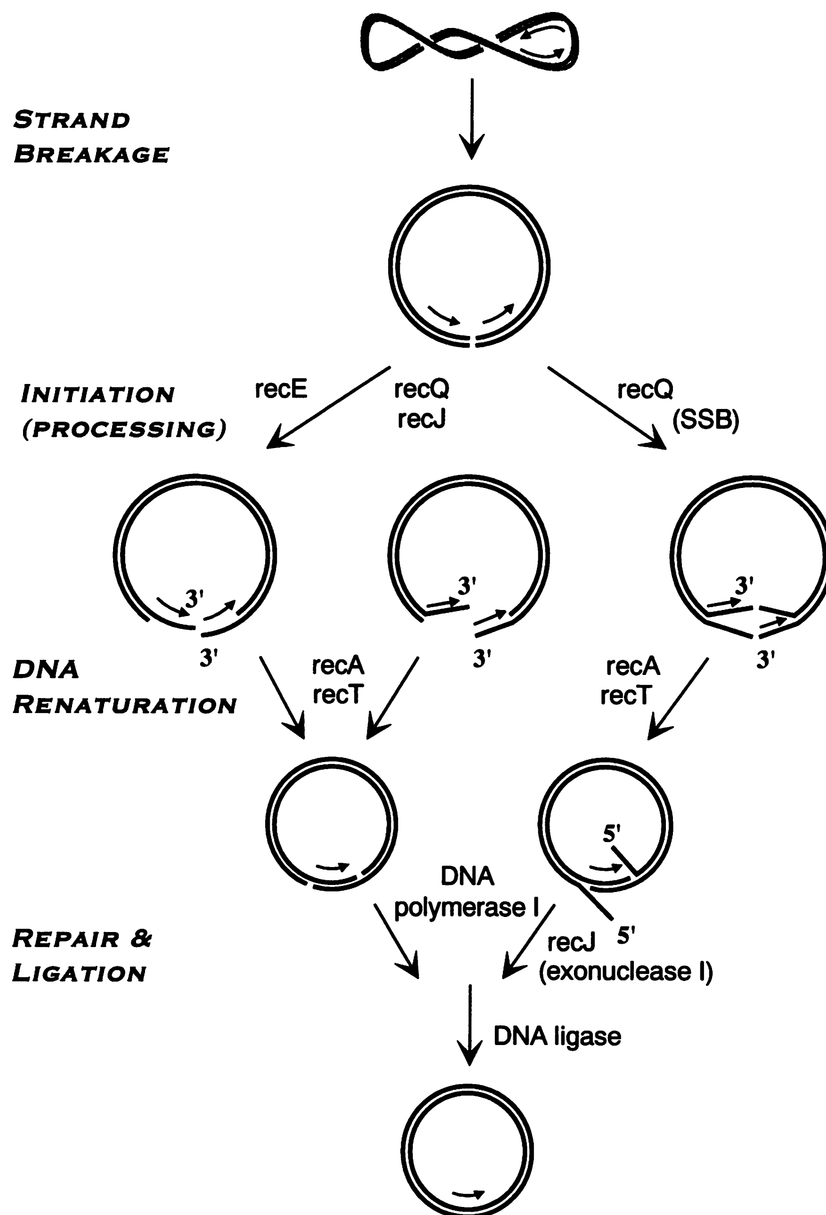

FIG. 3. Biochemical model for genetic recombination involving DNA reannealing. Details are discussed in the text.

protein is capable of DNA renaturation in vitro (86); however, the in vitro conditions required are distinctly nonphysiological, requiring high concentrations of spermidine or magnesium ion.

The protein-promoted DNA renaturation step depicted in Fig. 3 requires that both ends of the dsDNA break be processed to reveal ssDNA. If only one end is processed, renaturation cannot occur; instead, DNA strand invasion of the intact dsDNA end by the ssDNA end, mediated by RecA protein, could occur (613). The DNA strand invasion event would result in displacement of the identical DNA strand from the duplex DNA end, leading to formation of a paired molecule similar to that shown on the right-hand path in Fig. 3 but with just one ssDNA tail. Thus, DNA strand invasion and DNA reannealing are alternative means of generating intermediates of the type shown in Fig. 3, highlighting the probability that a given DNA substrate may be acted upon by a variety of different biochemical processes.

(iii) Repair and ligation. The final step requires repair of the annealed DNA followed by ligation. Since no Holliday junction is formed, resolution of such a structure is not required in the annealing pathway. Replicative repair (presumably by DNA polymerase I) is needed if resection by the nuclease progresses beyond the sequence overlap. Degradative repair by an ssDNA exonuclease, such as RecJ nuclease (shown in Fig. 3) or exonuclease I, of any ssDNA tails remaining after the annealing step is needed if degradation was insufficient to remove all of the ssDNA in the overlap region. An additional potential function of DNA polymerase I was disclosed by recent work which showed that DNA polymerase I can endonucleolytically cleave ssDNA at the junction with dsDNA, provided that the ssDNA tail has a free 5′ terminus (383); thus, DNA polymerase I may also process the tailed reannealing intermediates depicted in the right-hand path in Fig. 3. Subsequent ligation of these molecules would produce a product that contains a

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region of DNA heteroduplex and that is indistinguishable from one that arose by crossing over. This process, however, is nonconservative.

The DNA heteroduplex resulting from any recombination process is subject to mismatch repair; the process of mismatch repair is not covered in this review, but the reader is referred to a recent comprehensive review by Modrich (434a). A detailed description of each of the proteins referred to in the Introduction is presented below.

RecA PROTEIN

The *recA* gene of *E. coli* is indispensable to a number of processes which both maintain and diversify the genetic material of the bacterial cell (91, 179). *recA* mutations are remarkably pleiotropic, affecting not only recombination but also DNA repair, SOS mutagenesis, cell division, and chromosomal segregation. The RecA protein possesses a number of interesting biochemical activities which contribute to its biological functions, including DNA binding, ATP binding and hydrolysis, binding and cleavage of target proteins, helical-filament formation, and the pairing and exchange of homologous DNA strands. Given its important role in ensuring cell viability, it is not surprising that the RecA protein is both ubiquitous and well conserved among a range of prokaryotes (509). Additionally, proteins displaying homology to the RecA protein and possibly possessing similar DNA-pairing and exchange activities have been recently found in eukaryotic organisms (40, 562, 562a; see also references 225a, 306a, and 466a).

Biological Functions

Recombination and DNA repair. In 1965 Clark and Margulies first identified a class of *E. coli* mutations which completely lacked the ability to generate recombinants following bacterial conjugation (93). Isolation involved chemical mutagenesis of the F^- bacterial recipient followed by selection for the inability to yield recombinants after mating with an Hfr donor. The mutations responsible for the dramatic reduction in levels of recombination were mapped to a single locus, designated *recA*, located at approximately 58 min on the current map of the *E. coli* chromosome (411, 704). Subsequent genetic analysis further established that the *recA* gene is essential to all pathways of homologous recombination in the bacterial cell except *recET*-mediated plasmid recombination (see below); the recombination frequency of cells devoid of functional RecA protein is typically reduced by as much as 5×10^4 relative to that of the wild-type strain (91, 93, 179). Along with the dramatic recombination deficiencies observed following conjugal mating (39, 93), both specialized and generalized phage transduction events occur at very low frequencies in *recA* mutant strains (94, 224, 485, 486, 537, 662). Additionally, in the absence of both bacteriophage λ *red* (recombination) and *gam* activities, viable λ phage production is dependent on the host recombination system ($recA^+$) to produce circular dimeric or multimeric λ DNA substrates essential for packaging (for a review, see reference 574).

A functional relationship exists between homologous recombination and the repair of DNA damage, as evidenced by a relationship between the UV resistance of various mutants and their recombination proficiency (93, 96, 242, 674). Mutations in the *recA* gene sensitize cells to killing by UV, X-ray irradiation, and certain chemical mutagens (93, 196, 238, 242). Genetic evidence supports the hypothesis that radiation-induced DNA damage resulting in the cessation of DNA replication is repaired in part by genetic recombination (526, 586a). The finding that *recA uvrA* double mutants are more UV sensitive than either of the single mutants implied that genetic recombination, and specifically RecA protein function, played a direct role in the repair of UV-induced adducts (240). The *recA*-dependent repair of photoproducts requires DNA replication (586a), and genetic recombination is stimulated by UV-irradiated DNA that has undergone replication (241, 338, 527, 702). *recA uvrA* mutant strains are also highly sensitive to psoralen-DNA cross-links (100, 101). A mechanism for repair of psoralen-DNA cross-links was proposed to involve sequential incision by the UvrABC protein complex and DNA strand exchange by RecA protein beyond the lesion (102). The direct involvement of RecA protein in the repair of DNA cross-links was recently demonstrated by using an in vitro system which also incorporated the excision and 5′-exonuclease activities of purified UvrABC and DNA polymerase I proteins, respectively (571).

SOS induction. In addition to its direct role in the repair of damaged DNA, the RecA protein coordinates the cellular response to DNA damage through the SOS system (710). This SOS response is initiated by the RecA protein through its ability to recognize DNA damage and become "activated" for LexA repressor cleavage (236a, 344). Although it is still not absolutely certain what constitutes the cellular signal for DNA damage, various observations suggest that some form of ssDNA is almost certainly the activating agent. Activation of the RecA protein and SOS induction can involve either the RecBCD protein (77) or DNA replication (542). The molecular element common to each is ssDNA, which suggests that either the unwinding of dsDNA or ssDNA found at stalled replication forks leads to SOS induction (392). The binding of RecA protein to ssDNA results in its activation into a form that is now able to stimulate the cleavage of the LexA repressor protein (236a, 344). This leads to the transcriptional derepression of approximately 20 unlinked genes, *recA* included (467), which make up the SOS regulon (345, 402). The LexA protein is not the only target of RecA protein-promoted cleavage; the lytic repressor proteins of a variety of lambdoid bacteriophages (λ, φ80, 434, and P22) are also cleaved, resulting in prophage induction (162, 507, 543).

SOS mutagenesis. SOS mutagenesis is the process by which the lesions caused by DNA-damaging treatments such as mitomycin, methyl methanesulfonate, and UV radiation can be bypassed by an inducible error-prone replication process (663, 709). The roles of the RecA protein in the complicated process of SOS mutagenesis are only partially understood (612). The first is to induce the SOS response through the cleavage of LexA protein, which derepresses the expression of proteins UmuC and UmuD, which are essential to mutagenesis. The second role also involves the coprotease activity of RecA protein; however, the target protein is the UmuD protein, which is cleaved to produce the functional UmuD′ protein. A complex of the UmuC and UmuD′ proteins contributes to error-prone DNA repair, probably by helping DNA polymerase III bypass DNA lesions (495, 560). The third role of RecA protein in SOS mutagenesis was uncovered when cells that had both a defective LexA repressor, resulting in constitutive expression of the SOS genes, and a modified *umuD* gene, which encoded the processed UmuD′ protein, failed to display an SOS mutator phenotype (167a, 612). This suggests that RecA protein plays a direct role in the mutagenesis process, but its specific participation is unknown. One proposal is that RecA protein interacts directly with the UmuCD′ complex to rescue polymerase complexes stalled at DNA lesions (24, 715). A mutant RecA protein deficient in the third role of SOS mutagenesis is hypothesized to incorrectly interact

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with the UmuCD′ complex; it therefore fails to target this complex to the appropriate location on DNA (21). This mutant, RecA1730 protein, which is defective in SOS mutagenesis but not recombination when overexpressed, also appears to be defective in its ability to self-associate and to form helical filaments (151).

Induced stable DNA replication. DNA damage alters the mode of initiation of DNA replication such that it can occur in the absence of the usually required protein synthesis. This type of replication has been termed induced stable DNA replication (iSDR) (285, 286). iSDR results in the replication of the chromosome for many hours, is more resistant to UV irradiation than is normal DNA replication, and appears to be error prone (282, 323). Both derepression of the *recA* gene and, more importantly, activation of the RecA protein have been shown to be necessary and sufficient for induction of iSDR (392).

recB or *recC* mutants are defective in iSDR (392); in contrast, mutations in the *recD* gene actually enhance iSDR (17). This indicates that the activity required for iSDR is not the exonuclease activity of RecBCD enzyme, which is absent in *recD* mutants (476), but is most probably the helicase activity of the RecBC enzyme (see the section on RecBCD enzyme, below). The helicase activity of the RecBCD and RecBC enzymes presumably provides a function similar to its normal role in homologous recombination, namely, the production of ssDNA for use by the RecA protein in the formation of D-loop structures (141, 310, 510).

Since iSDR is independent of the DnaA protein (391), which is normally responsible for initiation of replication at *oriC* (231, 418), a mechanism which involves the formation of D-loops as the mode of initiation was proposed (17). RecA protein is required not only to induce its own synthesis but also to provide recombination activities; *recA* mutations which constitutively derepress the SOS regulon but which are recombinationally defective fail to promote iSDR (17). The well-known ability of the RecA protein to catalyze the formation of D-loop structures led to the proposal that RecA protein is involved in generating the structure which then serves as a initiation point for iSDR; however, the documented need for activated RecA protein remains an interesting mystery.

Constitutive stable DNA replication. *E. coli rnh* mutants, lacking RNase H_I activity, have an altered mode of DNA replication, known as constitutive stable DNA replication (cSDR). This type of replication can take place in the absence of protein synthesis, which is normally required for initiation at *oriC* (283). Initiation of cSDR replication occurs independently of *oriC* at multiple sites termed *oriK*, where persistent RNA-DNA hybrids, normally removed by RNase H_I, serve either directly as primers for initiation of DNA replication or indirectly as sites of open dsDNA structure where de novo priming by DnaG primase can occur. The timing and frequency of initiation at these sites are poorly regulated, leading to perturbations in cell growth of these strains. Thus, RNase H_I suppresses initiation at disperse *oriK* sites, ensuring initiation at *oriC*.

E. coli containing an *rnhA* mutation shows induction of the SOS system, suggesting that the ssDNA displaced from the resultant RNA-DNA hybrids can activate RecA protein to stimulate cleavage of the LexA protein. Mutations in either the *recB*, *recC*, or *sbcB* (exonuclease I) gene, in the presence of an *rnhA* mutation, result in growth defects, particularly when the cells are grown in rich media. Presumably, R-loops are removed through the combined action of exonuclease V (RecBCD enzyme), exonuclease I, and DNA polymerase I (284). This is taken to suggest that these R-loops act as lesions which stall replication forks and which, if not removed, lead to a decrease in viability. In the absence of these exonuclease activities, the *recA* gene, as well as the *recF*, *recN*, and *recJ* genes, becomes essential for cell growth. These observations suggest that R-loops are both formed by and repaired by a mechanism involving RecA protein and enzymes of the RecF pathway (284); RecA protein is envisioned to catalyze the formation of RNA-DNA hybrids, which are proposed to be key intermediates in this replication initiation process (67a).

Chromosome partitioning. An additional role for the RecA protein was recently proposed on the basis of the observation that many cells in a *recA* population have an abnormal number of chromosomes following the completion of ongoing rounds of DNA replication. The normal chromosome number is 2^n, n being an integer between 1 and 4; however, *recA* cells can be anucleate or contain an odd number of chromosomes (9, 569). These abnormalities were not due to either asynchronous initiation or incomplete replication, since the timing of initiation was precise, with 99% of the chromosome being replicated at the end of one cell cycle (731). These results suggested that the proper partitioning of the newly replicated chromosomes to daughter cells is defective in *recA* strains. Furthermore, a direct role for RecA protein function was argued by results demonstrating that the requirement for RecA protein was not as an inducer of the SOS system (731). It was proposed that RecA protein may be required for protection of replication forks that have stalled at *ter* sites. Additionally, RecA protein may be required for recombination at the same *ter* sites to produce concatamers of the daughter chromosomes which produces the tension necessary for proper chromosome partitioning (731). However, recent studies have provided strong evidence for a protective function of RecA protein (569a). In the absence of functional RecA protein, individual chromosomes are selectively and completely degraded by the RecBCD enzyme. These studies would suggest that the participation of RecA protein in synchronous segregation of chromosomes may be more indirect than was initially suggested (569a). Although the precise role of RecA protein in chromosomal segregation remains uncertain, the parallel to eukaryotic organisms, in which proper segregation requires recombination, is intriguing.

Biochemical Activities

The purified RecA protein possesses a number of biochemical activities. The 352-amino-acid, 37,842-Da RecA protein (236b, 533) forms regular helical filaments in both the presence and absence of DNA (136, 137, 154, 467, 499, 599, 600), binds both ss- and dsDNA (415, 416), and binds and hydrolyzes nucleoside triphosphates (NTPs) in a DNA-dependent manner (416, 424, 425, 467, 508). As a consequence of these fundamental biochemical properties, the RecA protein promotes a number of reactions significant to biological processes including the ATP-dependent formation of homologously paired joint molecules and exchange of complementary DNA strands (110–112, 124, 125, 413, 558, 588, 692–697; see references 109, 113, 210, 300, 303, 306a, 493, and 691 for reviews), the ATP-stimulated renaturation of complementary ssDNA strands (56, 412, 426, 689), and the ATP- and ssDNA-dependent cleavage of the LexA repressor, lambdoid repressor, and UmuD proteins (66, 116, 162, 344, 543).

DNA strand exchange. The RecA protein promotes the homologous pairing and exchange of DNA strands between a variety of DNA substrates in vitro (Fig. 4). The invasion of supercoiled DNA by linear ssDNA (Fig. 4A) produces homologously paired joint molecules containing D-loops (dis-

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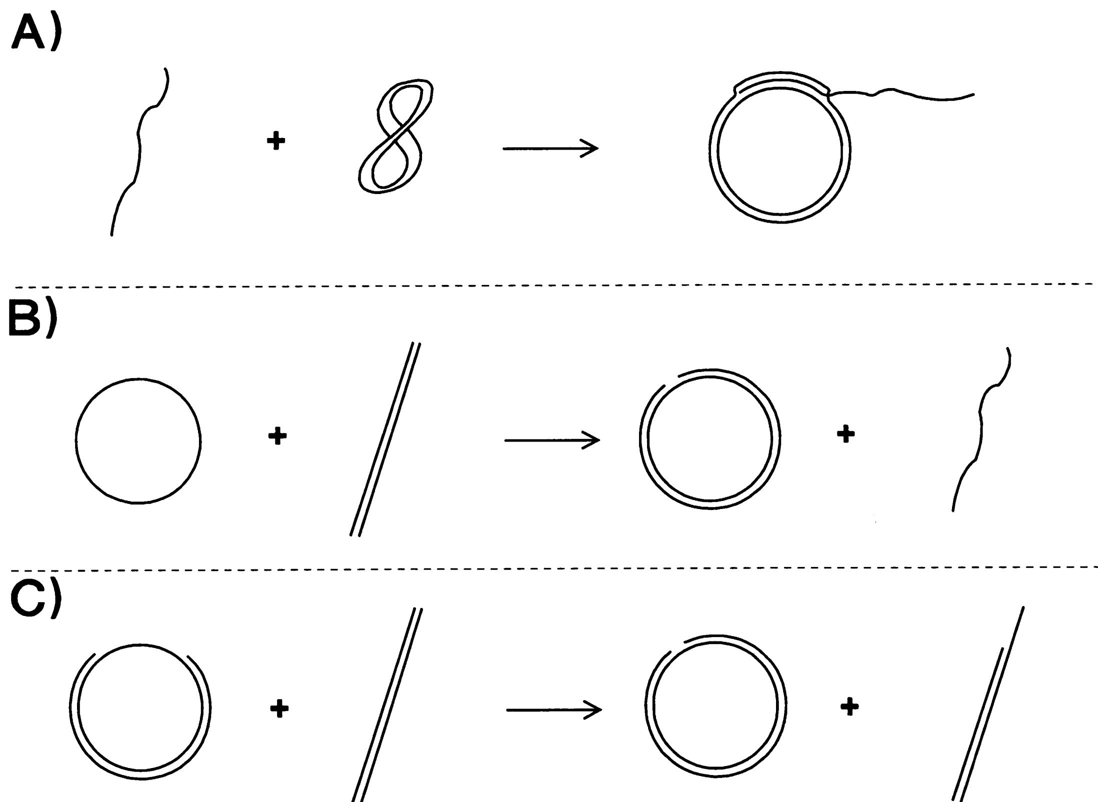

FIG. 4. DNA substrate pairs commonly used for RecA protein-promoted homologous pairing and DNA strand exchange reactions in vitro. (A) Supercoiled DNA and linear ssDNA; (B) circular ssDNA and linear dsDNA; (C) linear dsDNA and gapped circular dsDNA.

placement loops) (413, 558). The most commonly used pair of substrates in vitro, circular ssDNA and linear dsDNA (Fig. 4B), permit the complete exchange of DNA strands, resulting in the formation of nicked circular duplex and linear ssDNA products (112). The final pair of substrates (Fig. 4C), which are representative of reactions involving duplex molecules (71, 119, 693, 694), involves linear and gapped circular dsDNA molecules which are converted by the reciprocal exchange of DNA strands into products consisting of nicked circular dsDNA and linear dsDNA with a single-stranded overhang. Despite the differences in their structure, these three substrate pairs have a common set of required features. First, one DNA substrate of the pair is linear and thus has a free end (although the need for a free end can be obviated by the presence of a topoisomerase [120]), and second, one of the DNA substrates is at least partially single stranded. Although the net exchange of DNA strands normally requires that the obligatory region of ssDNA be homologous to a region within the dsDNA, homologous pairing between two fully duplex DNA regions can occur, provided that one of the DNA molecules contains a region of ssDNA and that the other is supercoiled (82, 83, 104, 105). The region of ssDNA need not be homologous, because it serves simply as a loading site for polymerization of RecA protein into the duplex DNA region that is homologous to its supercoiled DNA partner.

A number of different assays are used to characterize RecA protein-promoted homologous pairing and exchange of DNA strands. The formation of joint molecules can be detected by a nitrocellulose filter assay (25). In reactions utilizing the circular ssDNA and linear dsDNA substrates (Fig. 4B), the total amount of heteroduplex DNA formed within a joint molecule can be determined by monitoring sensitivity of labeled DNA to ssDNA-specific nucleases (e.g., S1 nuclease) (111). The complete DNA strand exchange reaction involving both three- and four-stranded substrates (Fig. 4B and C) can be visualized by agarose gel electrophoresis (111, 694). Because the reaction substrates, intermediates (joint molecules), and products are clearly resolved, the reaction between circular ssDNA and linear dsDNA is the most extensively studied (111, 260, 694). Therefore, the following discussion concerning the mechanism of homologous pairing and DNA strand exchange, although generally applicable to all of the aforementioned substrate pairs, will focus on the reaction involving circular ssDNA and linear dsDNA. Specific distinctions pertaining to either the four-stranded reaction or the formation of D-loop joint molecules will be highlighted.

The mechanism by which RecA protein promotes homologous pairing and exchange of DNA strands consists of a series of kinetically distinct steps (Fig. 5): presynapsis, synapsis, and the extension of DNA heteroduplex (183, 300, 494). The initial step, presynapsis, involves the stoichiometric binding of the RecA protein to ssDNA (111) or dsDNA containing a single-stranded gap (697) to form a nucleoprotein complex containing one RecA protein monomer for every 3 nucleotides or base pairs of DNA. Formation of a functional presynaptic filament normally requires an NTP cofactor, such as ATP or dATP. The bound ssDNA lies deep within the helical presynaptic filament (138, 156) and is extended with an axial spacing 1.5 times that of B-form DNA (137, 225, 596a). The presence of a single-stranded DNA-binding protein (such as the *E. coli* SSB protein or bacteriophage T4 gene 32 protein) facilitates formation of the presynaptic complex by removing secondary structure

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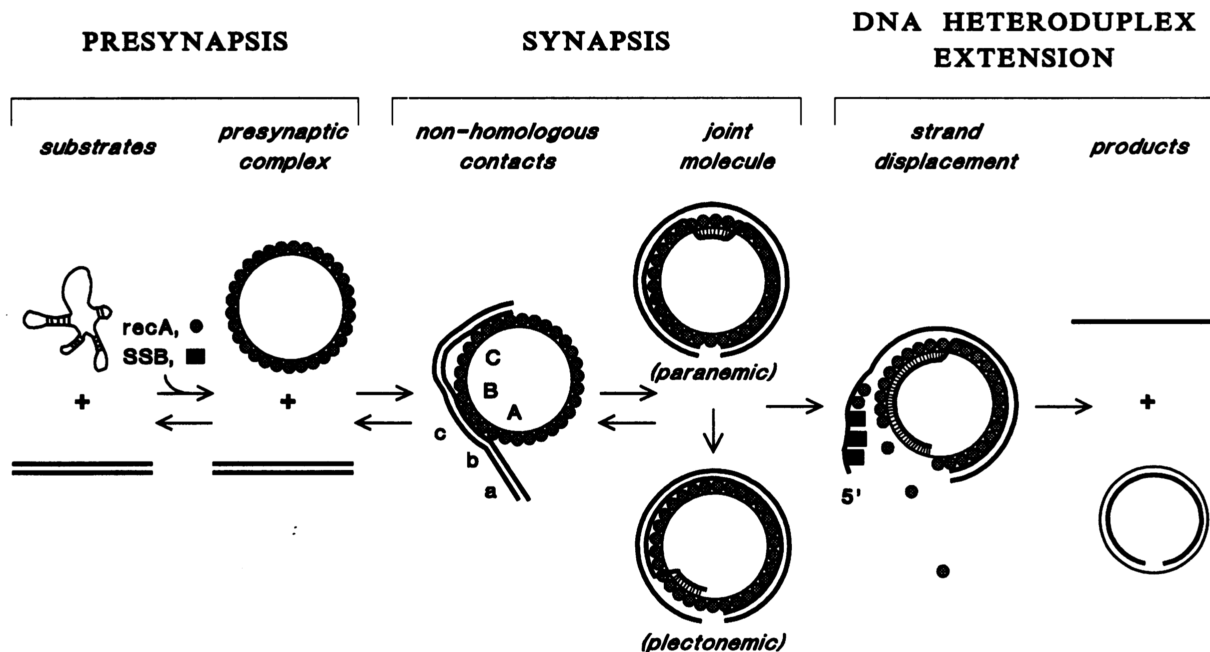

FIG. 5. Illustration of the kinetically distinct stages of the DNA strand exchange reaction catalyzed by the RecA protein. Depicted is the reaction between circular ssDNA and linear homologous dsDNA in the presence of the *E. coli* SSB protein. Upon deproteinization and the complete exchange of DNA strands, the products formed are linear ssDNA and gapped circular heteroduplex dsDNA. Adapted from reference 303 with permission of the publisher.

native to ssDNA (115, 307, 414, 454). The requirement for a single-stranded DNA-binding protein in presynapsis is obviated under conditions which either destabilize ssDNA secondary structure (low magnesium ion concentration) (647) or enhance RecA protein binding to ssDNA (331). It is also not required in the reaction involving two duplex molecules, provided that the ssDNA region of the gapped circular dsDNA is less than 300 nucleotides (104, 105, 341, 694–696).

The subsequent stage of DNA strand exchange, synapsis, consists of the search for and establishment of homologous contacts between the presynaptic complex and naked duplex DNA molecules. The process of synapsis is the least-understood aspect of RecA protein-promoted DNA strand exchange. The first dsDNA contacts encountered by the presynaptic filament are necessarily random and result in the formation of nonhomologously interacting DNA molecules termed coaggregates. It is proposed that coaggregates enhance the rate at which RecA protein locates homologous sequences by increasing the local concentration of potentially homologous molecules (646). Although nonspecific contacts are likely to play an important role in the homology search (204, 236), coaggregates (defined as macromolecular complexes capable of being sedimented by low-speed centrifugation) are not essential to RecA protein-mediated homologous pairing (308, 329, 483, 497). Heterologous contacts affect the conformation of the dsDNA, indicating that the presynaptic filament, itself in an extended conformation, causes the limited extension and unwinding of duplex DNA which stabilize transient heterologous contacts (522). Increasing the length of homologous dsDNA enhances both the rate constant and the extent of homologous pairing in reactions involving both three (259) and four (104) DNA strands. In contrast, the addition of heterologous DNA either has no effect on the homologous pairing reactions (104, 259, 483) or inhibits pairing when the heterologous DNA is supercoiled. Collectively, these results indicate that the mechanism of homologous pairing during synapsis consists of two kinetically distinct phases: the rapid formation of a homologously aligned intermediate followed by the rate-limiting conversion to a stable joint molecule (259, 303). Although homologous pairing has been observed over extensive lengths (~6 kb) (35, 45, 87, 125, 600), the initiation of pairing requires only 8 homologous nucleotide bases involving only two to three RecA protein monomers (105, 245).

Following homologous pairing, RecA protein initiates the exchange of DNA strands, resulting in joint molecule formation. This final stage of synapsis requires local denaturation of the dsDNA molecule and subsequent establishment of heteroduplex DNA through the exchange of ssDNA homologs. The RecA protein can form two types of joint molecules depending on the topological constraints imposed by the DNA substrates (Fig. 5). A plectonemic joint molecule is stable in the absence of RecA protein, requires the presence of a homologous DNA end, and results in the intertwining of the invading ssDNA around its complement. A paranemic joint molecule is unstable in the absence of RecA protein, does not require the presence of a homologous DNA end, and does not contain topologically linked DNA strands. Paranemic joints can form not only in the absence of a free homologous DNA end (i.e., circular ssDNA and closed duplex DNA) (35, 45, 87, 125, 503) but also between completely duplex regions of two DNA molecules (81, 104, 105).

After the formation of a plectonemic joint, the heteroduplex DNA is extended by a unidirectional branch migration step until the complete exchange of ssDNA strands is achieved. In reactions involving substrates of circular ssDNA and linear dsDNA (110, 260, 692) or gapped circular and linear dsDNA (692, 695), branch migration is polar, proceeding in a $5' \rightarrow 3'$ direction relative to either the incoming presynaptic filament or the displaced strand. Both postsynaptic stabilization of joint molecules and the end bias observed for strand invasion are

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proposed to result from binding of either SSB protein (85, 332) or RecA protein (84, 293) to the displaced strand. Because of topological constraints, the polarity of heteroduplex extension for D-loop joint molecules is not defined. However, the stable formation of joint molecules between linear ssDNA and supercoiled DNA displays a distinct bias with regard to the location of homology within the ssDNA molecule. Invasion of the supercoiled substrate by the 3′ end of the linear ssDNA is 10-fold more likely than invasion by the 5′ end and is accentuated by the presence of SSB protein (290, 291). This bias is a consequence of the initial random binding of RecA protein and its polarity of polymerization (5′ → 3′) onto ssDNA (500), which ensures that the 3′ end of ssDNA is much more likely to be coated with RecA protein than is the 5′ terminus. This results in the 3′ ends of ssDNA being substantially more invasive than the corresponding 5′ ends.

RecA protein-mediated exchange of DNA strands can tolerate DNA lesions (216, 347) and mismatched base pairs (36, 37, 123); for example, exchange can occur between M13 and fd phage DNAs, which are mismatched by 3% over their length (124). DNA strand exchange can bypass the insertion of heterologous sequences, with insertions in dsDNA (a 140-bp insertion reduces heteroduplex formation by approximately 60%) being more restrictive than those in ssDNA (a 1,000-nucleotide insertion reduces heteroduplex formation by approximately 60%) (37, 216). In addition to DNA sequence heterology, DNA-binding proteins can inhibit DNA strand exchange. The binding of a major *E. coli* chromosomal protein (HU) to linear dsDNA permits the formation of paranemic joint molecules while inhibiting their conversion to plectonemic joints (496). Similarly, linear dsDNA that is reconstituted with rat liver nucleosomes in the absence of H1 protein at an approximately physiological mass ratio of histone to DNA is a productive substrate for RecA protein-promoted joint molecule formation; however, these templates inhibit extension of DNA heteroduplex (497).

The requirement for ATP in RecA protein-mediated exchange of DNA strands led to the assumption that the energy derived from the hydrolysis of ATP was mechanistically required for DNA strand exchange. However, both the fact that DNA strand exchange is an isoenergetic process (i.e., the number of base pairs is conserved throughout the reaction) and the observation that the affinity of the RecA protein for ssDNA is modulated by ATP and ADP cofactors (422) inspired further characterization of the role of ATP hydrolysis in RecA protein-mediated DNA strand exchange (see below for further discussion). Studies with an essentially nonhydrolyzable ATP analog, ATPγS, as well as with a mutant RecA protein that is dramatically attenuated in its ATP hydrolysis activity, showed that efficient and extensive heteroduplex DNA formation (1.5 to 3.5 kb) can occur without significant NTP hydrolysis (421, 501, 518). Moreover, DNA strands can be readily and completely exchanged when shorter linear dsDNA molecules are used (54, 1,300 and 2,800 bp in length) (271, 309a, 518). Therefore, the chemical energy derived from the hydrolysis of ATP is not mechanistically required for the homologous pairing and exchange of DNA strands. ATP hydrolysis instead permits the conversion of the RecA protein from an ATP-bound form, which has high affinity for DNA, to an ADP-bound form, which has lower affinity for DNA. This conversion promotes RecA protein dissociation from the DNA (heteroduplex joint molecule) and leads to the net exchange of strands (303, 421, 501), although the ATP hydrolytic event is not obligatorily coupled to a dissociation event (303, 339, 340). DNA strand exchange can also occur in the presence of an allosteric effector, ADP · AlF_4^- (309), that lacks a high-energy phosphodiester bond but which can induce a conformation of RecA protein that mimics the activated ATP-bound form both structurally (726) and in the coprotease reaction (441). This observation demonstrates that an NTP is not essential in the exchange of DNA strands; rather, the allosteric transition to the active form of RecA protein is necessary and sufficient (309). Despite these findings, ATP hydrolysis by the RecA protein remains important to several aspects of the overall DNA strand exchange reaction. It is needed to permit unidirectional DNA heteroduplex extension (110, 293), to traverse regions of heterology encountered in the three-stranded reaction (Fig. 4B) (271, 519), and to exchange DNA strands in the four-stranded reaction (Fig. 4C) (272). ATP hydrolysis is also crucial for RecA protein turnover; in the absence of ATP hydrolysis, RecA protein would remain irreversibly bound to the first DNA site that it occupied (see reference 303 for a detailed discussion).

The structure of the RecA protein-promoted, homologously paired DNA intermediate is a subject of much discussion. Results of early studies advocated the formation of a three-stranded DNA structure stabilized by RecA protein (243). Later, it was suggested that RecA protein stabilized a transition state that consisted of three DNA strands positioned in such a way that they were predisposed to form products of DNA strand exchange upon removal (dissociation) of the RecA protein (421). It was also suggested that a stable DNA triplex involving non-Watson-Crick base pairing exists in the absence of RecA protein and that this structure is an intermediate in the exchange reaction (244, 498). Recently, however, chemical probing techniques ($KMnO_4$ and dimethyl sulfate sensitivity) clearly demonstrated that the disposition of the DNA strands in joint molecules contained within the RecA protein filament formed in the presence of ATPγS is essentially identical to that in the resultant products (i.e., DNA strand exchange had already occurred within the synaptic complex without the need for ATP hydrolysis) (6). Thus, the original base pairs of the substrate duplex molecule are disrupted within a joint molecule, suggesting that homologous pairing occurs through local exchange of DNA strands.

Renaturation of ssDNA. The renaturation of homologous ssDNA to form duplex DNA was the first pairing activity of RecA protein to be discovered (689). Renaturation offers a model system to characterize the mechanism by which the RecA protein searches for and aligns complementary sequences within DNA molecules. Optimal renaturation of homologous ssDNA molecules mediated by RecA protein occurs at subsaturating amounts of RecA protein, at protein/DNA nucleotide ratios (1:30) at which only 10 to 15% of the ssDNA is bound (56). Although stimulated by ATP, a significant amount of RecA protein-promoted renaturation proceeds in the absence of nucleotide cofactor (56). DNA renaturation in the absence of RecA protein is a second-order process, whereas renaturation promoted by the RecA protein in the presence of ATP is first order (56). As in the DNA strand exchange reaction, aggregates consisting of RecA protein and ssDNA molecules are formed (58). The proposed function of these intermediates in the mechanism of RecA protein-promoted renaturation is to increase the effective DNA concentration, thereby bringing complementary DNA sequences into proximity (58, 646). Aggregate formation parallels renaturation, occurring optimally at subsaturating RecA protein concentrations and being inhibited at stoichiometric protein concentrations (646). The idea that the same fundamental property of RecA protein is responsible for both the ATP-stimulated renaturation of complementary ssDNA and the exchange of DNA strands is supported by the fact that a

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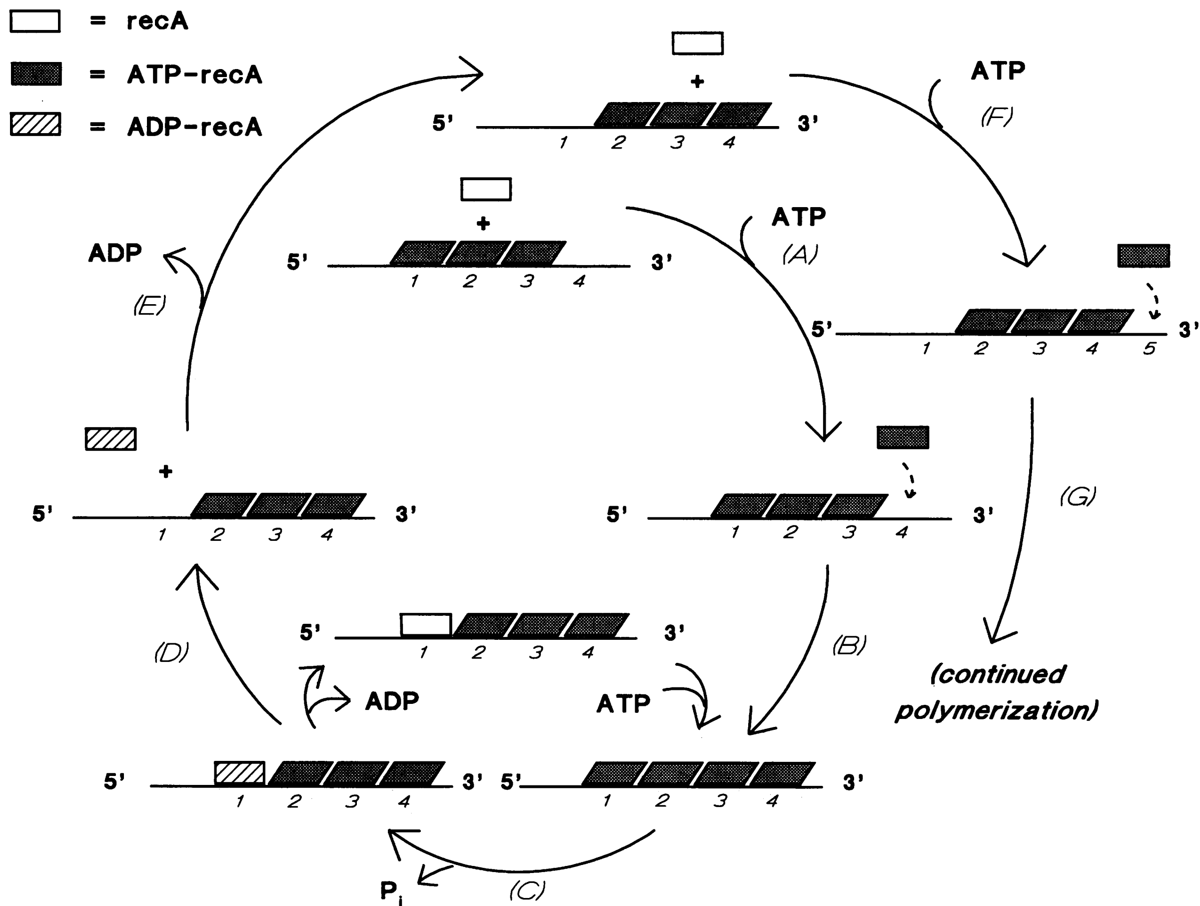

FIG. 6. Model for RecA protein association with and dissociation from ssDNA as regulated by the binding and hydrolysis of ATP (303, 422). As illustrated, RecA protein association and dissociation is polar (5' → 3') and results in net translocation of the protein filament. Represented by the inner cycle at step (C), the processive hydrolysis of ATP by the RecA protein occurs at high ATP concentrations; consequently, net protein polymerization on the ssDNA ensues (from reference 303 with permission of the publisher).

mutant RecA protein which is differentially affected in these two biochemical activities has yet to be identified (302). Thus, although DNA strand exchange activity is presumed to be the biologically relevant function of RecA protein, it remains possible that RecA protein-promoted renaturation activity also serves a physiological role. Lending further support to this possibility, the RecT protein, which possesses DNA renaturation activity (217, 218), can substitute for RecA protein in plasmid recombination (see the section on RecE and RecT proteins, below) (289).

Presynaptic complex formation. During the initial presynaptic stage of the DNA strand exchange reaction, RecA protein interacts with ssDNA to form a nucleoprotein filament. The functional presynaptic filament is a ternary complex consisting of RecA protein, ssDNA, and the nucleotide cofactor ATP. RecA protein binds to ssDNA in a nonspecific cooperative manner and forms a contiguous filament, in which the DNA molecule is completely saturated by RecA protein (114, 415, 422, 647, 697). Polymerization of RecA protein onto DNA occurs in a 5' → 3' polar fashion (500). Because initial nucleation of the RecA protein onto ssDNA is random, the 3' end of a linear ssDNA molecule is much more likely to be contiguously bound by RecA protein than is the 5' terminus. Because of the polarity and cooperativity of assembly, RecA nucleoprotein filament disassembly observes the same directionality but occurs at the opposite end (339, 340). The oligomeric state of the RecA protein species which associates with ssDNA is not certain, since free RecA protein consists of many aggregated species (29, 49, 50, 467). Furthermore, the kinetics of RecA protein association with ssDNA show a dependence on all the parameters (Mg^{2+}, pH, ionic strength) that affect RecA protein self-association, suggesting that the state of aggregation, or disaggregation, is important to the mechanism of RecA protein association with ssDNA (72).

The RecA protein-ssDNA-ATP ternary complex exhibits classic allosterism. The stability and structural conformation of RecA protein-ssDNA complexes are modulated by the presence of nucleotide cofactors. Compared with the affinity of RecA protein for ssDNA in the absence of any cofactors, ATP increases the equilibrium binding affinity of RecA protein for ssDNA, while ADP reduces the affinity (422, 424). Because of these opposing effects, the DNA-dependent hydrolysis of nucleotide cofactor by RecA protein can both regulate and facilitate its binding to and dissociation from ssDNA (303, 422, 424) (Fig. 6). This feature allows RecA protein to solve the paradox encountered by DNA-binding proteins: binding with sufficient affinity so that the lifetime on the DNA is long enough for the desired activity but also being able to dissociate from the DNA so that the enzyme can act catalytically.

Coincident with the ATP-induced increase in ssDNA-bind-

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ing affinity, a conformational change in the RecA protein-ssDNA complex occurs. Electron micrographs directly demonstrate that in the presence of ATP, the RecA nucleoprotein filament is extended with a helical pitch of 95 Å (9.5 nm); in contrast, a filament with a 75-Å (7.5-nm) pitch is seen either in the absence of cofactor or in the presence of ADP (137, 138, 225, 596a–600). A DNA-binding assay employing a chemically modified, fluorescent ssDNA substrate also detects the extension of the RecA protein-ssDNA complex elicited by ATP binding (422, 424). Therefore, the binding of ATP induces the functional high-affinity binding state of RecA protein, with its associated extended nucleoprotein filament structure, whereas the absence of nucleotide cofactor or presence of ADP produces a nonfunctional low-affinity binding state and a compact filament structure. The characteristic nucleoprotein filament formed in the presence of ATP, as defined by both the higher ssDNA-binding affinity and the extended structure, is the functional complex essential to all RecA protein-promoted activities. Mutant RecA proteins which display deficiencies in recombination are, without exception to date, defective in adopting the high-affinity state and as a result do not form a ternary complex competent for DNA strand exchange (302).

Many parallels exist when the binding of RecA protein to dsDNA is compared with its binding to ssDNA: the stoichiometry is similar when expressed as base pairs, and ATP binding yields a RecA protein-dsDNA complex with both an extended helical pitch and an increased stability (28, 137, 138, 225, 596a–600). The primary difference, however, is the slow kinetics of RecA protein binding to dsDNA. The binding of RecA protein to dsDNA is a complex process that involves a rate-limiting nucleation phase followed by rapid filament growth to produce a nucleoprotein filament that is accompanied by unwinding of the DNA duplex (305, 490). The length of the nucleation step is decreased by factors which destabilize the dsDNA (decreased magnesium ion concentration, higher temperatures) (305, 491) or enhance RecA protein association properties (the presence of ATP, lower pH, or the presence of attached ssDNA tails) (305, 490, 491, 697). An ssDNA gap or tail in dsDNA permits presynaptic filament formation by acting as an initial binding site for RecA protein and facilitating the efficient polymerization and stoichiometric binding of the RecA protein into the regions of dsDNA.

Optimal DNA strand exchange activity is achieved when the presynaptic filament is formed at a stoichiometry of 3 nucleotides per monomer; however, two distinct ssDNA-binding stoichiometries of the RecA protein (3 to 4 nucleotides versus 6 to 8 nucleotides per monomer) are observed. Direct measures of ssDNA binding by RecA protein yields values of 7 ± 1 nucleotides per monomer, yet under identical conditions, the ssDNA-dependent ATP hydrolysis activity of RecA protein saturates at 3 ± 1 nucleotides per monomer (325, 424). These differences suggest that the structure of the RecA protein-DNA complex is fundamentally asymmetric, that RecA protein binds to ssDNA in different modes, and that the protein can bind more than one strand of ssDNA (325, 617, 730). The different values for DNA-binding stoichiometry can be explained by proposing that RecA protein can bind two DNA molecules with an intrinsic site size of 3 nucleotides per monomer per DNA-binding site. At low DNA concentrations only one strand would be bound, with a resulting stoichiometry of 3 nucleotides per monomer, but as more DNA is added a second strand could be bound, leading to the value of 6 nucleotides per monomer (325, 617–620, 730). In the absence of any nucleotide cofactor, RecA protein binds DNA with a stoichiometry of 5 to 6 nucleotides per monomer. This type of complex is not convertible to the complex which binds with a stoichiometry of 3 nucleotides per monomer, suggesting that RecA protein may possess two modes of binding to DNA (523, 727).

An unusual feature of the RecA-ssDNA-ATP complex is its subunit nonequivalence with respect to ATP-binding affinity. In a saturated filament (three nucleotides per monomer), half of the RecA protein molecules bind ATP with a lower affinity than that for the remaining RecA protein molecules (325). The appearance of these nonequivalent species of RecA protein depends on the DNA/protein ratio; at a 7:1 DNA/protein ratio only one species is present, but at a 3:1 ratio two species are detected. This again suggests that there are two modes of RecA protein binding to ssDNA. The role of asymmetry in the RecA protein filament is not yet clear, but asymmetry could serve during DNA strand exchange to increase the rate of homology sampling between DNA molecules as a result of the lower affinity for DNA displayed by half of the RecA protein in the saturated filament. It could also serve to distinguish between incoming and displaced DNA strands during DNA strand exchange, perhaps giving directionality to branch migration.

The range of enzymatic activities displayed by the relatively small RecA protein are more easily understood when it is realized that RecA protein almost never exists as a monomeric species but is always found as higher-order oligomers (29, 49, 415, 467). The smallest oligomeric species formed is a trimer, with hexamers and dodecamers being the most prevalent species. These small oligomers are found under conditions which favor RecA protein disaggregation and do not support DNA strand exchange (no Mg^{2+}, 100 to 150 mM KCl) (50). It was concluded that RecA protein forms multiple discrete oligomers which are in reversible equilibrium with each other and that these species presumably are the building blocks which assemble into large RecA protein filaments (50). The addition of Mg^{2+} results in the association of these oligomers into extended filaments. Small-angle neutron scattering of these RecA protein filaments demonstrated that they were identical in helical pitch and dimension to those observed previously in electron micrographs of RecA protein-dsDNA complexes (137, 154). Therefore, the formation of regular helical filaments by RecA protein is not simply a result of protein binding to the inherently helical lattice of DNA but is an intrinsic property that is stimulated by, but is not dependent on, the presence of DNA. This realization readily explains the observations that both ATP hydrolysis (492) and LexA repressor cleavage (135) can occur in the complete absence of DNA, provided that the concentration of monovalent salt exceeds approximately 1.8 M. The high salt concentrations either simulate the polyanionic phosphodiester backbone of DNA or promote helical RecA protein filament formation by enhancing hydrophobic interactions. The latter hypothesis is supported by the fact that the large monomer-monomer interface within the helical RecA protein crystal structure consists of 63% hydrophobic amino acid residues (604).

The filaments formed by RecA protein depend on a number of solution conditions, the most important being as follows: (i) increasing Mg^{2+} concentration stimulates filamentation and extensive aggregation of the filaments; (ii) high salt concentrations inhibit filament formation; and (iii) ATP shifts the equilibrium toward smaller species. The kinetics of filament formation under conditions which support DNA strand exchange (10 mM Mg^{2+}, low ionic strength) show that RecA filament formation is biphasic. The first phase consists of RecA oligomers adding to either end of a growing RecA filament, and the second phase is a much slower "bundling" of these filaments (29). Consistent with these biphasic kinetics, the

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RecA protein displays two types of protein-protein interactions (185): an end-to-end interaction between monomers which is hydrophobic and which is thought to be important for the development of cooperative DNA binding, and a filament-filament interaction which is ionic.

Coprotease activity. Derepression of the SOS regulatory system and induction of various lambdoid prophages (λ, φ80, 434, and P22) require that the RecA protein becomes activated for cleavage of the LexA and lytic repressor proteins, respectively (162, 236a, 344, 345, 402, 507, 543). The proteolytic maturation of the UmuD protein, essential to mutagenesis, is also stimulated by activated RecA protein (66). In vitro, RecA protein-mediated cleavage of these target proteins requires formation of a ternary complex consisting of RecA protein, ssDNA, and either ATP or dATP, with an optimal stoichiometry of 3 nucleotides per monomer (117, 480). Because both RecA protein and ATP are present in uninduced cells, ssDNA is implicated as the limiting component responsible for activation of RecA protein. Consistent with this view and with the fact that the SOS system is repressed under normal growth, the in vitro cleavage of the LexA and λ *c*I repressors by the wild-type RecA protein is not effected by nucleic acids such as dsDNA, rRNA, or tRNA (117, 135, 480, 620, 684). The nonhydrolyzable ATP analog, ATPγS, promotes cleavage of LexA and λ *c*I proteins, demonstrating that hydrolysis of the NTP cofactor is not required (236a, 344, 480). The LexA repressor binds in the deep groove of the helical RecA protein-DNA filament, spanning the space between adjacent RecA protein monomers. The stoichiometry of LexA repressor binding to the RecA protein filament is estimated to be 1 LexA protein for every 2 RecA protein monomers (728).

The RecA protein was originally thought to be a classical protease, with catalysis proceeding via functional groups in an active site of the RecA protein. However, the discovery that the LexA and bacteriophage λ *c*I repressors undergo specific autodigestion under alkaline conditions demonstrated that the RecA protein indirectly stimulates the self-cleavage of target proteins under physiological conditions (342); consequently, this cleavage activity was redefined as a coprotease activity (132). The mechanism by which the LexA and λ *c*I proteins promote their own proteolytic cleavage is similar to that of a serine protease (270, 343, 572). Biochemical evidence suggests that activated RecA protein stimulates autodigestion through the binding and stabilization of a particular repressor conformation, lowering the apparent pK_a of a catalytic amino acid residue within the active site (for a review, see reference 343).

Structure-Function Relationships

RecA protein crystal structure. Recently, the three-dimensional crystal structure of the RecA protein in both the absence and presence of ADP was solved (606, 607). The structure with ADP is the inactive, low-affinity DNA-binding conformation; thus, it may have some significant local structural differences from the active, high-affinity DNA-binding form of the protein, although global structure should be preserved. The structure, in conjunction with genetic and biochemical data, permits assignment of regions proposed to bind DNA and nucleotide cofactor, to stabilize both filaments (monomer-monomer interactions) and higher-order aggregates (bundling of protein filaments), and to bind both repressor and UmuD proteins (Fig. 7A). Furthermore, the crystal structure provides a physical framework for understanding the biochemical behavior of mutant RecA proteins.

The RecA protein has a central domain consisting of a primarily parallel arrangement of eight β sheets (Fig. 7B). This domain contains regions that function in the binding and hydrolysis of nucleotide cofactor (ATP) and possibly the interaction with DNA. Although cocrystals with DNA are not yet available, two disordered regions (loop 1 and loop 2 at amino acid residues 156 to 165 and 194 to 210, respectively) are proposed to be involved in the binding of DNA. The assignment of these loops as possible DNA-binding sites is based on several observations. The residues within these regions are evolutionarily conserved (509) and are located within the interior of the RecA protein filament, which is the inferred site for DNA binding (138, 156). Additionally, several mutant RecA proteins (RecA1, RecA430, and RecA142) having alterations in these regions have defects related to DNA binding or to formation of the high-affinity DNA-binding state (see the discussion of mutant RecA proteins, below).

Diffusion of ADP into crystals of the RecA protein permitted the identification of regions that made up the nucleotide-binding site (606). The location of these residues and their respective functions are summarized as follows: Asp-100, Tyr-103, and Gly-265 make contacts with the base of the nucleoside and establish preferential binding of adenine containing nucleotides; the A-site motif of the RecA protein, 66-GPESSGKT-73, interacts with the PP_i moiety; Asp-144 within amino acid residues 140 to 144, constituting the B-site motif, coordinates the Mg^{2+} ion at the active site between β and γ phosphates; and Gln-96 may activate a water molecule through base catalysis for attack on the β,γ phosphodiester bond during hydrolysis of ATP. Structurally, the A-site of the RecA protein is nearly identical to those of adenylate kinase and of the GTP-binding eIF-Tu and ras p21 proteins (606). A number of biochemical facts coincide with the structural assessment. Tyr-264 is a residue that cross-links to azido-ATP (277) and lies near the nucleotide-binding cleft adjacent to the Gly-265 residue which makes contact with the adenine base (606). The dramatically attenuated NTP hydrolysis activity associated with the mutant RecA(K72R) protein indicates that the invariant Lys-72 of the A-site phosphate-binding loop is a component of the catalytic domain (501). Additionally, extensive mutagenesis of the phosphate-binding loop demonstrates that the conserved residues which make up the A-site motif are essential to biological function (364).

As a consequence of its $P6_1$ symmetry, the RecA protein crystallized as a single turn of a helical filament made up of six protein monomers (607); this structure is remarkably similar to the images derived from electron-microscopic examination of RecA protein-DNA filaments (154, 726). The number of RecA protein monomers per turn of the filament helix is similar (6.2 versus 6.0); the lower value is attributed to crystal-packing constraints. The helical pitch of the active extended filament is 95 Å (9.5 nm), whereas that of the inactive compact structure is 75 Å (7.5 nm) (136, 137, 154, 523, 727); the crystal structure has a pitch of 83 Å (8.3 nm), suggesting that crystal packing created some distortion of the RecA protein filament. Residues whose solvent-accessible surface area is reduced by more than 15 $Å^2$ (0.15 nm^2) upon protein oligomerization constitute regions responsible for interactions between RecA protein monomers; the interface between adjacent subunits of the RecA protein is considerable, accounting for 19.4% of the total exposed surface area of the monomer structure (607). A portion of this interaction interface includes the antiparallel packing of a β strand (residues 26 to 28) and α helix (residues 3 to 21), located in the small subdomain of the amino terminus, against a corresponding β strand (residues 114 to 118) and an α helix (residues 122 to 135) of an adjacent RecA protein monomer in the filament (607). The interactions involved in the monomer-monomer interface include both hydrophobic

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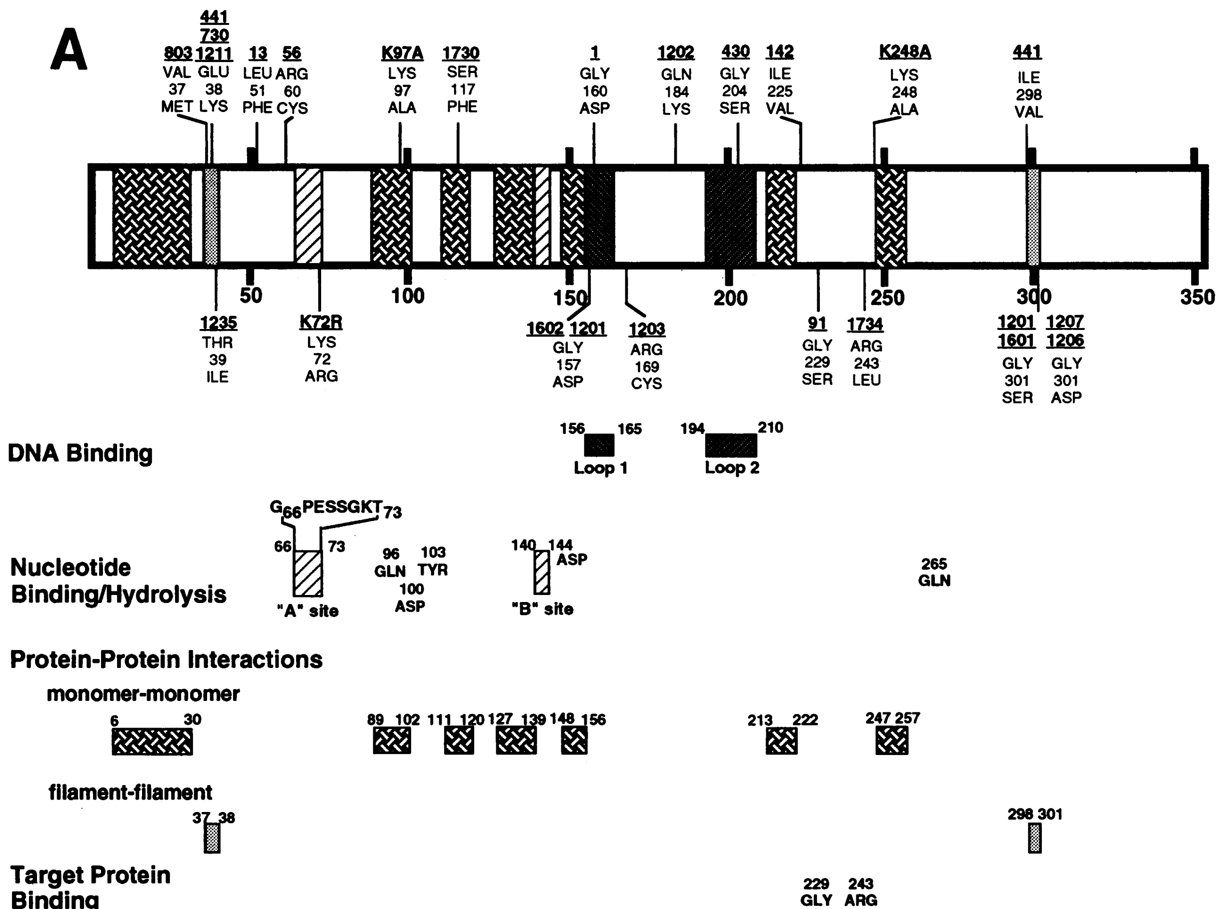

FIG. 7. (A) The primary sequence location of *recA* mutations and of likely functional domains based on the RecA protein crystal structure (606, 607). The references for the sequence data are as follows: *1, 430* (267); *13, 56* (92a); *K72R* (501); *803* (390); *441* (276); *1730, 1734, 142* (152); *91,* (465); *1201, 1202, 1203, 1206, 1207, 1211, 1601, 1602* (683); *K97A, K248A* (462). (B) Structure of RecA protein determined by X-ray crystallography (605–607), with functional domains assigned on the basis of the biochemical, genetic, and structural data discussed in the text.

and polar amino acid residues. This is in reasonable agreement with results of previous studies involving RecA protein affinity column techniques that suggested that the interaction between RecA monomers was primarily hydrophobic (185). Mutant RecA proteins which have changes in residues in this interface (Ser-117, His-97, Lys-248) display defective DNA-binding characteristics (21, 462), which in one case can be partially overcome by overexpression (21). Genetic analysis demonstrates that certain residues (Lys-216, Phe-217, and Arg-222) within a region of the RecA protein (residues 213 to 222) predicted to be involved in RecA protein monomer interactions can tolerate only highly conserved substitutions (Lys to Arg, Phe to Tyr, and Arg to Lys, respectively) (570).

The residues in the proposed polymer-polymer interface correspond to regions that are distant within a monomer subunit and involve the interaction between the carboxy-terminal domain of one filament (Ile-298) and the amino-terminal portion of a second filament (Glu-38). A number of mutations within these regions result in RecA proteins that have enhanced activities relative to that of the wild type (see below for a discussion of the RecA441, RecA803, and RecA730 proteins). Consequently, it was proposed that the observed bundling of RecA protein filaments (29, 155, 442, 523) not only may be biologically relevant but also may serve to moderate the activities of RecA protein (604, 607).

Relationship to RecA-like proteins from other organisms. Sequence comparison among 23 RecA proteins from eubacterial sources shows that this protein sequence is strongly conserved through evolution. In the core region of the RecA protein (residues 14 to 272), 136 residues (53%) are invariant or conserved (509, 605). In contrast, the carboxy-terminal 50 amino acids are highly divergent and appear to be dispensable since a truncated RecA protein lacking this portion remains functional both in vivo and in vitro (28, 237, 324). However, the

TABLE 2. Classes of *recA* mutations

Class	Mutations
Enhanced activities	*recA441, recA730, recA803, recA1202, recA1211*
Partially defective	*recA430, recA142*
Totally defective (null)	*recA1, recA56, recA13*
Differentially affected	
Coprotease activity	*recA1734, recA91*
Protease constitutive, recombination deficient	*recA1201, recA1203, recA1206, recA1207, recA1601, recA1602*
Filament formation defective	*recA1730, recA(H97A), recA(K248A)*

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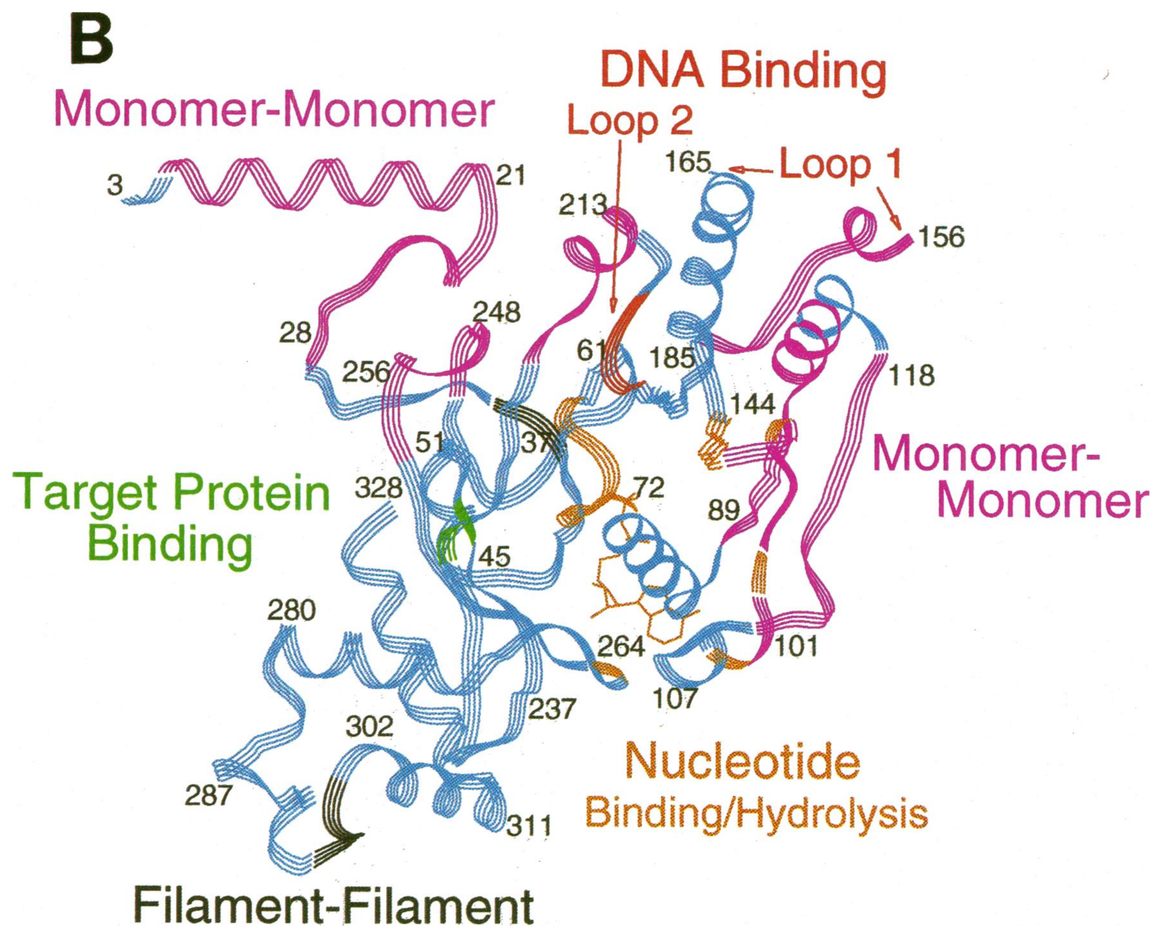

FIG. 7—*Continued.*

acidic carboxy tail appears to function as a regulatory domain that acts to prevent uncontrolled induction of the SOS response (237, 324).

The RecA protein also shares sequence similarity with recombination proteins from bacteriophage T4 (UvsX) (186) and *Saccharomyces cerevisiae* (Dmc1 and Rad51) (40, 562a). The similarity clusters around residues involved in ATP binding and hydrolysis and in the putative site for interaction with DNA. In addition, the large hydrophobic region which constitutes the core of RecA protein is conserved. Overall, of the 156 highly conserved or invariant residues in eubacterial RecA proteins, 87 (56%) are conserved in UvsX protein and 64 (41%) are conserved in Dmc1 protein (607).

The Rad51 protein has ATP-dependent DNA-binding characteristics that are similar to those of the RecA protein (562a). Residues 154 to 374 of Rad51 protein are 30% identical and 24% similar to residues 33 to 240 of RecA protein and include the A- and B-site nucleotide-binding motifs (562a, 664). The Rad51 protein-dsDNA filament is very similar to that of the RecA protein (468). It has a slightly greater helical pitch (99 versus 95 Å [9.9 versus 9.5 nm]) but has the same number of monomer units per helical turn (6.2), the same axial rise per base pair (5.1 Å [0.51 nm]), and the same apparent DNA-binding stoichiometry (3 bp per monomer) as the RecA protein filament. These results suggest that the Rad51, Dmc1, UvsX, and RecA proteins share a common ancestry and may share fundamental biochemical attributes.

Behavior of Mutant RecA Proteins

A remarkable range of mutant *recA* alleles have been described, and a number of mutant RecA proteins have been characterized biochemically (302) (Table 2; Fig. 7A). Some alleles show enhanced activity relative to the wild-type; these include *recA441* (273, 328, 329, 348, 378, 480, 515), *recA730* (330, 711), *recA803* (330, 389, 390), and *recA1202* and *recA1211* (634, 683, 684). Others, such as the *recA142* (42, 150, 304, 308, 506, 515, 542) and *recA430* (133, 378, 423, 440, 506, 515, 542, 661) alleles, are only partially defective. Furthermore, certain *recA* mutations display differential alterations. These include those which are constitutive for coprotease activity but are recombination defective (*recA1201*, *recA1203*, *recA1206*, *recA1207*, *recA1601*, and *recA1602* [634, 683]) and the alleles which show differential repressor and UmuD cleavage specificities (*recA1730*, *recA1734*, and *recA91* [152, 465]). Finally, there are those which show no activity in vivo such as the *recA1* (55, 57, 93, 326, 508, 528, 661, 704), *recA56* (19, 238, 326, 690), and *recA13* (326, 704) mutations.

RecA mutants with enhanced activities. Certain mutant alleles display elevated levels of *recA* function, both in vivo and in vitro. Many of these mutants were isolated on the basis of their ability to induce the SOS response or prophage constitutively (i.e., in the absence of DNA damage). The *recA441* mutation was originally identified by its ability to induce prophage at elevated temperatures (42°C) in the absence of

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DNA damage (202). Subsequently, it was also shown to cause thermally induced filamentous growth (273); thus, the allele is commonly referred to as *tif* (temperature-induced filamentation). Both constitutive prophage induction and filamentous growth result from constitutive coprotease activity at elevated temperatures (202, 273). The *recA730* allele, which is derived from *recA441*, exhibits constitutive SOS and prophage induction at all temperatures studied (711).

Both *recA441* and *recA730* enhance the frequency of recombination by at least two- to fivefold as measured by either P1 phage transduction or conjugation with an Hfr donor, suggesting that these mutant proteins are also more active than wild-type protein in recombination (711). In addition, the *recA441* allele shows enhanced frequencies of multiple exchanges (i.e., an increased frequency of exchanges per unit length), but no elevation in single-point crosses with distant markers (348) or in the recombination of λ *red phage* (71a) was detected; strain differences may account for these variations (711).

The *recA803* allele was identified as a mutation that could partially suppress the UV sensitivity of *recF* mutants and was hypothesized to act by increasing recombinational repair (657). *recA803* was found to partially alleviate both sensitivity to mitomycin and deficiencies in conjugal recombination in a *recF* (*recBC sbcBC*) strain (657).

Biochemical studies involving the Rec441, RecA730, and RecA803 proteins demonstrate that each can promote joint-molecule formation under conditions that are normally suboptimal for wild-type protein (329, 330, 389, 390). These results parallel the elevated recombination phenotype for each mutant. This elevated activity results from an enhanced ability of each mutant RecA protein to compete with SSB protein for DNA-binding sites. This heightened activity does not arise through an increase in the equilibrium ssDNA-binding affinity but, rather, from an increased rate of association of these mutant RecA proteins with ssDNA (328–330, 389). These observations offer an explanation for the constitutive protease activity observed for both *recA441* and *recA730* strains. Although studies in vitro indicate that the RecA441 and RecA730 proteins require the same ternary complex for stimulation of LexA protein cleavage as the wild-type protein does, their increased rates of association with ssDNA presumably permit these RecA proteins to compete with and displace SSB protein from ssDNA that exists naturally in the bacterial cell; therefore, they are constitutively activated for repressor cleavage (330). RecA803 protein also associates with ssDNA somewhat more rapidly than the wild-type protein does, but this difference appears to not be sufficient to produce the level of displacement required for a constitutive repressor cleavage phenotype (330).

The substitutions resulting in the mutant RecA441 and RecA803 proteins are located in the regions proposed from the crystal structure to be involved in filament-filament interactions (Fig. 7). Consequently, such mutations may increase the rate of association of the RecA protein with ssDNA by discouraging the formation of large RecA protein aggregates that decrease the rate of RecA protein binding to ssDNA (607).

An independently isolated class of *recA* mutations displaying constitutive coprotease activity were found to map in the two regions that also include the *recA441* and *recA803* mutations (amino acid residues 25 to 39 and 298 to 301) (635). A number of these mutations (represented by *recA1211* [Glu-38 to Lys]) lie within the regions implicated in filament-filament interactions, whereas a different set of mutations (represented by *recA1202* [Gln-184 to Lys] [683]) cluster within the major central domain (residues 158, 179, and 184), between the loops proposed to be involved in DNA binding (607) (Fig. 7). Both purified RecA1211 and RecA1202 proteins exhibit enhanced repressor cleavage activity, relative to the wild-type RecA protein, when the normally inactive NTP cofactors (dUTP, UTP, dCTP, CTP, dGTP, GTP, and TTP) or polynucleotide substrates (rRNA and tRNA) are used (682, 684). The proximity of these latter mutations to the putative DNA-binding site is consistent with their activation by RNA, suggesting that these mutations result in either increased affinity or reduced specificity for nucleic acid binding. Presumably, some of the mutations in the putative filament-filament interaction domain can produce the same net effect.

Partially defective RecA mutants. The *recA430* and *recA142* alleles are examples of defective *recA* mutations that retain partial function. The *recA430* allele is partially defective in recombination, SOS response, and prophage induction. In vitro the RecA430 protein is able to promote DNA strand exchange, although it is more sensitive to and requires higher protein and ATP concentrations. Furthermore, the RecA430 protein is less efficient in competing for ssDNA-binding sites with SSB protein. Consistent with these observations, the RecA430 protein has a lower binding affinity for ssDNA and a lower rate of association with ssDNA (423, 661). The mutated residue of the RecA430 protein (Gly-204 to Ser) lies in a region of the crystal structure implicated in DNA binding (loop 2 [Fig. 7]), lending credence to the assignment of loop 2 as a DNA-binding domain.

The *recA142* allele is completely defective in recombination and partially defective in prophage induction. As expected, the RecA142 protein is unable to catalyze DNA strand exchange in vitro. Unlike the fully defective alleles discussed below, the RecA142 protein does possess some activities: DNA-dependent ATP hydrolysis and reduced (~20% relative to wild-type RecA protein) LexA repressor coprotease activity. However, these activities are inhibited by SSB protein, indicating that RecA142 protein cannot compete with SSB protein for ssDNA-binding sites (304). The inability to promote DNA strand exchange and to compete with SSB protein is explained by the failure of RecA142 protein to assume the high-affinity DNA-binding state normally induced by ATP binding (304). The mutation in the RecA142 protein occurs at a highly conserved residue (Ile-225 to Val) in the hydrophobic core (607), and it may interfere with the ability of this protein to assume the high-affinity DNA-binding state.

Null alleles. The null alleles *recA1*, *recA56*, and *recA13* are phenotypically indistinguishable from a deletion of the *recA* gene (19, 118, 690). In vitro the proteins can bind ssDNA (55, 57, 467), but ATP decreases, rather than increases, the affinity (326, 661). The mutation in the RecA13 protein (Leu-51 to Phe) lies in the hydrophobic core of the protein, suggesting that disruption of the hydrophobic core packing by the bulkier phenylalanine residue leads to global changes in the protein (326). The mutation in RecA1 protein (Gly-160 to Asp) resides in the loop 1 region proposed to be involved in DNA binding (Fig. 7) and introduces a negative charge that may interfere with interactions with the phosphodiester backbone (604, 607). Replacement of this residue by an asparagine residue results in a less severe defect, suggesting that the negative charge is indeed detrimental (55, 447). The mutation in the RecA56 protein (Arg-60 to Cys) lies near the phosphate-binding loop and may interfere with the allosteric change induced by ATP or with DNA binding in the high-affinity conformation (326).

A number of defective full-length (e.g., RecA1 and RecA56 proteins) and truncated versions of the RecA protein inhibit the wild-type protein in vivo. This codominant inhibition was

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postulated to occur through the formation of mixed protein multimers which are "poisoned" for further activities (237, 324, 467, 551, 704, 719, 724). Biochemical investigation of this phenomenon by using the RecA56 protein provided quantitative evidence that the mechanism of inhibition is through heteromultimer formation (326). The defects seen with mixed RecA protein filaments display a range of sensitivities to increasing incorporation of the defective RecA protein into the filament. DNA strand exchange and competition with SSB protein are the most sensitive properties, and LexA repressor cleavage and ATP hydrolysis activity are the least affected. Thus, the entire RecA protein filament, rather than a protein monomer, should be viewed as the functional entity, with its overall behavior reflecting the composition of its monomer components (308, 326).

Differentially affected RecA mutants. The binding of repressor or UmuD proteins was proposed to occur at a cleft formed by adjacent RecA protein monomers (607). This suggestion was based on the existence of mutations (*recA1734* and *recA91*) which differentially affect the ability of RecA protein to cleave its target proteins but which remain recombination proficient. The RecA1734 protein can cleave the λ *c*I and LexA repressor proteins but has no activity toward ϕ80 repressor or UmuD protein (152), whereas the RecA91 protein can cleave the λ *c*I but not the ϕ80 repressor (152, 465). The *recA1734* and *recA91* mutations are substitutions of Arg-243 (to Leu) and Gly-229 (to Ser), respectively, defining a region which may be responsible for target protein binding.

The binding of RecA protein to ssDNA and ATP is normally required for both the activation of coprotease activity and the presynaptic stage of DNA strand exchange. Thus, RecA protein mutations which affect interactions with ssDNA and nucleotide cofactor typically affect protease and recombination activities similarly. However, a class of protease-constitutive mutants (i.e., activated in vivo in the absence of DNA-damaging agents) are deficient in recombination (634). These mutations cluster in two distinct regions which are proposed to be involved either in the binding of DNA (loop 1 residues 157 to 164) or in the formation of RecA protein filament bundles (residue 301) (607). The biochemical basis for this protease-constitutive, recombination-deficient behavior remains unknown. It is intriguing to speculate that this class of mutant RecA protein may be defective in binding of a second DNA molecule (and therefore unable to promote pairing and exchange) but may possess enhanced ssDNA-binding or filament formation properties that permit coprotease activity under normally nonpermissive conditions.

RecA mutants defective in protein filament formation. The *recA1730* mutation (Ser-117 to Phe) lies in a β sheet which is important for monomer-monomer interaction. This protein supports ϕ80 repressor, λ repressor, and UmuD protein cleavage but not LexA repressor cleavage. This protein is defective in recombination unless overexpressed (152) but remains defective in SOS mutagenesis, suggesting that the filament formation defect, but not the improper targeting of the UmuD'C complex, can be overcome at high protein concentrations (21).

Mutation of residues (His-97 and Lys-248 to Ala) that participate in monomer-monomer interaction result in proteins that bind poorly to ssDNA, have no ATPase activity, and fail to promote both the renaturation and the DNA strand exchange reactions (462). The Mg^{2+}-dependent aggregation normally seen with wild-type RecA protein is also severely reduced. These two residues, distant in the monomer, make up part of the large-subunit interface on opposing faces of adjacent RecA protein subunits within the helical filament (607). Thus, given the highly cooperative nature of RecA protein filament formation, it is not surprising that mutations which affect monomer-monomer contacts alter all of the fundamental activities of the protein.

RecBCD ENZYME (EXONUCLEASE V)

The RecBCD enzyme (exonuclease V) of *E. coli* is a large, multifunctional enzyme composed of RecB, RecC, and RecD subunits. Analogs of this enzyme have been found in both gram-positive and gram-negative bacteria (626, 632). This enzyme has both DNA helicase and nucleolytic activities that function in recombination and repair. The RecBCD enzyme is an essential component of the principal pathway for the recombination of linear DNA molecules in *E. coli*, the RecBCD pathway, and results of interspecies complementation studies suggest that this pathway is conserved among enteric bacteria (134, 417, 687). Genetic analysis and biochemical characterization of RecBCD enzyme and of corresponding genetic variants have provided considerable insight into the role that RecBCD enzyme plays in critical cellular functions (for reviews, see references 575, 577, and 626).

Biological Functions

Discovery. Isolation of the *recB* and *recC* genes was facilitated by the fact that mutations in either of these genes reduce conjugational and transductional recombination by 10^2- to 10^3-fold (166). Mutants were isolated by screening chemically mutagenized cells for the inability to act as the F^- recipient in an Hfr cross and ensuring that F' transfer was not affected (93). *recB* was also independently isolated as a partially defective mutant (*rorA*) which displayed X-ray but not UV sensitivity (198, 199). The mutations defined two complementation groups at 61 min on the *E. coli* chromosome (166, 703a). Null mutations in either group have similar phenotypes (Table 3): decreased conjugational, transductional, and phage recombination (75, 240, 703a); sensitivity to DNA-damaging agents, including UV and gamma irradiation (166, 240); decreased transformation efficiency (232, 470); decreased cell viability (68); and the absence of ATP-dependent nuclease activity, resulting in "cautious" degradation of chromosomal DNA upon exposure to UV light (240, 242, 703a).

The *recD* gene was identified only after the recognition that a third subunit, the RecD polypeptide, was an integral component of the holoenzyme (11). Mutations in the *recD* gene turned out to be a subset of a previously identified class of mutations referred to as *recBC*‡ (76). Strains with these mutations have a phenotype quite distinct from that of *recB* or *recC* mutants; the cells are proficient in conjugal recombination (38, 76), and, in wild-type (χ^0) bacteriophage λ crosses, *recD* strains are hyperrecombinogenic (76, 637). *recD* cells are resistant to UV and gamma irradiation (47), have normal levels of cell viability (76), and display plasmid instability (38). Like *recBC* mutants, however, *recD* mutants lack ATP-dependent nuclease activity and are not stimulated by χ, a DNA sequence which enhances recombination occurring by the RecBCD pathway (76) (see below).

Physical characterization of the *recBCD* locus. Cloning and sequencing of the *thyA-argA* region of the *E. coli* chromosome revealed the presence of four genes (11, 153, 229, 540): *recC* (178), *ptr* (which encodes protease III [177]), *recB* (176), and *recD* (175). The *recB* and *recD* genes constitute an operon which initiates transcription upstream of *recB* (11, 176, 540). *recC* is transcribed independently, although in the same orientation (178, 540). Regulation of these genes is not affected by

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TABLE 3. Summary of mutant RecBCD enzyme properties[a]

Mutational class	Gene(s) affected	Representative alleles	DNA helicase[b]	ssDNA and dsDNA exonuclease	hDNA formation[c]	χ activation[d]	Recombination[e]	UV sensitivity	SOS induction[f]	Cell viability	References
Wild type	None		+	+	+	+	+	Resistant	+	High	10, 233, 626
Null[g]	B, C	*B21, C22*	−	−	−	−	−	Sensitive	−	Low	75, 166, 229, 233
Δ*recD*	D	NA	±[h]	−	ND	−	ND	ND	ND	ND	140, 298, 404, 476
‡	D, C	*D1011, C1010*	±[h]	−	+	−	+	Resistant	+	High	11, 76, 139, 376, 626, 637
*	C	*C1003*	+	+	+	−	±	Resistant	ND	High	233, 548
Tex	B, C	*B344, C343*	+	+	ND	±	+	Resistant	ND	High	382, 548
Class I	B	*B2109*	+	+[i]	−	−	−	Sensitive	ND	Low	10, 157, 158, 233, 577
Class II	B, C	*B2154, C2145*	+	+	−	−	−	Sensitive	ND	Low	10, 233
Class III	B	*B2153*	+	+	−	+	−	Sensitive	ND	Low	10, 233
Class IV	ND	*rec2148*	+	±	+	−	±[j]	ND	ND	Low	10, 233
RorA	B	*rorA1*	ND	+[k]	ND	ND	+	Resistant	ND	ND	198, 199

[a] See text for more complete descriptions. Adapted from references 10, 233, and 577. Symbols: NA, not applicable; ND, not determined; +, activity present; ±, activity reduced; −, activity absent.
[b] Determined with crude cell extracts.
[c] Heteroduplex DNA formation in λ-infected cells.
[d] Determined by both stimulation of recombination in λ crosses and in vitro χ cutting.
[e] Proficiency determined by Hfr conjugation and P1 transduction.
[f] Induced by nalidixic acid.
[g] Includes nonsense, insertion, and deletion alleles.
[h] Determined by using purified enzyme; see the text and references for details.
[i] Activity present at high ATP concentrations (>1 mM).
[j] From 3 to 25% of wild-type activity observed in λ recombination.
[k] ssDNA exonuclease activity is unknown.

SOS induction (228, 230), and the level of the enzyme in the cells is low (~10 copies per cell), presumably because of poor consensus transcription and translation initiation signals as well as slightly higher than average usage of rare codons (175, 176, 178, 230). The *recB*, *recC*, and *recD* genes encode proteins with calculated molecular masses of 134, 129, and 67 kDa, respectively (175, 176, 178).

Genetic recombination. RecBCD enzyme is important in at least four types of recombination events: conjugation, transduction, transformation with broken chromosomal DNA, and recombination of λ *red gam* phage. Each of these processes is related in that the DNA is, at some point, a linear duplex molecule. During conjugation, ssDNA enters the recipient cell and is rapidly converted to a duplex form by lagging-strand synthesis. Broken chromosomal DNA is, of course, linear. Transduction is characterized by the injection of linear cellular dsDNA into the cell. The packaging and viability of λ *red gam* phage requires dimerization of the phage genome, a process which requires host recombination functions and is initiated by a dsDNA cut at the *cos* site. Each of these processes provides dsDNA ends that can serve as entry sites for RecBCD enzyme (187, 628). Other processes, such as intrachromosomal recombination and plasmid recombination, are independent of RecBCD enzyme activity, presumably because no duplex DNA end is available to serve as an entry site for RecBCD enzyme (376, 729). Certain chromosomal rearrangements (e.g., inversions or circle integration) in *Salmonella typhimurium* seem to occur by a two-step mechanism in which an intermediate joint molecule is initially formed by a *recBC*-independent event that produces a broken chromosome; this intermediate is then repaired by a second *recBC*-dependent step (396).

Repair of damaged DNA. The repair of damaged DNA involves the RecBCD enzyme through its direct participation in recombinational repair and its indirect participation in SOS induction (77, 420, 674). dsDNA breaks are repaired by a *recA-recBC*-dependent mechanism (674, 676). Presumably, RecBCD enzyme can act on the dsDNA break along with the RecA protein and other enzymes to carry out recombination coupled with DNA repair (311, 455, 539, 674). Although it is unknown if RecBCD enzyme is directly involved in the repair of ssDNA breaks, it is presumed that irreparable ssDNA damage subsequently leads to dsDNA breaks that require RecBCD enzyme for repair (674).

The mechanism by which RecBCD enzyme mediates induction of the SOS response is uncertain, but a reasonable scenario is at hand (see also the section on RecA protein, above). RecBCD enzyme is required for induction by agents such as nalidixic acid but not by others such as UV irradiation (214, 420). A possible explanation involves the creation of dsDNA breaks, which may result from aberrant dsDNA cutting by nalidixic acid-inhibited topoisomerase II (DNA gyrase); these breaks represent entry sites for RecBCD enzyme. It is most likely that the helicase activity, and not the nuclease activity, of RecBCD enzyme is involved in the induction of the SOS response through the production of ssDNA, since nalidixic acid also induces the SOS response in nuclease-deficient *recBCD*‡ mutant cells (20, 77).

Degradation of foreign DNA. RecBCD enzyme contributes to the degradation of foreign DNA entering the cell. The nuclease activities of RecBCD enzyme are involved directly, and its helicase activity is involved indirectly by creating ssDNA which is acted upon by other nucleases (504). For example, the transformation of wild-type cells with linear dsDNA is only approximately 4% as efficient as the transformation with circular DNA; mutations in *recD*, however, allow linear molecules to transform at least 10-fold more efficiently (376, 529).

Another means by which foreign DNA enters the cell is during phage infections. Many phages encode specific inhibitors of RecBCD enzyme. These inhibitors utilize different mechanisms to protect the phage genome from the degradative activity of RecBCD enzyme. If the genes encoding these

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protective functions are mutated, the infecting phage DNAs are rapidly degraded into oligonucleotides (471, 568, 652). The γ (Gam) protein of bacteriophage λ inhibits the activities of RecBCD enzyme by directly binding to the protein (265, 456, 487) and possibly interacting with the RecD subunit (403). This inhibition is necessary to protect the λ chromosome from RecBCD enzyme degradation during rolling-circle (σ) replication of wild-type phage. The phage T4 gene 2 product is another inhibitor of RecBCD enzyme, but it presumably binds to the ends of the linear phage genome, preventing the binding of RecBCD enzyme (471). *Salmonella typhimurium* phage P22 encodes a function, designated *abc* (anti-RecBC), which inhibits the nuclease activity of RecBCD enzyme (171, 487). The region encoding *abc* function consists of three open reading frames that potentially encode polypeptides with molecular masses of 10.9, 11.6, and 6.6 kDa (457). Although the precise mechanism of protection is not known, *abc* activity depends on the 11.6-kDa polypeptide and, to a lesser extent, on the 10.9-kDa polypeptide (457). Finally, RecBCD enzyme inhibits rolling-circle (σ) replication of plasmids by degrading the newly synthesized DNA (97, 168). This degradation is overcome by having a correctly oriented χ sequence within the plasmid DNA (121), suggesting that the dsDNA exonuclease activity, rather than the DNA unwinding activity, is directly responsible in the degradation process (see below).

Stimulation of recombination by χ. Bacteriophage λ encodes its own recombination machinery (54, 562b), the *red* system, that consists of the λ exonuclease and β proteins. In *red gam* phage infections, θ replication prevails, producing monomeric circular genomes (168). These genomes cannot be packaged without further processing provided by the *red* system in the wild-type phage. In the absence of λ recombination functions, *red gam* phage are dependent on the activities of the RecBCD pathway to generate the required packageable concatemeric intermediate. These phage, when devoid of χ, rarely package their genome as a result of inefficient recombination by the RecBCD pathway, resulting in a low titer or small plaque size.

Spontaneous mutations in λ *red gam* that resulted in large-plaque formation were discovered (222, 320). Subsequent studies determined that these mutations, called χ (for crossover hot-spot instigator [320]), create DNA sites that enhance recombination occurring via the RecBCD pathway 5- to 10-fold (320, 419, 593, 593a). Stimulation is polar (594) and is dependent on the orientation of the χ site relative to the λ *cos* site (278). The polar effect of χ is detectable for more than 10 kb from the site (80, 167, 593), although the stimulation falls by a factor of 2 for every 2.2 to 3.2 kb (80, 167). χ occurs naturally in the *E. coli* chromosome with a frequency of once approximately every 5 kb (169, 399). Studies demonstrated that recombination during conjugation and transduction occurs preferentially at and downstream of χ, suggesting that nearly all conjugal recombination events in *E. coli* involving the RecBCD pathway are χ mediated (143). Detailed mutational analysis, based on about a dozen mutations creating χ activity and a similar number abolishing χ activity, provided the sequence of χ (581, 582, 584, 589, 641); the precise sequence is 5′-GCTGGTGG-3′, its complement, or both (582). Most single-base-pair alterations within this sequence eliminate the stimulatory activity of χ, although certain single-base-pair changes leave 6 to 38% activity (78, 79).

Because a dsDNA break is necessary to activate recombination at χ in phage λ, Stahl et al. (596) proposed that a factor was required which could bind to the DNA end and translocate until it reached χ (see also references 585 and 591). Since stimulation by χ was dependent on the *recB* and *recC* genes, the RecBCD enzyme was presumed to be the factor. Subsequent biochemical studies confirmed a direct interaction between χ and RecBCD enzyme (see below). Until recently, there was no direct proof of whether χ acted early (i.e., before RecA protein-dependent DNA heteroduplex formation) at an initiation step or whether it acted late (i.e., after DNA heteroduplex formation) at a resolution step, in recombination (Fig. 2). Recent experiments designed to address this question demonstrate that χ stimulates recombination by enhancing the yield of heteroduplex DNA three- to sevenfold; this result was interpreted to suggest an early initiation function for χ (233). It was pointed out that these experiments, however, do not allow unequivocal determination of whether χ and RecBCD enzyme promote the formation, stabilization, or resolution of intermediates in recombination (215), but a counter-argument was presented (233a). Nevertheless, recent biochemical studies demonstrate an enhancement of DNA heteroduplex formation by χ (see below). Thus, although χ may affect other steps in the recombination process, the collective genetic and biochemical data argue for, minimally, an initiation function for both RecBCD enzyme and χ (Fig. 2). Furthermore, attempts to demonstrate a Holliday junction resolution activity for RecBCD enzyme were negative (448, 629).

A specific model presented by Smith and coworkers, incorporating the known genetic and biochemical properties of RecBCD enzyme and χ, proposes their involvement in the initiation of recombinational events (579, 585; see reference 578a for a recent overview). Through its helicase activity, RecBCD enzyme initiates the process by unwinding dsDNA; upon encountering a χ sequence, the enzyme specifically nicks the ssDNA to the 3′ side of χ (484, 627). Continued translocation produces a ssDNA tail containing a 3′-hydroxyl end, which is a suitable substrate for DNA pairing by the RecA protein. The RecA protein is envisioned to bind to the ssDNA tail and carry out DNA strand invasion of a recipient DNA molecule (Fig. 2). This model is supported by genetic studies which suggest that the RecBCD enzyme can act early in the recombination process (233, 486, 720) and by the known biochemical activities of RecA and RecBCD proteins (see the section on RecA protein, above; also see below). Nearly 80% of conjugational recombination events are proposed to occur at the ends of the transferred DNA by means of this mechanism (578).

Biochemical investigations have established specific tenets of this model (see below). Recombination reactions reconstituted in vitro show that the RecBCD enzyme can initiate recombinational events by creating ssDNA, which can be used as a substrate for RecA protein-dependent DNA heteroduplex formation (310, 510, 515, 679). DNA heteroduplex formation in vitro is enhanced when χ-containing DNA is used as the donor DNA species (i.e., the dsDNA which is being unwound by RecBCD enzyme and which is the source of ssDNA for strand invasion) (141). Stimulation of homologous pairing occurs downstream from χ and, as predicted (579), initiates at the 3′ end generated by cleavage at χ (303a).

An alternative view suggests that the RecBCD enzyme acts late in recombination, most probably in the resolution of recombination intermediates (39, 356). The nuclease activity of RecBCD enzyme was postulated to resolve Holliday junctions (592); however, direct experiments designed to test this premise have failed to uncover any ability to cleave specifically at cruciform DNA or Holliday junctions (448, 629). Moreover, a protein with the required properties for resolution of recombination intermediates has been identified in *E. coli* (see the section on RuvC protein, below).

A variation of the resolution model was suggested by Thaler

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et al. (636, 637) to explain the unique genetic properties of *recBCD*‡ mutations. The authors propose that the RecBCD‡ protein is equivalent to the χ-activated RecBCD enzyme, by virtue of the RecD subunit being removed from the holoenzyme through its encounter with χ. The wild-type RecBCD enzyme is considered to be an antirecombinogenic resolvase that disrupts paranemically paired recombination intermediates by virtue of its helicase and nuclease activities, whereas the χ-activated RecBCD enzyme (the proposed RecBC protein) can translocate nondisruptively to the intertwined region and productively resolve the junction. Recently, Stahl et al. proposed a modification of the original Thaler et al. hypothesis (595). They proposed that exonuclease V activity is fully manifest and degrades the parental DNA, until the RecD subunit dissociates upon interaction with χ; this exonucleolytic degradation upstream of χ results in frayed DNA ends that are reactive in RecA protein-dependent processes. A model that is similar up to this point, referred to as a split-end model, was subsequently elaborated to describe recombination events at double-strand breaks and at χ (517). In the Stahl model, the altered enzyme, devoid of the RecD subunit, is envisioned to continue traveling without net unwinding of DNA until it encounters a crossover structure which it resolves, whereas, in the split-end model, the enzyme is converted into a helicase. Positive biochemical or genetic tests of these resolution hypotheses are yet to be reported; however, the notion that interaction with χ alters the RecBCD enzyme by attenuating its nuclease activity but not its helicase activity was already demonstrated in vitro (141, 142), and results of recent work imply involvement of the RecD subunit in this alteration process (140). Furthermore, genetic experiments on the physical structure of "patched" recombinants suggest that both DNA strands downstream of χ participate in the recombination events that lead to hybrid DNA formation, arguing that both strands can be invasive (215a). The degree to which these models are consistent with the biochemical behavior of RecBCD enzyme is elaborated in the next section.

An interesting facet of χ behavior is the observation that SOS induction produces a partial inhibitor of χ activation which does not affect overall recombination levels (505). This inhibitor is the product of the *ruvA* and *ruvB* genes (661a), which together form a complex that binds specifically to Holliday structures and possesses ATPase, helicase, and branch migration activities (see the section on RuvAB proteins, below). Because overproduction of the RecD subunit alleviates this inhibition (505), it was proposed that the RuvAB complex modifies RecBCD enzyme via interaction with the RecD subunit to produce a "χ-independent" recombination enzyme. This alteration may be similar to that elicited by the productive interaction of the RecBCD enzyme with χ, which results in either removal or functional inactivation of the RecD subunit (140) (see below).

Biochemical Activities

Properties of the RecB, RecC, and RecD polypeptides. Early studies reported that the purified exonuclease V was composed of two polypeptides (163, 201, 716). These polypeptides were the products of the *recB* and *recC* genes (229), and hence the nuclease was alternatively referred to as the RecBC enzyme (201). Incubation of the enzyme in high salt concentrations (4 M NaCl) resulted in dissociation of the holoenzyme complex into two fractions, termed α and β, that had estimated molecular masses of ca. 60 and 170 kDa, respectively (336, 337). The individual fractions lacked ATP-dependent nuclease activity, but nuclease activity was restored when the α and β fractions were mixed. The β fraction contained the RecB and RecC polypeptides, suggesting that the functional form of RecBC enzyme consisted of three subunits (RecB, RecC, and an unknown 60-kDa protein) (337). Subsequent work discovered that the α fraction contains a third polypeptide encoded by the *recD* gene (11). Hence, exonuclease V is now referred to as the RecBCD enzyme.

Knowledge of the functions for each of the individual polypeptides within the RecBCD holoenzyme is incomplete; however, an increasing number of facts are becoming available. The amino acid sequences of the RecB and RecD subunits contain consensus nucleotide-binding domains (175, 176), and both these can bind the ATP analog, 8-azido-ATP (44, 258). The RecB protein sequence also contains a helix-turn-helix motif characteristic of a DNA-binding domain at residues 63 to 86 (176). The individual RecB, RecC, and RecD proteins contain neither nuclease nor processive helicase activities (43, 404), but the RecB protein can hydrolyze ATP in a DNA-dependent manner (44, 298) and can unwind a 22-bp region of duplex DNA in an oligonucleotide displacement helicase assay (44). The apparent polarity of translocation for this weak RecB protein helicase activity is seemingly 3′ → 5′ relative to the ssDNA to which it is bound (44, 404). Purified RecB and RecC subunits can be reconstituted to produce a protein that has ATP-dependent helicase but little or no nuclease activity (140, 298, 404, 476); the observed specific activity of RecBC enzyme is low because of either inefficient reconstitution or a low apparent affinity (i.e., high K_m) for dsDNA ends (140). Genetic data suggest that RecC and RecD subunits associate because mutations with the ‡ phenotype map to both *recC* and *recD* (11). The individually purified RecB, RecC, and RecD subunits can associate in vitro to give enzymatic activities that are indistinguishable from those of the wild-type RecBCD enzyme, albeit with lower specific activities (43, 44, 404). To date no biochemical activity has been directly associated with the RecC protein; however, it is related antigenically to an endo-exonuclease from *Neurospora crassa* and its carboxyl terminus (residues 813 to 1120) is reported to have 32% similarity to the *Saccharomyces cerevisiae* endo-exonuclease, NUC1 (184), suggesting that the RecC subunit may contain an active site for nuclease activity.

DNA helicase properties. The ability of the RecBCD enzyme to unwind dsDNA was suggested by the nature of the dsDNA exonuclease reaction. Degradation of duplex DNA requires ATP hydrolysis, and the intermediates produced are dsDNA with long single-stranded tails (384, 632). Direct evidence for and measurement of RecBCD enzyme-catalyzed DNA unwinding was obtained from electron microscopy. Those studies showed the production of two unique partially unwound DNA intermediates: "twin loops" and "loop tails" (625, 633). Figure 8A illustrates a twin-loop structure which consists of two unwound ssDNA loops of equal size that are flanked on each side by dsDNA. Loop-tail structures (Fig. 8B) are composed of two ssDNA tails of different lengths and an ssDNA loop. In this structure, the DNA strand containing the shorter tail and loop is the one having the 3′ terminus at the enzymatic entry site (46); the sum of these two lengths equals that of the longer ssDNA tail. The loops grow with distance from the dsDNA end and enlarge at a rate of approximately 100 bp per s (625, 628). These studies demonstrated that the enzyme entered at a dsDNA end and unwound DNA at a constant rate; they also suggested that the unwinding was highly processive (at least 20 kb per binding event).

Detailed kinetic analysis of the helicase activity of RecBCD enzyme was accomplished through the use of a continuous, fluorescent DNA unwinding assay (514). This assay takes

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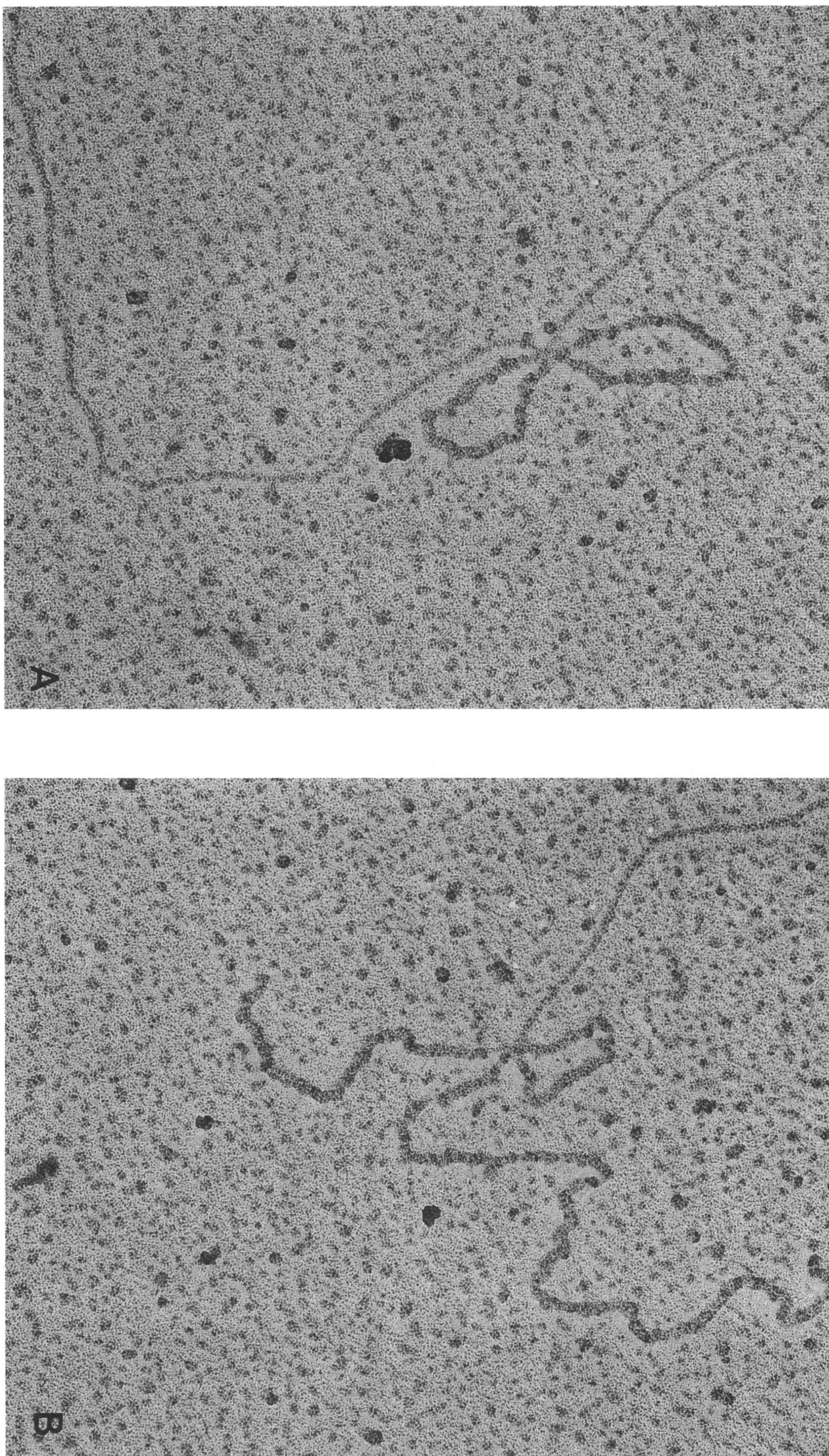

FIG. 8. DNA intermediates formed by RecBCD enzyme unwinding. Linear dsDNA from phage T7 was reacted with a cell extract from a strain overproducing RecBCD enzyme in the presence of ATP, magnesium, calcium, and SSB protein; the unwound molecules were fixed and visualized by electron microscopy. (A) A twin-loop structure. (B) A loop-tailed structure. The thinner lines are dsDNA, and the thicker lines are SSB protein-coated ssDNA. Reprinted from reference 579 with permission of the publisher.

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advantage of the quenching of the intrinsic fluorescence of SSB protein upon binding to the ssDNA created by DNA unwinding. Under optimum conditions, the RecBCD enzyme can processively unwind large tracts of dsDNA (average of 30 kb per binding event) at rates of up to 1,000 to 1,500 bp per s at 37°C (158, 511, 514). The apparent K_m values of RecBCD enzyme for DNA ends and ATP are 1.2 nM and 130 μM, respectively (158, 514). These values confirm the significance of the helicase activity in vivo since they are similar to or below the presumed in vivo concentrations of these substrates (514). Matching results are also observed by using an unwinding assay based on fluorescent DNA-binding dyes as reporters of DNA unwinding (160).

The unwinding of DNA by RecBCD enzyme requires a linear molecule that has a flush or nearly flush (less than ≈25 nucleotides of ssDNA) dsDNA end (628). The RecBCD enzyme binds to the ends of a DNA molecule with high affinity ($K_d \approx 0.5$ nM) in the presence of Mg^{2+} ions (628). Although the specific site(s) of DNA interaction is not known, both the ATPase and nuclease activities of RecBCD enzyme are inhibited by covalent modification with pyridoxal 5-phosphate in a manner consistent with its location in the DNA-binding site of the enzyme (12, 187). UV cross-linking studies indicate that the RecB subunit interacts with the end of the 3′-terminal strand, while the RecC and RecD subunits interact with the end of the 5′-terminal strand (187). Thus, the inability of RecBCD enzyme to unwind DNA molecules with ssDNA overhangs greater than 25 nucleotides in length (628) may be due to an inability of the RecB, RecC, and RecD subunits to simultaneously interact with the appropriate DNA ends.

Under optimal conditions, DNA unwinding is accompanied by the hydrolysis of 1.7 to 3 molecules of ATP per bp unwound (513). Helicase activity is not as sensitive to changes in the ionic environment (e.g., magnesium and calcium ion concentrations) as the dsDNA exonuclease activity (see below). Although DNA unwinding is dependent on the presence of Mg^{2+}, similar rates of unwinding are obtained at Mg^{2+} concentrations ranging from 1 to 10 mM; the presence of 1 to 5 mM Ca^{2+} results in a slight decrease (20 to 30%) in the rate of unwinding (514).

To determine the effect of DNA-binding proteins on the ability of RecBCD enzyme to unwind DNA, the suitability of chromatin DNA to serve as a substrate for RecBCD enzyme helicase activity was tested (159). Although this particular template is not encountered by RecBCD enzyme in vivo, the enzyme may face similar blocks to unwinding in *E. coli* since the prokaryotic chromosome is condensed by histone-like proteins such as HU and IHF to form a "nucleoid" structure (reviewed in reference 146). Despite the necessarily heterologous nature of the assay system, RecBCD enzyme is capable of unwinding the nucleosomal DNA, whether this DNA is isolated as native chromatin from HeLa cells or is reconstituted in vitro (159). The processivity of unwinding is reduced to a greater extent (greater than 20-fold) than is the rate of unwinding (about 3-fold), suggesting that the processivity of translocation by RecBCD enzyme in vivo may be attenuated by the presence of DNA-binding proteins, as suggested by Roman et al. (511).

A model for DNA unwinding by RecBCD enzyme incorporating current observations is shown in Fig. 9. RecBCD enzyme initiates unwinding by binding to the dsDNA termini. On the basis of their ability to bind ATP, the RecB and RecD subunits are proposed to act as translocating subunits which couple unwinding and ATP hydrolysis. Although the specific role of each subunit in the holoenzyme is unknown, it is possible that only one subunit functions as an active "helicase" with the other being simply a "translocase" (513). The RecB subunit translocates along the top strand of the DNA with a 3′ → 5′ polarity at a different rate than does the RecD subunit, which moves along the bottom strand with a 5′ → 3′ polarity. The outcome of having the RecB and RecD subunits moving at different rates while being bound together in the holoenzyme complex results in the accumulation of a loop that grows with distance in front of the RecB subunit. Thus, in the presence of stoichiometric amounts of SSB protein, a loop-tailed structure is formed, whereas if SSB protein is limiting, the strands can reanneal to form a twin-loop structure (624). This model requires that the RecD subunit possess translocation activity as part of the holoenzyme; while this supposition remains unproven, mutagenesis of the ATP-binding site of the RecD subunit demonstrates that its proper function is not essential for RecBCD enzyme helicase activity (296–298). This mutation, however, lowers the processivity of unwinding by the holoenzyme by an undetermined mechanism. It is now clear that the helicase activity of the RecB subunit is sufficient for unwinding by the RecBC enzyme (140, 298); however, it is not clear whether loop structures are formed.

Nonspecific nucleolytic properties. The RecBCD enzyme can degrade both ds- and ssDNA, with the products ranging from oligonucleotides to ssDNA fragments thousands of bases in length (201, 264, 384, 716). The hydrolysis of DNA can occur as a result of ATP-dependent dsDNA exonuclease, ATP-dependent ssDNA exonuclease, and ATP-stimulated ssDNA endonuclease activities. This ability to degrade linear dsDNA can be viewed as a protective mechanism to guard the cell from foreign DNA invasion, yet its existence appears contradictory to the need for RecBCD enzyme in homologous recombination.

RecBCD enzyme (exonuclease V) was originally identified as the only NTP-dependent dsDNA exonuclease in *E. coli* (716). Exonuclease V-like enzymes have been identified in many bacterial species (396, 417, 583, 632, 686), although an ATP-dependent DNA nuclease is yet to be found in eukaryotes. The digestion of dsDNA by this activity is processive, requires both a dsDNA end and continued ATP hydrolysis, and is blocked by the presence of a psoralen-DNA cross-link (163, 201, 263, 264, 384, 716). Thus, the dsDNA exonuclease activity is coincident with DNA unwinding, which suggests that degradation occurs as a result of the endonucleolytic cleavage of ssDNA during DNA unwinding (264, 384, 385, 632, 633). The RecBCD enzyme degrades duplex DNA asymmetrically, with the DNA strand that is 3′ at the entry site being degraded to a greater extent than that containing the 5′ end (141, 142).

In vitro reaction conditions have a dramatic effect on the dsDNA exonuclease activity of RecBCD enzyme, yet they leave the helicase activity relatively unaltered. The ratio of magnesium ion concentration to ATP concentration is the crucial parameter that dictates the level of dsDNA exonuclease activity in vitro (157, 163). Maximal dsDNA exonuclease activity is observed when the ratio of Mg^{2+} concentration to ATP concentration is above 0.75, and the activity is dependent only on the free Mg^{2+} concentration (provided that the ATP concentration exceeds the K_m for ATP) (157). Optimal production of acid-soluble nucleotides (i.e., smaller than 12 to 15 nucleotides) occurs at high concentrations of magnesium ion and low concentrations of ATP (e.g., 10 mM Mg^{2+}, 25 to 50 μM ATP) (163, 201, 716). Production of acid-soluble nucleotides by the dsDNA exonuclease activity is inhibited under conditions of low magnesium ion concentration (<0.5 mM) or millimolar concentrations of ATP (163, 201, 469, 716). Under these conditions, reaction intermediates were partially duplex DNA molecules with single-stranded regions ranging from

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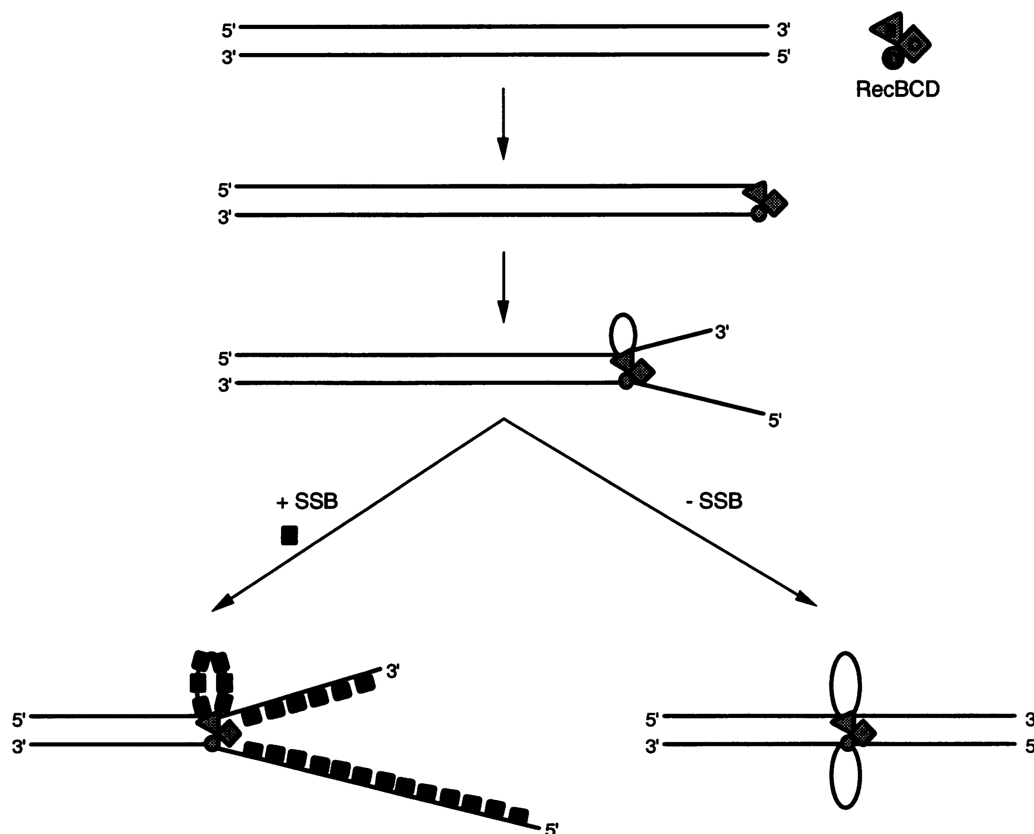

FIG. 9. Model for dsDNA unwinding by RecBCD enzyme. Details are discussed in the text. The RecB, RecC, and RecD subunits are represented by the triangle, diamond, and circle, respectively; SSB protein is represented by shaded squares. A loop-tailed structure is shown to the left, and a twin-loop structure is shown to the right. Degradation of the DNA strands is not shown (see Fig. 11 for an illustration of combined nuclease and helicase action).

2,000 to 4,000 nucleotides in length (264, 384). On the basis of these findings, it was suggested that the dsDNA exonuclease and helicase activities are separate activities but occur concurrently. Other agents that inhibit the dsDNA exonuclease activity are Ca^{2+}, Mn^{2+}, and Cd^{2+} (516, 716); the presence of 1 mM Ca^{2+} in reaction mixtures suppresses the dsDNA exonuclease activity by more than 83%. However, the physiological relevance of inhibition by Ca^{2+} is unclear since concentrations of free Ca^{2+} in *E. coli* are below 0.1 μM (188). Finally, SSB protein also inhibits degradation of dsDNA, presumably by protecting the unwound ssDNA from postunwinding degradation (385).

The simplest mechanism for dsDNA exonuclease activity of the RecBCD enzyme envisions cleavage of DNA occurring as an endonucleolytic scission on the ssDNA behind the point of dsDNA unwinding. The unwinding and nucleolytic activities can be viewed as separate but intertwined functions that determine the size of degradation products produced. The intrinsic frequency of cleavage (i.e., number of scissions per second, k_{cat}) is dictated by the experimental conditions, with the frequency increasing as the free Mg^{2+} concentration increases. The size of the ssDNA fragments produced, in turn, depends on the rate of RecBCD enzyme translocation (i.e., number of base pairs unwound per second), which determines how far the enzyme will travel before a cleavage occurs and which is quite sensitive to the ATP concentration ($K_m \approx 130$ μM [514]). The average size of ssDNA fragments produced is therefore the average rate of unwinding divided by the average rate of cleavage, which yields the average length of ssDNA produced before cleavage occurs (i.e., number of base pairs per scission event). Consequently, under conditions of free Mg^{2+} concentration in excess (>1 mM, where k_{cat} for cleavage is constant [157]), the size of the degradation products will depend on the rate of unwinding. At very low, nonphysiological ATP concentrations (i.e., 25 to 50 μM), the low rates of unwinding (514) result in production of short acid-soluble fragments; at higher, physiologically relevant ATP concentrations (i.e., >1 mM ATP), the higher rate of unwinding results in much longer fragments that are, on average, in the kilobase size range (384, 385, 632). The seemingly complex dependence on the [Mg^{2+}]-to-[ATP] ratio is easily reconciled by the dual role of Mg^{2+} in dsDNA exonuclease activity: it is required to form a complex with the ATP needed for helicase activity, and it is required in its free form for nucleolytic activity (157).

Through its ssDNA endonuclease activity, the RecBCD enzyme can cleave ssDNA circular molecules (200, 201), ssDNA gaps within duplex DNA (264, 385, 488, 628, 704a), and D-loop structures (701a, 704a). The rate of cleavage of gapped dsDNA increases in proportion to the size of the ssDNA gap (628). This activity does not require ATP, but ATP stimulates cleavage approximately sevenfold to a rate of approximately 0.7 scission per min (157, 201). The turnover number for this activity is so small that its physiological significance is uncertain.

In contrast to the ssDNA endonuclease activity, the ssDNA exonuclease activity is ATP dependent, produces oligonucleo-

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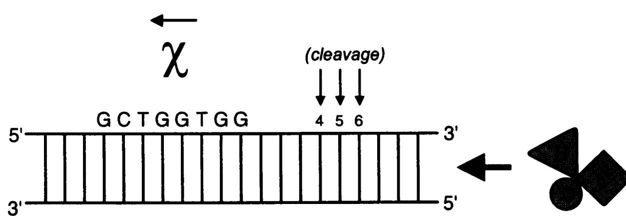

FIG. 10. Interaction between RecBCD enzyme and χ. The arrow above the χ site indicates the direction that RecBCD enzyme must approach for recognition of χ. The nick generated at χ occurs 4 to 6 nucleotides to the 3' side of χ on the strand containing the χ sequence (5'-GCTGGTGG-3'). RecBCD enzyme is represented by the triangle-diamond-circle.

tides 4 to 5 nucleotides in length (201), and is believed to occur processively (200). The ssDNA exonuclease activity, although dependent on ATP, is not influenced by ATP concentration in the manner exhibited by dsDNA exonuclease activity (163, 201). One explanation for this behavior is that ssDNA exonuclease activity does not require ATP for enzymatic translocation; instead, ATP serves as an allosteric effector necessary for both ssDNA end binding and cleavage approximately 5 nucleotides from the end.

RecBCD enzyme and χ sites. In vitro, the χ site causes RecBCD enzyme to generate a ssDNA break in the vicinity of the χ sequence (Fig. 10) (484). This nick occurs 4 to 6 nucleotides to the 3' side of the χ sequence on the DNA strand containing the χ sequence to generate 5'-phosphate and 3'-hydroxyl termini (627). Cleavage at χ happens only during dsDNA unwinding, since χ-dependent nicks are not detected with circular dsDNA or with ssDNA molecules (484), and is highly specific—a single-base-pair change in the χ sequence reduces χ nicking from 10- to over 20-fold (79). The strand cleavage seen at χ occurs at a frequency of 25 to 40% of the RecBCD enzyme-χ encounters (142, 630). As predicted by genetic studies, this interaction with χ in vitro is orientation dependent; RecBCD enzyme must approach the χ sequence from the 3' side to create the specific nicks seen at χ (627).

In addition to defining the locus for a nick, χ is a unique regulatory element which acts to attenuate the dsDNA exonuclease activity of RecBCD enzyme (141, 142, 303a). Upon interaction with χ, degradation of the 3'-terminal DNA strand (the strand containing the χ sequence [see Fig. 11]) is reduced at least 500-fold, yet the helicase activity of RecBCD enzyme is essentially unaffected (141, 142). Continued unwinding by RecBCD enzyme generates an intact ssDNA molecule downstream from the χ site that is preferentially used in RecA protein-dependent homologous DNA-pairing reactions in vitro (141). Attenuation of strand degradation activity is manifest for at least 10 kb downstream from χ (142). This effect renders RecBCD enzyme incapable of cleaving DNA not only nonspecifically (141, 142) but also at a second, properly oriented χ site on the same DNA molecule (630). As discussed for the nonspecific dsDNA exonuclease activity, the pattern of χ-dependent ssDNA fragment production is dependent on the free Mg^{2+} concentration (139). At higher free Mg^{2+} concentrations, only the fragment downstream of χ is detected, because of excessive degradation of the upstream fragment (141, 142). At lower free Mg^{2+} concentrations (with an optimum at an [Mg^{2+}]-to-[ATP] ratio of 0.25 [139]), both upstream (3'-side) and downstream (5'-side) fragments are recovered (484, 627, 630) but with a net bias of about 5- to 10-fold toward recovery of the downstream fragment (139). It appears that the yield of χ-specific fragments is governed by a compromise between the frequency of nonspecific cleavage and the probability of cleavage by the enzyme while paused at the χ sequence (see below).

The interaction with χ is accompanied by a pause of RecBCD enzyme at the χ site (140, 142). This brief cessation of DNA unwinding is estimated to be on the order of 1 s and may provide a molecular explanation for cutting at χ. The view of the dsDNA exonuclease activity of RecBCD enzyme described in the previous section proposes that cleavage occurs approximately randomly on the ssDNA which is produced coincidentally with unwinding (163, 201, 513, 626, 632, 716). Thus, it follows that the consequence of any pause by RecBCD enzyme would be an increased probability of a nucleolytic event at the site of pausing. Because of attenuation of the nuclease activity after interaction with χ, the observed nick at χ can be viewed as the last cleavage event that is made by the unaltered enzyme; this explanation easily rationalizes production of the downstream χ-specific fragment (142). χ-specific fragments originating from the upstream side of χ are detected only when the probability of cleaving the ssDNA between the entry site and the χ sequence is low; this occurs when the distance to the χ site is relatively short and the nucleolytic activity of RecBCD enzyme is moderated by the presence of low free Mg^{2+} concentrations (139). The pause at χ may also reflect the molecular event (i.e., inactivation or loss of the RecD subunit) that is responsible for the χ-dependent attenuation of the nuclease activity of RecBCD enzyme (140). This view of enzymatic pausing offers an alternative view to the idea that the DNA cutting at χ is a site-specific endonucleolytic event (484, 627) but does not deny that pausing, nuclease inactivation, and χ-specific nicking in vitro and χ stimulation of recombination in vivo are all interrelated events which are manifestations of the same primary event, recognition of the χ sequence.

Once the RecBCD enzyme has exited or dissociated from the χ-containing DNA molecule that produced the attenuation of nuclease activity, full activity of the enzyme can be restored; i.e., the enzyme can become fully active for nuclease (both χ specific and nonspecific) and helicase activities. However, the free Mg^{2+} concentration also affects this reactivation step. At high free Mg^{2+} concentrations, the activities of RecBCD enzyme are immediately restored when it exits the χ-containing DNA molecule, and subsequent unwinding and degradation of other DNA molecules occur in a completely catalytic fashion (141, 142). In contrast, at low free Mg^{2+} concentrations, an interaction with χ reversibly inactivates the enzyme, so that neither DNA unwinding, nucleolytic degradation, nor χ fragment production occurs on subsequent DNA molecules (140, 630); inactivation of nucleolytic activity requires that assay conditions which are low in free Mg^{2+} concentration (i.e., the ATP concentration exceeding the Mg^{2+} concentration) be maintained (140). Under these conditions, unwinding is noncatalytic, with the extent of unwinding being dependent on the amount of RecBCD enzyme relative to χ-containing DNA (140, 630); catalytic action is restored by the addition or presence of excess Mg^{2+} (140–142). The precise mechanism of χ inactivation is not yet known, but, on the basis of the ability of the reconstituted RecBC protein (i.e., without the RecD subunit) to mimic the χ-attenuated RecBCD enzyme, it is surmised that the alteration involves inactivation or loss of the RecD subunit (see below) (140).

Manifestation of χ-dependent inactivation is also inferred from in vivo work. Placement of a χ sequence upstream of a second χ diminishes the ability of the downstream χ to stimulate recombination (595, 718). The ability of χ to protect

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DNA downstream, but not upstream, from itself against degradation by RecBCD enzyme was also deduced from in vivo work. Genetic studies involving recombination of bacteriophage λ showed that in crosses with one λ phage being χ^0 and the other being χ^+, complementary χ^+-containing recombinants are recovered as frequently as the χ^0 reciprocal recombinants only if the χ^+ parent present is in excess of the χ^0 parent (595). This observation suggests that the formation of each χ^+ recombinant involves two χ^+ parents; genetic information upstream of χ from one of the χ^+ parents is lost as a result of RecBCD degradation, thereby requiring two recombination events to produce viable recombinant progeny. In support, recent findings involving viable-plasmid production via a rolling-circle replication mechanism demonstrated that the presence of χ in the proper orientation with respect to the replicated DNA end results in the formation of high-molecular-weight linear multimers, whereas when the orientation of χ is reversed, RecBCD enzyme-dependent degradation ensues (121). These findings suggest that RecBCD enzyme-dependent degradation up to χ is responsible for the loss of genetic information and that the attenuation of nuclease activity is a crucial aspect of χ stimulation of recombination in vivo.

Behavior of Mutant RecBCD Enzymes

Examination of mutant forms of RecBCD enzyme permits elucidation of the physiological function for each enzymatic activity. Various mutations in the *recB*, *recC*, and *recD* genes were identified, and the enzymatic activities of these mutant enzymes were examined. The biochemical and genetic properties of such mutations are summarized in Table 3.

***recBCD*‡ mutations and RecBC enzyme.** *recBCD*‡ mutants were originally isolated on the basis of their ability to support the growth of phage T4 gene *2* phage (76). The majority of ‡ mutations, which are presumed to be nonsense mutations, map to the *recD* gene, with the exception of one that maps to *recC* (11, 76). Cell extracts of various *recD*‡ strains are devoid of full-length RecD polypeptide, yet it is not known if a truncated RecD polypeptide is an active component of the RecBCD‡ enzyme (11). Because the *recC1010* allele does not affect the synthesis of a RecD polypeptide, it is thought that this mutation alters the RecC polypeptide such that it cannot interact with RecD protein (11).

Phenotypically, *recBCD*‡ cells are very similar to wild-type cells (see above) in that they are fully viable, resistant to DNA-damaging agents, and recombination proficient (76). Recombination in these strains is different, however, in that levels of conjugal recombination and P1-mediated transduction are comparable to or are elevated relative to wild-type strains (76, 376), and these processes are dependent on the presence of an active *recJ* gene product (376). Enhanced plasmid recombination is also seen in ‡ strains (38, 376), but it is dependent on *recJ*$^+$ to a lesser extent (376). For recombination of λ *red gam* phage, *recD* mutants are hyperrecombinogenic, are *recJ* independent, and do not support χ activation of recombination beyond the already elevated levels (76, 637). These findings led to the hypothesis that the RecBC enzyme, which lacks the RecD subunit, is equivalent to the RecBCD enzyme after it has encountered a χ sequence and is functionally χ-activated (636, 637).

The most noticeable biochemical difference between ‡ mutants and wild-type RecBCD enzyme is that the mutant enzymes are devoid of any detectable ds- or ssDNA nuclease activities in vivo and in vitro (11, 76, 139, 140, 626). These mutants also have either no, or a markedly lower, ability to produce a χ-specific DNA fragment relative to the extent seen with the wild-type enzyme (139, 626) and do not promote χ-dependent joint-molecule formation in vitro (139). Although cell free extracts of RecBCD‡ mutants do not show any detectable DNA-unwinding activity (11, 515a), a purified preparation of the RecBCD‡1011 enzyme does have modest (ca. 20 to 30% of the wild-type activity at equimolar functional enzyme concentrations) although uncharacterized helicase activity (139). The difficulty in detecting unwinding activity in crude extracts of *recD*‡ cells may suggest the presence of additional factors that can inhibit the helicase activity of RecBCD‡ enzyme, or it may simply reflect the difficulty of detecting low levels of helicase activity in unpurified preparations. However, recent in vivo experiments support the in vitro characterization of RecBC enzyme. Degradation of intracellular phage DNA occurs via a *recB*- and *recC*-dependent process that requires *recJ* and *xonA* function. The authors concluded that RecBC protein is capable of DNA unwinding in vivo but not of DNA degradation and that degradation requires the ssDNA exonuclease activities of RecJ protein and exonuclease I (504).

A purified mutant enzyme from a strain containing an insertion (Tn*1000*) in the *recD* gene shows no RecD polypeptide, but maxicell analysis of this construct shows a polypeptide somewhat larger than RecD protein; the species is presumed to be a fusion product between RecD and the Tn*1000* insertion (476). This purified RecBC protein has no detectable ds- or ssDNA exonuclease activities, yet it can unwind DNA (at a rate 1 to 2% of the wild-type rate [calculated from data reported in reference 476]) and has reduced ds- or ssDNA-dependent ATPase activity (two- to fourfold less than the wild-type enzyme) (476).

Cloning of the individual *recB*, *recC*, and *recD* genes and purification of each subunit permit study of the RecBC protein devoid of the RecD subunit (43, 404). In agreement with the results for the RecBCD‡ mutant enzyme, reconstitution of the RecBC protein from the individual subunits yields no DNA exonuclease or χ-nicking activities but does yield modest levels of helicase and DNA-dependent ATPase activities (43, 140, 298, 404). The reconstituted RecBC protein possesses helicase activity (as much as 50% of the wild-type enzyme activity [90a, 140]) only under conditions of high magnesium ion concentrations (e.g., 10 mM Mg^{2+}, 5 mM ATP), whereas unwinding is not detected at low magnesium ion concentrations (e.g., 1 mM Mg^{2+}, 5 mM ATP) (140). These results mirror the behavior of the RecBCD enzyme once it has been modified by interaction with χ and suggest that the RecBC protein is enzymatically similar to the χ-activated wild-type enzyme (see above).

***recBC**D mutations.** The *recC** mutations were isolated as pseudorevertants of a presumed missense mutation in the *recC* gene that partially or completely regained various aspects of wild-type enzyme activity (548). These mutants are moderately recombination proficient and somewhat sensitive to UV irradiation; they do not, however, stimulate recombination at χ sites (548). Crude cell extracts of RecBC*D mutant enzymes have wild-type levels of dsDNA exonuclease activity and do not produce χ-specific fragments (484, 548). A purified preparation from a *recC1003* strain shows that this enzyme is composed of the RecB, RecC*, and RecD subunits of the expected sizes and possesses wild-type levels of dsDNA exonuclease activity but no detectable χ fragment production (139). Although this mutant enzyme is a potent helicase with rates of unwinding comparable to those of the wild-type enzyme, it is unable to promote χ-dependent joint-molecule formation in vitro (139). These findings suggest that the χ recognition domain of RecBCD enzyme resides in the RecC subunit; however, other mutations (e.g., some class II mutations [see

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below]) that do not exhibit χ stimulation map to the RecB subunit, suggesting that χ recognition responsibility may be shared between the RecC and RecB subunits. To explain the ability of these mutants to promote modest levels of recombination in the absence of χ recognition, it is proposed that they are capable of recognizing and stimulating recombination at sequences other than 5′-GCTGGTGG-3′, although the alternate recognition sequence is yet to be determined (548).

***texA* mutations.** The *tex* class (for transposon excision) of mutations are defined by their enhanced frequency of precise excision of transposons Tn*5* and Tn*10* from the chromosome (382). Mutations resulting in the *texA* phenotype map to both the *recB* and *recC* genes (382), and strains containing these mutations are proficient in promoting recombination but show reduced levels of χ stimulation (i.e., *texA* mutants display a twofold increase in recombination at χ, whereas wild-type cells exhibit a sixfold increase). *texA* strains are not appreciably more UV sensitive than are tex^+ strains (382). Extracts of *texA* mutants possess higher levels of dsDNA exonuclease activity (120 to 140% of wild-type activity) (382) and show reduced but detectable χ fragment production in vitro (484). Other activities, such as ssDNA endo- and exonuclease and helicase activities, have not been tested. Thus, *tex* mutations appear to affect the ability of RecBCD enzyme to behave properly at χ sites (i.e., the frequency of productive interactions may be reduced relative to that of wild-type enzyme).

Class I mutants. In a search for mutations that differentially alter the activities of the RecBCD enzyme, modifications that gave rise to five phenotypically distinct classes of *recB* or *recC* mutations were isolated (10). The class I mutants originated both as a spontaneous mutation (*recB2152*) and as the result of chemical mutagenesis (*recB2109*) (10). These mutants are recombination deficient (a frequency of 0.1% compared with the wild type in conjugal assays) and fail to be activated by χ (10). It was also observed that although the DNA of T4 gene *2* phage (see above) is not degraded to acid-soluble oligonucleotides in vivo, the mutant T4 phage is unable to survive on these cells whereas λ *red gam* phage produce large plaques.

The product of the *recB2109* allele, the $RecB^{2109}CD$ enzyme, was purified and extensively characterized (157, 158). In vitro, the enzyme displays a two- to threefold reduction in most activities of the wild-type enzyme, including helicase, ds- and ssDNA exonuclease, and ssDNA endonuclease. The site of the mutation in the RecB subunit is unknown, but the mutation appears to affect its interaction with nucleotide cofactor, since the K_m for ATP of several activities examined is significantly higher (6- to 14-fold) than that of the wild-type enzyme (10, 157). Despite its ability to degrade dsDNA in vitro, this enzyme does not display χ-specific fragment production (10, 157). It is not clear whether this defect arises from an inability to recognize the recombination hotspot sequence itself or whether the process by which attenuation of the 3′-strand-specific nuclease activity occurs is defective.

It was proposed that the inability of this enzyme to productively interact with χ (i.e., to attenuate its nuclease activity) results in a mutant enzyme that is a constitutive dsDNA exonuclease which degrades the 3′-terminal strand required for pairing by RecA protein (157, 158). This interpretation is consistent with the observation that regardless of its ability to unwind DNA, the $RecB^{2109}CD$ enzyme does not form heteroduplex DNA in vivo (233) or efficiently in vitro (158). The apparent discrepancy between the in vivo and in vitro results regarding its dsDNA exonuclease activity can be resolved if this mutant enzyme is unable to produce acid-soluble oligonucleotides (i.e., fragments <16 nucleotides) in vivo but is able to do so in vitro (10, 157).

Class II mutants. Class II mutations, which occur in the *recB* as well as in the *recC* gene, exhibit many of the same phenotypes and activities as those of the class I mutants (10). Unlike the class I mutants, however, these strains degrade T4 gene *2* phage DNA to oligonucleotides in vivo and do prevent plaque formation by λ *red gam* phage. On the basis of their similarity to the class I mutants, it is possible that they are alleles with the same fundamental defect (i.e., a failure to act properly at χ, resulting in constitutive expression of dsDNA exonuclease activity) but with a more severe manifestation of the class I defect.

Class III mutants. Class III mutations are represented by a single allele, *recB2153* (10). On the basis of genetic analysis and biochemical characterizations with cell extracts, this mutant enzyme appears to possess all of the activities of the wild-type protein (i.e., helicase, dsDNA exonuclease, ssDNA exonuclease, and χ recognition), yet this mutation confers a recombination-deficient phenotype in both conjugal and λ recombination assays. Although able to cleave at χ, this class III mutant does not make heteroduplex DNA in vivo (233). Explanations for these observations include the possibility that the level of any of these enzymatic activities may be quantitatively reduced (e.g., processivity of unwinding) or that some currently unappreciated facet of RecBCD enzyme biochemical behavior is affected (10). Alternatively, another interpretation of this phenotype could be that the mutant enzyme does not fully attenuate its dsDNA exonuclease activity as a result of a defective interaction with χ (233). This would result in continued cleavage and degradation of the ssDNA that normally serves as the substrate for RecA protein.

Class IV mutants. Strains bearing *rec-2123*, *rec-2140*, or *rec-2148* alleles were created by mutagenizing a plasmid containing the $thyA^+$-$recBCD^+$-$argA^+$ region of the *E. coli* chromosome (10); the specific alleles have not yet been assigned to a particular gene(s). These cells are phenotypically recombination deficient. In crude extracts, these mutant enzymes possess significant helicase activity (estimated at 50 to 90% of wild-type levels). The levels of ssDNA and dsDNA exonuclease activities of these mutants is highly variable (*rec-2123* has no detectable nuclease activities, *rec-2148* has no detectable dsDNA exonuclease but has ~35% of wild-type levels of ssDNA exonuclease, and *rec-2140* has both dsDNA and ssDNA exonuclease activities [14 and 47%, respectively]). These assays were conducted at the low ATP concentration (25 μM) that failed to support the dsDNA exonuclease activity of the $RecB^{2109}CD$ protein, however. Thus, it is possible that at more physiological ATP concentrations (≥1 mM), these mutants will demonstrate nuclease activity. None of these mutants possesses χ fragment-producing activity (10). Although the three alleles are not equivalent in most genetic assays, in many respects they are similar to the class I mutants. The class IV mutants, however, are able to form heteroduplex DNA in vivo in both the presence and absence of χ (233). These observations imply that in vivo the mutant RecBCD enzymes are able to unwind DNA, and they may suggest that the enzymes are defective in performing a step required for resolution of a recombinant intermediate (233). Alternatively, the behavior of these mutants may be explained if the mutant enzymes, while unwinding DNA, nick the DNA less frequently (compared with the wild-type enzyme). Upon encountering χ, these enzymes pause at the χ sequence with the same duration as that of the wild-type enzyme, but, because they nick less frequently, the probability that a cut would occur in the vicinity of the χ sequence would be lower. If attenuation of the already diminished dsDNA exonuclease activity is incomplete, nicks downstream of χ would go unnoticed in in vitro experiments,

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but the ssDNA tracts produced in vivo would be sufficient for DNA heteroduplex formation.

***rorA* mutation.** *rorA1* (for Roentgen-resistant) designates a mutation in the *recB* gene that causes UV resistance but X-ray sensitivity (198, 199). This mutant enzyme retains dsDNA exonuclease activity both in vivo (199) and in vitro (655). The DNA-dependent ATPase activity is increased relative to the nuclease activity, suggesting that either the frequency of nicking of the DNA during unwinding is reduced or the efficiency of ATP hydrolysis (normally 2 to 3 molecules hydrolyzed per bp unwound [513]) is decreased. On the basis of the difference in phenotype caused by *rorA* versus *recB* or *recC* mutations, it was proposed that the *ror* mutation affects the interaction of RecBCD enzyme with other proteins (199). Enzymatic results with extracts suggest that the RecB subunit interacts with DNA polymerase I (223, 614), although direct physical evidence is lacking. It should be noted that the *ror* phenotype is also exhibited by cells which overproduce the RecD subunit (48).

Other RecBCD enzyme mutations. A mutation generated in vitro in the consensus phosphate-binding motif of the RecB protein results in a mutant RecBCD enzyme, RecB(K29Q)CD enzyme, that has essentially no dsDNA-dependent nuclease or ATPase activities with dsDNA (246). However, this enzyme has both ssDNA-dependent ATPase and ssDNA exonuclease activities but at reduced levels (decreased five- to eightfold and twofold compared with wild-type levels, respectively). If the inference is that the mutation eliminates ATP hydrolysis by the RecB subunit, these findings imply that the RecD subunit within the holoenzyme can hydrolyze ATP, a feature not detected for the individual subunit (43, 404). Although helicase activity was not examined directly, the low level of dsDNA-dependent ATPase activity implies that the mutant enzyme also cannot unwind dsDNA. This deduction suggests that the RecB polypeptide is the essential helicase subunit of the holoenzyme.

In contrast to this mutation in the RecB subunit, mutation of the analogous phosphate-binding motif of the RecD subunit results in a mutant RecBCD enzyme [RecBCD(K177Q) enzyme] that retains many of the activities of the wild-type enzyme although to a lesser extent (295–297). Exonucleolytic degradation of ds- and ssDNA is reduced 4- to 14-fold (296), as is dsDNA-dependent ATP hydrolysis (K_m and k_{cat} are decreased 5- and 8-fold, respectively) (296); an approximately 90% decrease in the helicase activity of RecBCD(K177Q) enzyme is also observed (calculated from data in reference 296). The most dramatic defect exhibited by the mutant enzyme is in the processivity of DNA unwinding. The RecBCD(K177Q) enzyme can unwind DNA processively approximately 1.5 kb before dissociating (297), whereas the wild-type enzyme can unwind up to 30 kb (511). Pending the assumption stated in the previous paragraph, these findings suggest that the holoenzyme does not need two actively translocating subunits to unwind DNA and demonstrate that DNA unwinding catalyzed by the RecB subunit when it is part of the holoenzyme is far greater than the limited unwinding observed for the isolated RecB subunit (44, 404). Furthermore, they suggest that the RecD subunit may be a processivity factor that acts either directly through interactions with the holoenzyme complex or indirectly through, for example, interactions with the 5′-terminal DNA strand during unwinding (187).

Nuclease Attenuation Model for Recombination Promoted by RecBCD Enzyme and χ

A model for the initial steps of homologous recombination occurring by the RecBCD pathway is shown in Fig. 11 (303a). This model is an economical view that reconciles most features of RecBCD enzymatic and genetic behavior, and it incorporates important elements of previously described models (141, 585, 591). RecBCD enzyme initiates the recombination process by binding the end of the dsDNA molecule which contains a χ sequence (Fig. 11A). While unwinding the dsDNA, the dsDNA exonuclease degrades the 3′-terminal strand preferentially, producing ssDNA fragments, while the 5′-terminal strand is cut much less frequently; RecA and SSB proteins immediately bind to the unwound DNA strands (Fig. 11B and C). χ recognition happens only if RecBCD enzyme encounters χ in the proper orientation (Fig. 11D). This recognition event results in both a pausing of the unwinding activity and a heightened probability of a cut in the DNA strand containing the χ sequence (Fig. 11D). The interaction between RecBCD enzyme and χ also results in modification of the RecBCD enzyme that dissociates or functionally inactivates the RecD subunit, thereby producing the nuclease-attenuated helicase that is functionally equivalent to the RecBC enzyme (unshaded RecD subunit in Fig. 11). The specific biochemical event responsible for inactivation of the RecD subunit is unknown and may be either a dissociation event or simply a conformational change. Continued unwinding of the dsDNA (for at least 10 kb) produces a ssDNA fragment that is preferentially utilized by the RecA protein to promote homologous pairing (Fig. 11E to G) (the predominant plectonemic joint molecule detected in vitro is shown; any paranemic species formed would not have been undetected). In the presence of elevated magnesium ion concentrations (i.e., $[Mg^{2+}] > [ATP]$), the RecBCD holoenzyme is reactivated (or reassembled) upon exiting the DNA (Fig. 11H). Catalytic unwinding of subsequent DNA molecules can ensue. If the magnesium ion concentration is limiting, then reactivation is slow, the χ-altered enzyme remains inactive, and χ affords protection to other DNA molecules (i.e., *trans* protection by χ occurs).

The biochemical steps that lead to formation of a Holliday junction are still unclear, but pairing of the remaining complementary strands (i.e., the displaced strand in the joint molecule and the complementary strand from linear DNA) would result in the formation of a Holliday junction (Fig. 2). The resolution of the Holliday junction to produce recombinant progeny can occur through the actions of the RuvABC proteins and/or RecG protein (see the sections on RecG, RuvAB, and RuvC proteins, below) (353, 698). The discovery that χ down-regulates RecBCD enzyme nucleolytic activity resolves the paradoxical question of how RecBCD enzyme can be a potent nuclease while simultaneously being essential to recombination in wild-type cells. Modification of the holoenzyme through the interaction with χ, by alteration of the RecD subunit, changes the functionality of RecBCD enzyme from that of a helicase with nuclease activity to that of an enzyme which is simply a helicase. Although certain details of this model must be elaborated, these biochemical steps are likely to represent events important to genetic recombination in vivo.

RecE (EXONUCLEASE VIII) AND RecT PROTEINS

The *sbcA* mutations (suppressor of *recBC*) were isolated as suppressors of the mitomycin-sensitive, recombination-defective phenotype resulting from mutations in the *recB* or *recC* genes (23, 633a). Suppression resulted from activation of the normally unexpressed *recE* gene (23, 633a). This mode of suppression differs from that of the other well-known suppressor of the *recBC* phenotype, *sbcB*, which results in the partial inactivation of the exonuclease I protein (see the section on SbcB protein, below). The *recE* gene is activated by many types

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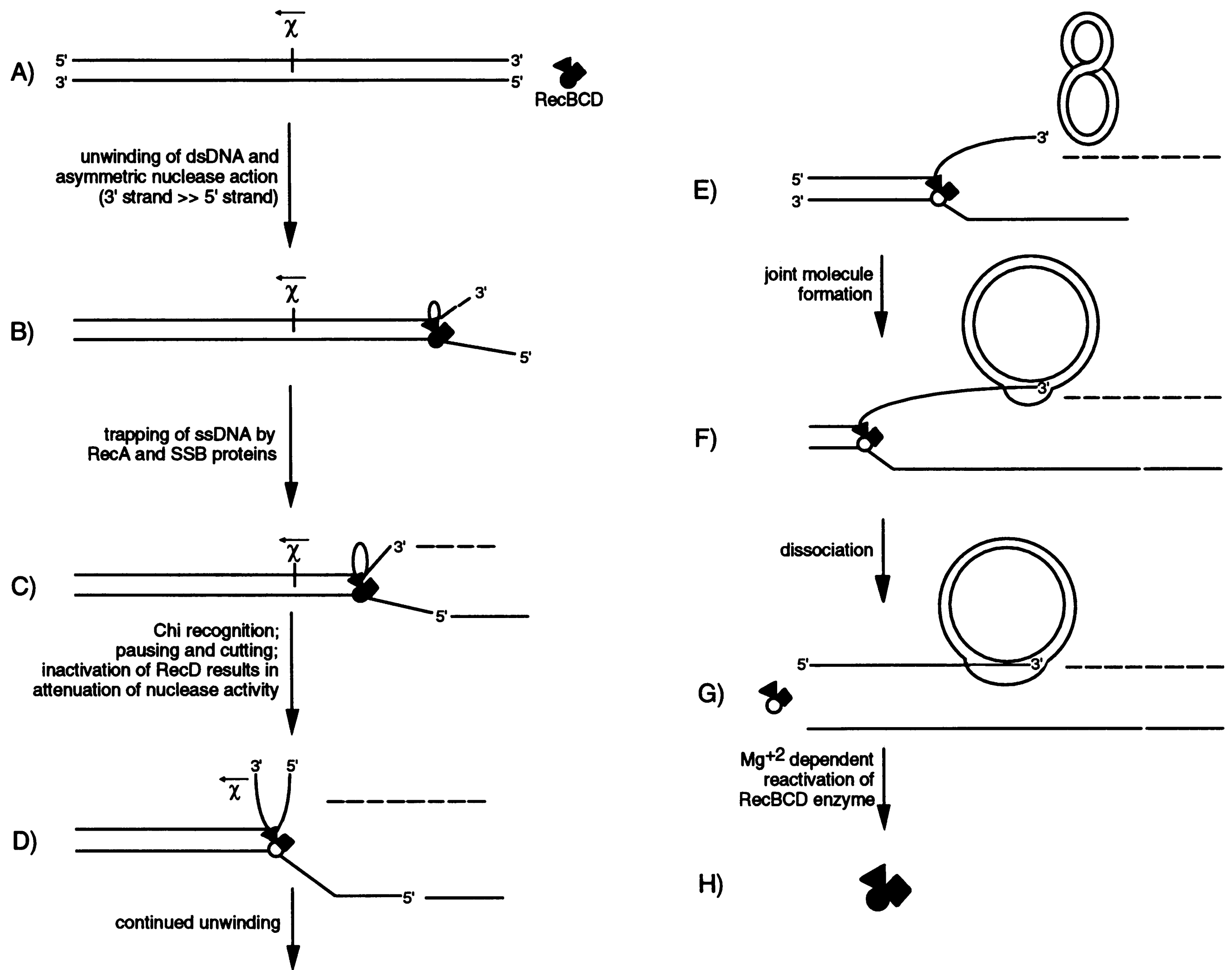

FIG. 11. Nuclease attenuation model for the initiation of homologous recombination by RecBCD enzyme. Details are discussed in the text. The linear dsDNA molecule containing χ represents the donor DNA, and the supercoiled DNA molecule represents the recipient DNA. The RecB, RecC, and RecD subunits are represented by the triangle, diamond, and circle, respectively. Prior to interaction with χ, the helicase and nonspecific nuclease activities of RecBCD enzyme unwind the dsDNA and, in parallel, degrade the 3'-terminal strand more extensively than they degrade the 5'-terminal strand. Upon encountering χ, RecBCD enzyme pauses; the pause enhances the probability that a cleavage occurs in the vicinity of χ. The interaction with χ causes a functional alteration in the RecBCD enzyme, leading to inactivation or dissociation of the RecD subunit (open symbol). The nuclease-attenuated RecBC(D) protein continues unwinding to produce ssDNA downstream from χ that is used by RecA protein to promote joint molecule formation. At the completion of unwinding, the RecBCD holoenzyme is normally reactivated and can commence binding and unwinding on another DNA molecule. RecA and SSB proteins are bound to the ssDNA but are not shown. Modified from reference 141 with permission of the publisher.

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of mutations, including simple point mutations, insertion mutations, and large external deletions (105 and 140 kb) which result in translational fusions with upstream genes that often remove substantial portions of the N-terminal *recE* coding sequence (89, 94, 394). This gene, located at approximately 30 min on the *E. coli* chromosome map, produces an exonuclease (exonuclease VIII) that is derived from the cryptic rac prophage present in many but not all *E. coli* strains (195a, 255, 316a, 707).

Recently, it was realized that the *recE* locus actually consists of two open reading frames, with the second, smaller gene being out of frame with the first gene (95, 217, 289). This second gene, designated *recT*, encodes a 30-kDa protein; the upstream gene, *recE*, encodes a 96-kDa protein (217). The RecT protein promotes DNA renaturation and subsequent DNA strand exchange reactions (217, 218). Because of both their close genetic linkage and their functional similarities to the bacteriophage λ *red* system α and β genes, the RecE and RecT proteins are discussed together in this section.

Biological Functions

An interesting aspect of RecE pathway-mediated plasmid recombination is that it can occur independently of RecA protein function (181, 381). This observation spurred many attempts to account for the ability of an exonuclease to stimulate *recA*-independent recombination. As early as 1983, Joseph and Kolodner speculated that the activation of exonuclease VIII might occur simultaneously with the activation of a protein that had attributes similar to those of RecA protein

(256). They reasoned that the biochemical similarity of exonuclease VIII to λ exonuclease and the similarity of the *recE* locus to the λ *red* region might point toward the activation of an analog of the λ *red* β gene, which has a DNA renaturation activity similar to that possessed by *E. coli* RecA protein (56, 58, 274a, 453, 689). The fact that *red* mutations can be complemented by the *recE* allele lent further credence to this speculation (196). Additionally, it was shown that at least two types of recombination occur via the RecE pathway, independently of RecA protein, that resulted in progeny which could not be explained without proposing some type of RecA protein-like pairing activity (279, 379). The recent identification and purification of the RecT protein showed that the *sbcA* mutation results in the activation of both exonuclease VIII (RecE protein) and a ≈33-kDa protein (RecT protein; apparent size derived from sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE]) which has activities similar to those of the β protein of λ (217). The *recE* gene lies upstream and overlaps the *recT* coding sequence by 5 nucleotides, presumably forming a transcriptional unit (95). Although the RecE and RecT proteins are biochemically similar to λ exonuclease and β protein, respectively, they differ in molecular weight, are antigenically distinct, and do not appear to show any significant sequence similarity (95, 255, 289a).

RecT protein, like the λ β protein, catalyzes the renaturation of complementary ssDNA strands (217). The combined facts that β protein can substitute for RecA protein (33) in plasmid recombination and that the RecT protein is biochemically similar to the β protein provide a ready explanation for the observation that, in some cases, activation of the *recET* locus is sufficient to produce recombination without participation of the RecA protein (381). As expected, RecT protein can substitute for the λ β protein in plasmid recombination in strains which are Δ(*recA*) *recBC sbcBC* (95). In the absence of *recT*, plasmid recombination stimulated by the action of RecE protein alone is only 3% of that observed in the presence of *recT* (95). Thus, the *recA* independence of plasmid recombination can be traced to the pairing activity of RecT protein.

Complementation tests show that both *recE* and *recT* are also required for conjugational recombination in a *recBC* $sbcB^+$ ($recA^+$) genetic background (95). Deletion analysis of the *recET* region shows that the removal of *recT* function alone lowers both UV irradiation and mitomycin survival and decreases conjugational recombination frequency, indicating that *recT* is required for both repair and recombination. Deletion of both *recE* and *recT* leads to a further decrease in UV irradiation and mitomycin survival but does not further affect conjugal recombination, indicating that the nuclease activity is involved in recovery from DNA damage (95).

Biochemical Activities

Purified RecE protein (exonuclease VIII) is reported to be a 140-kDa polypeptide which appears to act as a trimer or tetramer (255). RecE protein is very tolerant of extra amino acids, since the deletion which removes 140 kb of DNA results in the production of a fortuitous fusion polypeptide larger than the wild-type RecE protein (394). The RecE protein also seems to accommodate large deletions of coding sequence, since suppression of a frameshifted defective *recE* allele resulted in polypeptides that showed high levels of exonuclease activity despite having much of their N-terminal sequence deleted (89, 378a, 708). Western immunoblot analysis and partial purification detected truncated RecE protein products that ranged in size from 62 to 39 kDa and that retained exonuclease activity; one of these (pRAC3) was purified to homogeneity, with the same results (378a). Collectively, these results suggest that only 39 kDa of the carboxyl terminus of the RecE protein (of a total of 140 kDa) is required for exonuclease activity and that the C-terminal region may exist as a distinct structural and functional domain. It is not yet clear what role the N-terminal domain of RecE protein plays. However, further analysis of the *recE* coding region showed that the protein predicted by the open reading frame would have a molecular mass of only 96.2 kDa (95). The fact that it appears on SDS-PAGE as a 140-kDa species has been attributed to anomalous migration behavior (95, 289).

The RecE protein is a dsDNA-specific exonuclease activity that preferentially degrades the 5′ terminal strand (195a, 255, 256, 316a). Linear duplex DNA with either 5′ or 3′ overhangs of 4 bp and blunt-ended DNA are degraded equally well. The activity of exonuclease VIII is not inhibited by 3′ ssDNA tails but is inhibited by 5′ ssDNA tails longer than about 250 nucleotides (256). The RecE protein shows no activity toward gapped, supercoiled, or nicked duplex molecules, and, despite the unusual property of removing the terminal 5′ nucleotide from ssDNA, it shows very little exonuclease activity toward ssDNA (256). The nuclease activity is highly processive and leads to the removal of 10,000 to 20,000 nucleotides from the 5′-terminal strand per binding event; the reaction products are both 5′ mononucleotides and full-length ssDNA (218a).

The RecT protein can bind to ssDNA (but not to dsDNA) and can promote the ATP-independent renaturation of complementary ssDNA (217). Recent in vitro experiments extend these observations to show that the RecT protein can also promote homologous pairing between circular ssDNA and linear dsDNA (which are the traditional substrates for the RecA protein-promoted DNA strand exchange reaction), provided that the linear dsDNA is resected by an exonuclease to expose homologous ssDNA (218). In the absence of nuclease activity, no pairing is detected. The RecE protein can provide this needed nuclease function; however, apparently any other strand-specific dsDNA exonuclease can substitute in vitro. This RecT protein-promoted reaction requires no ATP and at least one RecT monomer per 13 nucleotides of ssDNA (218). The pairing reaction probably initiates via reannealing of the homologous ssDNA regions, consistent with the known renaturation activity of RecT protein. Heteroduplex formation is not limited to the region of resected DNA, since ssDNA is displaced from the duplex region of the linear dsDNA. This strand displacement or DNA heteroduplex extension is RecT protein specific, since a protein proficient in DNA renaturation (histone H1 protein) can promote the initial pairing (reannealing) step but cannot produce DNA heteroduplex products possessing displaced ssDNA (218). These properties are sufficient to explain the ability of *recE* and *recT* functions to complement *recA* defects in recombination reactions involving plasmid and phage λ (289). It is presently unknown whether recombination in these cases occurs via the DNA strand invasion mechanism or the DNA-reannealing mechanism depicted in Fig. 2 and 3, respectively (567a), and why *recET* fails to complement *recA* defects in conjugational and transductional recombination; perhaps plasmid recombination occurs primarily by the annealing mechanism whereas conjugational and transductional recombination occur by the DNA strand invasion mechanism.

RecF, RecO, AND RecR PROTEINS

The ability of *E. coli* to carry out recombination in the absence of a functional RecBCD enzyme led to the discovery of other genes whose products establish the existence of

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independent pathways of recombination. Specifically, in a *recBC sbcBC* background, the products of the *recF*, *recO*, and *recR* genes are necessary for recombination via the RecF pathway. Although the enzymatic functions of these genes are not well understood, current studies suggest that the RecF, RecO, and RecR proteins work together and may be involved in the initiation of recombinant events; hence, here they are discussed as a group.

Biological Functions

RecF. The *recF* gene was isolated as a UV-sensitive and recombination-deficient mutation in strains containing a *recBC sbcBC* background (238, 352). It is located at 83 min on the *E. coli* chromosome in the midst of a number of genes involved in DNA replication (498a). The role of the *recF* gene product in recombination and related processes is varied. *recF* is necessary for inter- and intramolecular recombination between circular plasmid molecules in wild-type backgrounds (98, 180, 253, 288) and for intramolecular recombination of circular and linear plasmids in a *recBC sbcBC* background (90, 381). *recF* function is needed for conjugational recombination in strains containing *recB* or *recC* mutations (238, 355, 356, 395) but not in strains containing *recD* mutations (358, 376). *recF* is also not required for intramolecular recombination of plasmids in *recBC sbcA* strains (381).

Mutations in the *recF* gene affect the repair of damaged DNA. The RecF gene product is required for *recA*-dependent postreplication recombinational repair (521, 680), plasmid-based mismatch repair (180), and UV-induced recombination (446). RecF activity is also required for efficient induction of the SOS response to DNA damage and for λ prophage induction (41, 238, 420, 532, 541, 542, 658, 714), as well as for the adaptive response to alkylation damage (656). Expression of some of the genes involved in the RecF pathway of recombination (*recN*, *recQ*, and *ruv*) is under SOS control (357, 458, 563), yet others (*recF*, *recJ*, *recO*, and *recR*) are not regulated by the *recF*-dependent SOS response (41, 375, 397, 445).

Defects in *recF* that yield a UV-sensitive phenotype can be partially relieved by extragenic suppressors that map to the *recA* gene (657). These suppressors of *recF* (called *srf*) allow recombination and DNA repair by the RecF pathway to occur in the absence of functional RecF protein. Extensive genetic analysis of the mutant alleles *recA720*, *recA730*, *recA750* (672), *recA441* (144, 276, 541, 640, 659, 672, 711), *recA801* (640, 658, 673, 677), *recA803* (386, 390), and *recA2020* (673, 677), together with biochemical characterization of the mutant proteins RecA730 (330), RecA441 (328–330), and RecA803 (330, 386, 389, 390), established that these *srf* mutations increase the ability of RecA protein to promote DNA strand exchange under suboptimal conditions. The underlying basis for this enhanced activity results from the increased ability of the mutant RecA proteins either to utilize ssDNA containing secondary structures or to displace the SSB protein from ssDNA (see the section on RecA protein, above). From these observations, it was postulated that one function of RecF protein is to enhance the behavior of wild-type RecA protein in a like manner (386, 389, 390), although direct in vitro tests of this hypothesis have failed to demonstrate stimulation of RecA protein activity that is specific to RecF protein (387, 649) (see below).

RecO and RecR. The *recO* (288) and *recR* (398) genes were originally identified by their effects on the RecF pathway of recombination. Mutations in these genes exerted effects similar to *recF* mutations when tested for intramolecular plasmid recombination (90, 288, 381, 398), conjugational recombination (288, 355, 356, 398), and recombinational repair (672). The *recO* and *recR* genes are located at approximately 55 and 11 min on the *E. coli* chromosome and encode proteins of 26 and 22 kDa, respectively (397, 398, 445, 725).

Biochemical Activities

The *recF* gene encodes a 40-kDa protein (41, 209, 387). The RecF protein binds ssDNA in the absence of magnesium ions and nucleotide cofactor; this binding is preferential for the ends of linear ssDNA (209). Under conditions that support RecA protein-dependent joint molecule formation (e.g., 10 mM Mg^{2+}, 1 mM ATP), ssDNA binding is enhanced; this binding is nonspecific, appears cooperative, and saturates at approximately 15 nucleotides for every RecF protein monomer (387). The RecF protein has an NTP-binding domain that is similar to those contained within a superfamily of proteins which include RecN, MutH, HexA, the phage T4 gene 46 protein, and the yeast Rad50 protein (205), suggesting that it might bind ATP. In agreement, RecF protein can be cross-linked with 8-azido-ATP (388). ATP binding is enhanced by the binding of either ssDNA or dsDNA to RecF protein, and the stable binding of RecF protein to dsDNA complexes requires the presence of either ATP or ATPγS (388). The physiologically important property of the RecF protein is presumably dependent on ATP binding or hydrolysis, since mutations in the phosphate-binding motif of the ATP consensus sequence within RecF protein result in a *recF* mutant phenotype (536).

Preliminary biochemical characterization shows that the RecO protein binds ssDNA and promotes renaturation of complementary ssDNA molecules (380, 649a) and that RecR protein binds dsDNA (360a). These observations suggest a possible role for the RecO and RecR proteins in facilitating early (e.g., presynaptic complex formation) and/or intermediate (e.g., homology search and DNA strand invasion) steps in recombination.

Functional Interaction among RecF, RecO, and RecR Proteins

Three separate lines of genetic analyses suggest that the products of the *recF*, *recO*, and *recR* genes function together at the same step of recombination, most probably at an initiation step that is independent of the RecBCD enzyme function. First, analysis of conjugational recombination and UV repair determined that the *recF*, *recO*, and *recR* genes belong to the same epistasis group (354, 398). Second, certain mutant *recA* (*srf*) alleles that suppress *recF* deficiencies also suppress *recO* and *recR* mutations (92, 672). Finally, investigation of phage λ recombination determined that λ possesses a single open reading frame (referred to as the *orf* gene) that encodes a functional analog of *recO*, *recR*, and *recF* (i.e., it can suppress mutations in any of these genes) (544).

On the basis of biochemical investigation of mutant RecA proteins conferring the *srf* phenotype, it was postulated that RecF protein facilitates recombination by assisting in the assembly of the RecA protein-ssDNA presynaptic filament (389, 390). Direct examination of this proposal in vitro demonstrated that purified RecF protein, by itself, inhibits both the ssDNA-dependent ATPase and DNA strand exchange activities of the RecA protein, when SSB protein is present (387, 649); this inhibition occurs at a stoichiometry of $\approx$1 RecF protein monomer per 20 nucleotides (calculated from reference 387). However, this inhibitory effect of RecF protein is overcome by the addition of purified RecO and RecR proteins

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(649). In fact, RecF protein, when used in conjunction with RecO and RecR proteins, actually stimulates RecA protein-dependent joint-molecule formation when conditions are suboptimal for RecA protein (i.e., when SSB protein is prebound to ssDNA) (649). This inhibitory effect of SSB protein on presynaptic-complex formation and subsequent joint-molecule formation is overcome either by RecO and RecR proteins or by RecF, RecO, and RecR proteins. Maximal stimulation under these conditions occurred at a RecO-to-RecR molar ratio of approximately 1, did not depend on the presence of RecF protein, and was partially inhibited by RecF protein concentrations that exceeded the RecO and RecR protein concentrations by more than two- to threefold. These proteins stimulated the rate of initiation of joint molecule formation but did not affect the rate of DNA strand exchange once homologous pairing had initiated. This result suggests that RecO and RecR proteins overcome the inhibition to presynaptic complex formation normally caused by SSB protein and facilitate the binding of RecA protein to ssDNA that is complexed with SSB protein. However, the role of the RecF protein in this reaction is unclear since RecO and RecR proteins, in the absence of RecF protein, are sufficient for stimulation of joint-molecule formation. Perhaps, in vivo, RecF protein may be involved in a step not experimentally tested by using this in vitro reaction (649).

RecG PROTEIN

The *recG* locus was identified as a mutation (*recG162*) that made wild-type cells moderately recombination deficient and UV sensitive (603). Studies on this gene languished until mapping of a recombination-deficient transposon insertion mutant (*rec-258*) (398) reidentified this locus (353).

Biological Functions

The most interesting phenotypic effect of *recG* mutations is that *recG*, unlike any other known recombination-related gene except *recA*, appears to operate in all three "pathways" of recombination. Although the effects of *recG* are not nearly as severe as those of *recA*, noticeable recombination deficiencies are observed in *rec*$^+$, *recBC sbcA*, and *recBC sbcBC* strains. In wild-type cells, these effects include sensitivity to UV, ionizing radiation, and mitomycin, as well as decreased levels (three- to fivefold) of conjugal and transductional recombination (353). These results suggest that *recG* is involved in recombinational repair of both dsDNA breaks and ssDNA gaps; it is not involved in excision or error-prone repair.

To further define the role of *recG* in recombination, studies were conducted in which a *recG* mutation was combined with mutations in other genes involved in recombination to look for an enhancement of the defect which might suggest overlapping functions. Notable effects were seen in combination with *recB* and *recJ* mutations but not in concert with *recD*, *recF*, *recN*, or *recQ* mutations (353). The most significant effect, however, was observed in *ruv* (*ruvA*, *ruvB*, or *ruvC*) *recG* double mutants (350). Mutants with single mutations in either *ruvA*, *ruvB*, or *ruvC* have approximately similar phenotypes and are fairly recombination proficient (see below). When the *ruv* mutation is present in combination with *recG*, however, both conjugational and transductional recombination and UV resistance are dramatically reduced (30- to 500-fold greater than in the mutants with single *ruv* mutations), arguing that the *ruv* and *recG* genes define components of alternative recombination (resolution; see below) pathways active in wild-type cells (350). The *ruv* set of genes and *recG* can be phenotypically distinguished, however, because *recG* mutations in a *rec*$^+$ background do not affect recombination between defective tetracycline genes in a circular plasmid dimer (350, 353).

The *recG* gene maps at 82 min on the *E. coli* chromosome, between the *pyrE* and *dgo* genes (262, 353). It is part of the *spo* operon and is immediately downstream of *spoU*. *recG* forms a 2,079-nucleotide open reading frame, previously known as *spoV*, which encodes a protein of 76,438 Da (262, 359). Transcription of *recG* most probably originates upstream of the *spoS* gene and proceeds through *spoT* and *spoU* before reaching *recG*, although there is also a weak promoter upstream of *spoU*. The *spo* operon promoter is not regulated by the LexA repressor, and therefore *recG* is not inducible by the SOS response. The cellular levels of RecG protein appear to be low, since the *recG258* mutation can be complemented by plasmid constructs which do not express RecG protein at a level (estimated to be less than 10 copies per cell) which can be visualized by maxicell analysis (359).

Biochemical Activities

The amino acid sequence of RecG protein displays similarity to the DEXH class of DNA and RNA helicases (262, 359). All seven motifs defined for helicases are present (206), with highest homology found in the nucleotide-binding regions (motifs I and II; amino acids 288 to 304 and 394 to 400, respectively). This fact, in combination with the genetic evidence stated above which suggested that RecG protein and either the RuvAB complex or RuvC protein have overlapping functions, led to the testing of RecG protein for activity on model Holliday junctions possessing 12-bp homologous core sequences. Lloyd and Sharples found that the purified RecG protein most closely resembles the RuvAB complex in terms of activity (361). Like RuvA protein (see the section on RuvAB proteins, below), RecG protein binds specifically to Holliday structures (361), but it appears to bind as a monomer, rather than as two tetramers like RuvA protein (362). Similar to RuvB protein, RecG protein possesses both DNA-dependent ATPase activity (with a turnover of 3,500 ATP molecules per min per protein molecule on supercoiled DNA) (361) and the ability to dissociate immobile model crossover junctions (both X- and Y-shaped dsDNA junctions) (362). To achieve 50% dissociation of a synthetic crossover junction, significantly less RecG protein ($\sim 10^3$-fold) than RuvAB complex is required; in addition, the kinetics of dissociation are approximately 10-fold faster with RecG protein (362). The two proteins with branch migration activity also differ in their ability to unwind DNA. Whereas the RuvAB complex can unwind limited lengths of duplex DNA (see the section on RuvAB proteins, below), RecG protein is unable to unwind conventional helicase substrates, which led to the proposal that the RecG protein has enhanced specificity, relative to the RuvAB complex, for crossover or branched structures (362).

Although *recG* mutations also display heightened recombination and repair deficiencies in the presence of a *ruvC* mutation, no Holliday junction cleavage activity, similar to that catalyzed by the RuvC protein, has been detected by using the purified RecG protein. This result may suggest that RuvC protein serves as the cleaving activity for Holliday junctions bound by RecG protein as well as for those bound by the RuvAB complex (see below) or that a second cleavage activity remains to be identified. An alternative proposal is based on the observation that RecG protein displays a greater tendency to inhibit RecA protein-promoted DNA strand exchange than to facilitate further DNA strand exchange (699a). It was suggested that reversal and disruption of recombination inter-

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mediates is, in fact, the biological role of the RecG protein. Such reversal provides an alternative to Holliday junction cleavage as a means for dealing with recombination intermediates which permits gene conversion without crossing over (699a).

RecJ PROTEIN

The *recJ* gene maps at approximately 62 min on the *E. coli* chromosome and encodes a 63-kDa protein that possesses a 5′ → 3′ exonuclease activity on ssDNA (374, 377).

Biological Functions

recJ is needed in both the RecF and RecE pathways of recombination (373, 375, 381). In the absence of a functional RecBCD enzyme, mutations in *recJ* decrease conjugational recombination by 1,000-fold and result in the sensitization of cells to UV radiation (238, 678). *recJ* is also required for chromosomal recombination and UV repair in cells possessing *recD* mutations, mutations that result in a loss of exonuclease V activity which is characteristic of the RecBCD enzyme (see the section on RecBCD enzyme, above) (376). In wild-type cells, in which the RecBCD pathway is the predominant means of generating recombinants, mutations in the *recJ* gene have little effect on conjugal recombination (374). Plasmid recombination, however, is highly dependent on *recJ* function, even in a strain which is *recBC*$^+$; mutations in *recJ* result in a 4,000-fold decrease in the level of plasmid recombination (288). The *recJ*, *recD*, and *recN* genes appear to be functional equivalents since single mutations in any gene have little effect on conjugational recombination and double mutations have only modest effects; however, when all three genes are mutated, there is a 50- to 100-fold reduction in the recovery of recombinants and a greater sensitivity to UV light (354). *recJ* and *recG* mutations appear to be synergistic with respect to UV sensitivity, suggesting overlapping roles for these two proteins (353), although recent studies demonstrate that the RecG protein is not a nuclease but, rather, has the ability to bind and disrupt synthetic Holliday junctions (361, 362) (see the section on RecG protein, above). Suppression of *recF* mutations by either *recA441* or *srf* alleles requires a functional *recJ* gene (640).

recJ, in addition to *uvrD* (helicase II), is required for the process known as indirect stimulation of recombination, in which recombination between a chromosomal site and a phage site is stimulated by the coinfection of a second phage which has been irradiated (546). This phenomenon appears to be dependent on the *recJ* gene regardless of the *sbcA* and *sbcB* genotype. This result suggested that the two modes of *recJ*-dependent recombination, direct and indirect (tele-activation), were quite different (546).

Before the purification and characterization of RecJ protein were achieved, a genetic result hinted that RecJ protein might be a nuclease. In *recD* cells conjugational recombination and UV repair are approximately normal even though the exonuclease activity of the RecBCD enzyme is undetectable (76, 376). In these cells, in addition to the requirement for *recB* and *recC* genes, there is a requirement for *recJ* (376). These observations were explained by hypothesizing that the missing nuclease activity caused by the *recD* mutation was being replaced by an activity of RecJ protein (377).

In contrast to exonuclease I (*sbcB*, *xonA*), which appears to have an antagonistic role with respect to recombination (see the section on SbcB protein, below), RecJ protein nuclease activity in the appropriate genetic background seems to have a constructive role in recombination because inactivation of RecJ protein leads to a reduction in recombination. The most obvious difference between RecJ protein and exonuclease I are their polarities of ssDNA hydrolysis: RecJ protein degrades 5′-terminal ssDNA, leaving 3′-terminal ssDNA tails intact, whereas exonuclease I degrades 3′-terminal ssDNA, leaving 5′-terminal ssDNA tails intact. This suggests that 3′-terminal ssDNA is a better substrate for a step in the recombination process (Fig. 2); this bias toward 3′-terminal ssDNA coincides with the substrate preference displayed by RecA protein in DNA strand invasion reactions (see the section on RecA protein, above). The only instance when RecJ function may play a destructive role is in the stability of cosmid clones carrying foreign DNA; the presence of a wild-type *recJ* gene in a strain that is also *recBC sbcBC* leads to insert instability (249).

Biochemical Activities

The RecJ protein has been overproduced and purified (377). It possesses a ssDNA-specific, ATP-independent exonuclease activity which acts in the 5′ → 3′ direction (377). RecJ protein can digest, at its maximal rate, 1,000 nucleotides per min per protein molecule. The processivity of degradation and the size of the reaction products have not yet been determined. RecJ protein has neither detectable double-stranded exonuclease activity nor endonuclease activity on ss- or dsDNA. The nuclease activity is thought to function in recombination by one of the paths depicted in Fig. 2 and 3 (377).

RecN PROTEIN

The *recN* (*radB*) gene is one of a class of genes (the others being *rorA*, a *recB* allele [199], and *rorB* [126]) which, in *recBC*$^+$ cells, displays sensitivity to mitomycin and ionizing radiation but appears to be UV resistant and repair proficient (40 to 50% of wild-type levels) (288, 357, 538). The *recN* gene was isolated as a mutation (*rec-259*) which was induced by DNA damage in *recBC sbcBC* cells (357), whereas *radB* was isolated as an X-ray-sensitive mutant of *xthA14* cells (538). The gene is located at ≈56 min on the *E. coli* chromosome (288, 481, 538).

Biological Functions

A *recN* mutation reduces both conjugational and transductional recombination approximately 100-fold in *recBC sbcBC* cells (357, 538); its effect in *recBC sbcA* cells is moderate (3-fold). Reflecting its importance in the RecF pathway, the expression of *recN* is induced 20- to 30-fold in *recBC sbcBC* strains (481). Unlike mutations in other genes of the RecF pathway (*recF*, *recJ*, *recO*, and *recQ*), *recN* mutations do not restore resistance to "thymineless death" (460), a poorly characterized phenotype of *E. coli* cells which is displayed as a sensitivity to the absence of thymine. Like *recQ* mutations, however, *recN* mutations cause cells to form filaments when exposed to DNA-damaging agents (481).

The repair of DNA damage is thought to occur by a UV-inducible mechanism termed postreplication repair. Two types of postreplication repair processes exist: one repairs ssDNA gaps and is *recF* dependent, and the other heals double-strand breaks and is *recB* dependent. The RecN gene product appears to be required for the latter process, i.e., for RecBCD-dependent repair of nearly blunt-end, double-strand breaks which arise as a result of DNA damage (481, 538, 539, 678). *recN* also appears to be involved in the intramolecular recombination of linear and circular DNAs by the RecF pathway (90, 249), although it is not required for circular

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plasmid-by-plasmid recombination (288, 381). In addition, *recN* is required for recombination of linear (but not circular) dimer plasmids in *recBC*$^+$ *recD* cells but not in *recBC sbcA* or *recBC sbcBC* cells (376).

Biochemical Activities

The *recN* gene is 1,701 nucleotides in length and encodes a protein of 567 amino acids, with an estimated M_r of 63,599 (173, 482, 520). The protein has a pI of ~5.8 and appears to exist as several variants by two-dimensional gel electrophoresis (173); whether this is due to some in vivo modification of the protein has yet to be determined. The sequence of RecN protein contains a consensus nucleotide (NTP)-binding fold at amino acids 27 to 43 (520). As expected from both genetic (357) and biochemical (173) studies, the *recN* promoter contains two SOS boxes (520), which allows expression of the gene to be tightly regulated by LexA protein. Such regulation appears to be important because overproduction of RecN protein is detrimental to cells (i.e., expression of RecN protein from a multiple-copy-number vector increases the UV sensitivity of *recN*$^+$ strains). This observation, however, may be an indirect effect caused by the presence of multiple copies of the *recN* promoter, resulting in redistribution of LexA protein from the promoters of other SOS-inducible genes (482).

At this time, the protein has not been purified and no biochemical activity has been demonstrated for it. Lloyd et al. (355) postulated that RecN protein is involved in generating or making accessible ssDNA with a 3′ terminus which can be utilized in DNA strand exchange. This proposal is based on the genetic observations that in *recBC sbcA* cells, in which 3′-tailed ssDNA molecules can be continually generated by the action of exonuclease VIII (the *sbcA* gene product), the requirement for *recN* is modest, but in *recBC sbcBC* cells, in which no nuclease activity is available to generate such a substrate, *recN* is absolutely required. This view is consistent with the need for *recN* for linear-plasmid recombination in *recBC*$^+$ *recD* cells. Thus, RecN protein may promote the initial steps of recombination by stabilizing 3′ ssDNA tails (e.g., protecting from nuclease activity or trapping after helicase activity); assignment of its actual function in recombination awaits biochemical characterization.

RecQ PROTEIN

The *recQ* gene was identified as a mutation that increased resistance to thymineless death (458). For unknown reasons, certain mutants with mutations in the RecF pathway of conjugal recombination display enhanced resistance to thymineless death. In an attempt to isolate additional genes which might be active in the RecF pathway, Nakayama et al. screened mutagenized cells for increased resistance to thymineless death (458). One such recessive mutant, which restored partial resistance to thymineless death, was designated *recQ1*. Subsequent analysis of Tn*3* insertions in *recQ* indicated that *recQ1* represented the null phenotype (459). This gene mapped at 86 min between *pldA* and *pldB*, which encode enzymes involved in phospholipid degradation (458, 459).

Biological Functions

In *recBC sbcB* cells, the *recQ1* mutation displays enhanced sensitivity to UV (20-fold) and decreased conjugational recombination efficiency (20- to 70-fold), suggesting that it is part of the RecF pathway (458). Unlike *recF* mutations, however, *recQ1* does not enhance the UV sensitivity of *recBC*$^+$ cells; this was interpreted to suggest that the repair pathway active in *recBC*$^+$ cells requires the *recF* gene product but not that encoded by *recQ* (458). Recent studies show that although mutations in *recQ* do not affect the levels of conjugal recombination in wild-type (*rec*$^+$) cells, *recQ*, along with other genes of the RecF pathway (*recF*, *ruv*, *recJ*, and *recN*), does affect the frequency of exchanges within a unit length of DNA (322). *recQ* is also a component of the RecE pathway, as well as being involved in circular dimer plasmid recombination in a *recBC sbcA* background (381). The RecQ gene product is not required for cell viability, although its overexpression is detrimental (459).

Biochemical Activities

Cloning of the 1,827-nucleotide *recQ* gene revealed several means by which the expression of RecQ protein may be regulated (248). First, the *recQ* promoter contains a LexA repressor binding site, and *recQ*::*lacZ* fusion studies confirm that the *recQ* gene is in fact under SOS regulation. The accessibility of the *recQ* promoter may also be affected by its overlap with the termination sequence of the upstream *pldA* gene. Finally, the presence of both a noncanonical Shine-Dalgarno sequence (AGG) and a GTG initiation codon may further reduce expression. Since four possible initiation codons were identified by sequence analysis, determination of the correct amino terminus awaited purification and amino acid sequencing of the RecQ protein; the second putative initiation codon is used to yield an estimated 67-kDa protein (651).

Since a consensus ATP-binding site was found in the sequence of the *recQ* gene (residues 44 to 60) (248), the purified protein was tested for, and in fact possesses, both DNA-dependent dATPase and rATPase and DNA helicase activities (651). The ATPase activity (≈0.1 nmol of ATP hydrolyzed per min per ng of RecQ protein or ≈6,800 molecules hydrolyzed per min per protein molecule, using circular ssDNA) has a distinct preference for ssDNA substrate, although limited activity (10- to 50-fold less) is observed with various forms of dsDNA. The hydrolysis of either ATP or dATP is required for RecQ protein to unwind dsDNA as measured by oligonucleotide displacement assays. At low concentrations of enzyme (2.2 nM), a ssDNA region is required and the apparent polarity of movement on the bound ssDNA appears to slightly favor the $3' \rightarrow 5'$ direction. However, at 10-fold-higher concentrations of enzyme, this bias disappears and the enzyme is capable of unwinding short (143-bp) blunt-ended DNA fragments.

In the absence of SSB protein, the "efficiency" of DNA unwinding, as measured by the yield of displaced oligonucleotide, is low; an annealed fragment of 71 nucleotides is displaced to a maximum of approximately 75% in 30 min, and the displacement of a 143-nucleotide fragment is even less (651). Additionally, a vast excess (in terms of helicase molecules per substrate molecule) of RecQ protein is required (e.g., an enzyme-to-DNA ratio of 58 is required for 50% unwinding of a 71-bp partial duplex in 10 min). More recently, studies have shown that this perceived inefficiency can be alleviated by the inclusion of an ssDNA-binding protein such as SSB protein or T4 gene 32 protein (650). SSB protein does not interfere with the ATPase activity of the RecQ protein and in fact stimulates its DNA helicase activity. SSB protein must act during the elongation, rather than the initiation, of DNA unwinding by binding to the unwound strands, because the unwinding of blunt-ended substrates is also stimulated (~threefold) by SSB protein. In the presence of SSB protein, catalytic amounts of enzyme are sufficient to unwind a 71-bp partial duplex molecule, although the processivity of the enzyme is still quite low; under conditions for maximal unwind-

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ing, when ~90% of the 71 bp partial duplex is unwound, only ~25% of a 343-bp partial duplex is unwound.

The putative role of RecQ protein in the RecF pathway is derived from comparison with the RecBCD enzyme, which is both a helicase and a nuclease. Although RecQ protein does not possess the various nuclease activities of RecBCD enzyme, the helicase activity of RecBCD (or RecBC) enzyme appears to be the recombinationally important activity (see the section on RecBCD protein, above). Consequently, the helicase activity of RecQ protein may serve a similar function: to separate duplex DNA into its component single strands. The resultant 3′-terminal ssDNA would be bound by RecA protein in a step preceding homologous pairing and DNA strand exchange (Fig. 2 and 3). Given the bias shown by RecA protein in the DNA strand invasion reaction (see the section on RecA protein, above), the DNA strand having the 3′ end at the entry site should be the preferred substrate. This preference for 3′-terminal DNA may be further enhanced by the activity of another RecF pathway component, RecJ protein; this exonuclease, which degrades 5′-terminal ssDNA preferentially (see the section on RecJ protein, above), may allow efficient loading of RecQ protein onto a 3′ ssDNA tail. Alternatively, a helicase such as RecQ protein may be involved in branch migration of a Holliday-type structure.

RuvAB PROTEINS

The *ruv* locus was initially identified on the basis of sensitivity to mitomycin (473) and was later isolated as a mutation which reduced intracistronic recombination (590). Strains containing these mutations were also 10^2- to 10^4-fold more sensitive to UV and ionizing radiation than wild-type cells and tended to form multinucleate filaments when exposed to UV light or DNA-damaging agents. Suppression of the filamentation defect by mutations in either *sfiA* or *sfiB* does not increase the UV survival of *ruv* mutants (351, 474). Mutations at the *ruv* locus mapped at 41 min (473).

Biological Functions

Although conjugal recombination proficiency is only mildly affected by *ruv* mutations in a wild-type background (473), that a requirement for *ruv* in recombination is manifest in *recBC sbcA*, *recBC sbcBC*, or *recG* backgrounds (350, 351, 355). In these cells, recombination is reduced by 30- to 300-fold. Recovery of F′ transconjugants is much reduced in *ruv recBC sbcA* or *ruv recBC sbcBC* cells, and the mobilization of nonconjugative plasmids is inhibited by *ruv* mutations in the recipient. Since these inhibitions can be bypassed by a *recA* mutation (31), this result indicates that *ruv* is required late in the pathway of recombinational repair of DNA damage, after the formation of recombination intermediates which cannot then be resolved (31).

Sequencing of the *ruv* locus revealed the presence of an operon containing two genes (32, 561). The upstream gene was designated *ruvA*, and the downstream gene was designated *ruvB*. DNA damage induces *ruv* expression, which suggests that *ruv* is regulated by LexA (357, 563). As expected, sequences for the regulation of expression by LexA repressor were found upstream of *ruvA* (32, 561); *lacZ* fusion and footprinting analysis determined that these SOS boxes are not tightly bound by LexA protein, however (561). This finding explains the high basal rate of transcription observed in uninduced cells and the modest levels of induction in mitomycin-treated cells (563).

Biochemical Activities

RuvA protein. The *ruvA* gene encodes a 203-amino-acid protein with an expected M_r of 22,172, although it migrates on SDS-PAGE as a 27-kDa species (18). The purified RuvA protein binds preferentially to DNA containing a synthetic crossover, or four-way, structure in the absence of both nucleotide cofactor and Mg^{2+}; no homology within the four arms of this structure is necessary for binding (251, 477, 555, 643). Lower-affinity binding (≤20-fold less) is also detected with circular ssDNA, supercoiled DNA, and linear duplex DNA (555). In solution, RuvA protein forms a stable tetramer (556, 643), and it exhibits maximal binding to a synthetic crossover structure at a stoichiometry of 3.4 RuvA tetramers per junction (478).

RuvB protein. The *ruvB* gene encodes a protein of 336 amino acids with an expected M_r of 37,177, although it has an apparent migration of 41 kDa (18, 250, 555, 643). RuvB protein alone at high concentrations binds ss- or dsDNA, with a significantly higher affinity for dsDNA than for ssDNA (250, 452). Binding to dsDNA requires either ATP or ATPγS and saturates at an apparent stoichiometry of 1 RuvB dimer per 12 nucleotides of DNA (452). The RuvB protein sequence contains a nucleotide-binding fold at residues 60 to 76 and has an additional helicase motif of the DEXH family (645). Consistent with the presence of these motifs, the RuvB protein binds 1 ATP molecule per monomer with a K_d of 7.1 μM and possesses DNA-dependent ATPase activity, but this activity is extremely weak (k_{cat} < 1/min) (250, 643). Surprisingly, RuvB protein binds ADP more tightly than ATP (K_i = 0.65 μM), which may explain the observed substrate inhibition even at high concentrations of ATP (250). By itself, the RuvB protein has no detectable helicase activity; however, when it is complexed with RuvA protein, the resulting RuvAB protein can unwind dsDNA (see below). Although RuvB protein was initially reported to be monomeric (250) or dimeric (556, 643), recent work indicates that it forms double hexameric rings on DNA (600a).

The most remarkable property of the RuvB protein (and of the RuvAB complex [see below]) is its ability to stimulate branch migration of Holliday structures. When it is added to RecA protein-mediated recombination intermediates, branch migration of the Holliday junction intermediates is maximal at a stoichiometry of 1 RuvB protein dimer per 12 nucleotides, a value which is reduced when RuvA protein is present (451).

RuvAB protein. All of the activities of RuvA and RuvB proteins are enhanced when both proteins are present in a reaction, suggesting that the functional form is indeed a RuvAB protein complex (251, 451, 452). The active (DNA-binding) form of the RuvAB complex appears to be a RuvA tetramer associated with a RuvB hexamer (433a). Although RuvA protein can bind to mobile synthetic four-way structures (i.e., synthetic crossover structures with a homologous core region) in the absence of RuvB protein, addition of RuvB protein stabilizes by ~fourfold the binding of RuvA protein to the junction DNA (251) and reduces the stoichiometric need for RuvA protein to 1 tetramer per junction (478). Reciprocally, addition of RuvA protein stimulates by 10-fold the weak ATPase activity of RuvB protein with synthetic crossover DNA (478) and reduces by 50-fold the requirement for RuvB protein in an ATP-dependent branch migration reaction with recombination intermediates (644).

The ability of RuvAB protein to promote branch migration is almost certainly the physiological function of the RuvAB protein in recombination (698). The protein complex possesses branch migration activity on three types of substrate reported

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to date: supercoiled plasmid with extruded cruciforms, synthetic four-way (or crossover) junctions, and RecA protein-generated recombination intermediates. Each of these processes requires nucleotide binding and Mg^{2+}. In the presence of ATP, the RuvAB complex catalyzes the renaturation of the extruded cruciforms (555). Likewise, incubation of RuvAB proteins with synthetic mobile structures results in dissociation of the molecule into pairs of component strands. This reaction requires that only the central core region, and not the flanking arms, be homologous (251, 644) and that a high stoichiometry of RuvA tetramer and RuvB dimer (~10 each per junction) be used (478).

The most interesting reaction catalyzed by the RuvAB complex occurs on intermediates formed through the action of RecA protein (251, 451, 452, 643, 644). RecA protein alone can promote branch migration on the crossover structures in whose formation it participates; if, however, the RuvAB complex is added to an ongoing strand exchange reaction containing bona fide Holliday junctions in which both the region of the crossover and the flanking arms are homologous, the accumulation of intermediates is reduced because of the enhanced rate of (bidirectional) branch migration (644). The increased rate of both substrate and product appearance suggests that the RuvAB complex is capable of catalyzing branch migration in both directions (i.e., toward both the formation of product and the regeneration of starting substrates), which is a notable difference from the RecA protein-promoted branch migration reaction (see the section on RecA protein, above). This process requires approximately one RuvB hexamer and three RuvA tetramers per 1,500 to 3,600 nucleotides (451) and occurs at higher ATP and lower Mg^{2+} concentrations than does the RuvB protein-mediated reaction. In addition, RuvAB protein-mediated branch migration, unlike that promoted by RecA protein, is able to bypass lesions in the DNA, suggesting an important role for the RuvAB complex in postreplication repair (644).

The recognition that the ability of the RuvAB complex to catalyze branch migration of crossover structures may involve unwinding of a duplex molecule led to an examination of its DNA helicase activity. The protein complex is able to displace annealed fragments in an ATP-dependent reaction with an apparent $5' \rightarrow 3'$ translocation polarity on the ssDNA leader (645). The ability to displace an annealed oligonucleotide is inversely related to the length of the duplex region (e.g., at optimal RuvA and RuvB protein concentrations, 90% of a 52-nucleotide fragment is displaced in 25 min while less than 5% of a 558-nucleotide fragment is displaced), suggesting that the processivity of unwinding is limited. Because the concentration of RuvA protein required is proportional to the total DNA concentration, it was suggested that RuvA protein acts as a factor that targets the complex to DNA and crossover structures while RuvB protein, which hydrolyzes ATP, serves as the driving force by which branch migration, a result of ATP-dependent DNA unwinding followed by reannealing, occurs (645). Given the helicase activity of the protein complex, it is possible that the RuvAB complex also displaces bound proteins, so that branch migration can proceed efficiently.

RuvC PROTEIN

Upon reexamination of a series of mutations with the *ruv* phenotype, a third gene, *ruvC*, was identified (553). The *ruvC* gene is present in an operon (with *orf-26*) located upstream of the *ruvA* and *ruvB* operon. Its expression, unlike that of *ruvA* and *ruvB*, is not regulated by LexA repressor (554, 616). The RuvC protein both binds to Holliday junctions and, in the presence of Mg^{2+}, symmetrically cleaves the junction (106).

Biological Functions

In wild-type cells, *ruvC* mutations display the complex phenotype exhibited by *ruvA* and *ruvB* (see the section on RuvAB proteins, above); they sensitize cells to UV and gamma irradiation as well as to DNA-damaging agents, but they have a modest effect on conjugation and transduction efficiencies (553). Recombination is more strongly affected in *recBC sbcA* (355, 381), *recBC sbcBC* (351), and *recG* (350) cells, however.

Biochemical Activities

The enzymatic activity of the RuvC protein was uncovered during a biochemical search for an activity that might cleave Holliday junctions (107). Although synthetic four-way junctions constructed by annealing partially homologous oligonucleotides were useful in identifying cleavage activities in both bacteriophage and eukaryotic organisms, similar assays were unsuccessful in isolating a Holliday junction-cleaving activity from *E. coli*. These substrates were possibly not optimal for two reasons: the crossover was fixed in position because of nonhomology in each of the arms (i.e., it was immobile), and the substrate was devoid of recombination proteins, proteins which might help target recognition of the structure by the cleavage enzyme (107). Consequently, West and coworkers decided to use a more physiological substrate—a RecA protein-coated recombination intermediate (i.e., Holliday junction) involving two dsDNA molecules, one a linear molecule and the other a gapped molecule (107). An extract from a strain deficient in several known nucleases was partially purified, and the fractionated protein was added to an ongoing DNA strand exchange reaction (107). This assay yielded a faint signal which was characteristic of a cleaved molecule. Further experiments indicated that this activity could be readily detected by using a modified synthetic Holliday structure in which the central core was homologous, conferring limited mobility within the crossover (106). Gel filtration of the partially purified fraction suggested that the molecular weight of the activity was ~20,000, and so extracts of several recombination- or repair-deficient strains with a molecular weight of <40,000 were tested for the absence of this activity. This process led to the identification of the Holliday junction-cleaving activity as the product of the *ruvC* gene (106).

RuvC protein has been overproduced and highly purified (149, 252, 554). Despite the similarity in phenotype of the three *ruv* genes, only the RuvA and RuvB proteins appear to have a direct physical and functional interaction. RuvC is a homodimeric protein (252) composed of subunits with an estimated M_r of 18,747; it is also quite basic (pI = 9.6) (554). RuvC protein shares no strong homology with any known protein, including the rather promiscuous DNA crossover junction-cleaving enzymes, T4 endonuclease VII and T7 endonuclease I (554, 616).

In the absence of Mg^{2+} or ATP, RuvC protein is able to bind specifically to DNA containing a Holliday junction, although it will bind with low affinity to either linear or supercoiled duplex DNA or to ssDNA (149). This junction may take the form of a synthetic four-way structure consisting of four oligonucleotides with a homologous core sequence (106, 149), an extruded cruciform (252), or a recombination intermediate formed through the action of RecA protein on two duplex DNA molecules (107, 149). The presence of a homologous (i.e., mobile) core sequence increases the efficiency of RuvC binding. Reduction of the length of homology in a

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synthetic four-way structure from 12 to 6 bp reduces binding (and consequently cleavage) of the junction by at least 10-fold (149), although a nonhomologous synthetic junction can be bound at high concentrations of RuvC protein (30). On RecA protein-free DNA strand exchange intermediates, resolution of these structures is unbiased toward either parental (patch) or recombinant (splice) products, but the presence of RecA protein during an ongoing DNA strand exchange reaction biases the formation of splice products over patch products (107, 149). The reaction is seemingly not catalytic, and the apparent specific activity is low: optimal cleavage of the RecA protein-mediated recombination intermediate requires 1 RuvC monomer per 80 nucleotides of DNA (149).

Further dissection of the cleavage reaction catalyzed by RuvC protein has demonstrated that the process involves three distinct, separable steps (30). First, RuvC protein binds to the specific crossover structure in a cation-independent manner. The affinity of the protein for this particular structure is $\sim10^3$- to 10^4-fold greater than its affinity for B-form duplex DNA. Following binding, the protein distorts the DNA within the nucleoprotein complex such that the DNA displays enhanced sensitivity to hydroxyl radicals. This change is dependent on the presence of a cation, although no difference is detected whether Mg^{2+} or Ca^{2+} is used. These first two steps are not dependent on the presence of homology within the crossover structure. In the third step, however, the RuvC protein carries out strand cleavage in an Mg^{2+}- and homology-dependent manner. The ssDNA nicks made by the enzyme are symmetric, occur in strands of like polarity, and are found exclusively on the 3′ side of thymine residues. Following nicking, the ends can be religated by either *E. coli* or T4 DNA ligase (30, 252).

On the basis of both biochemical and genetic evidence, it seems certain that RuvC protein is the elusive *E. coli* Holliday junction-cleaving enzyme which is responsible for the resolution of recombination intermediates. In agreement, purification of a phenotypically defective RuvC protein, the RuvC51 protein (Gly-15 to Asp), yields an altered protein that can still bind but cannot cleave synthetic Holliday junctions, arguing that cleavage activity is essential to biological function (554a). Genetic studies demonstrating the enhanced recombination deficiency in *ruvC recG* double mutants (350) suggest that the RecG protein may serve a similar role, but, biochemically, RecG protein is functionally equivalent to RuvAB complex in that it carries out branch migration exclusively (see above). A solution to this seeming contradiction may be forthcoming: it appears that the RecG protein functions in conjunction with a previously unknown gene product defined by the *rus* (*ruv* suppressor) locus (400). The *rus* mutation, which is not allele specific, is capable of suppressing both the mitomycin and UV sensitivity and the recombination deficiency of *ruv* mutations in either *recBC sbcBC* or *rec*$^+$ *sbc*$^+$ strains. Suppression requires *recG*, suggesting that a gene product defined or controlled by the *rus* mutation works with the RecG protein to effect suppression, probably by providing a more effective alternative mechanism for Holliday junction processing (400).

SbcB PROTEIN (EXONUCLEASE I)

The *sbcB* gene, which encodes the exonuclease I protein, is located at 44 min on the *E. coli* chromosome linkage map and is also known by the alternate name *xonA* (316, 317, 633a).

Biological Functions

The *sbcB* allele was first identified as a suppressor of the recombination- and DNA repair-deficient phenotype of *recBC* strains (317). A mutation in the *sbcB* gene restores conjugational recombination and resistance to DNA-damaging UV light and mitomycin to near wild-type levels (317). Suppression of the *recBC* phenotype also requires the presence of a distant second-site mutation designated *sbcC*. This gene has not been well characterized to date (see the section on SbcC and SbcD proteins, below). Suppression of the *recBC* phenotype by *sbcB* alleles is presumed to result from inactivation of the destructive 3′ → 5′ exonucleolytic activity of exonuclease I. This allows the persistence of single-stranded 3′ tails that can be used by the RecF pathway of recombination (238, 675).

Although probably alleles of the same gene, *xonA* and *sbcB* mutations differ in that *xonA* mutations suppress the UV sensitivity but not the recombination deficiency of *recBC* strains (in such strains, recombination frequencies for *xonA* mutants are 60- to 140-fold lower than for *sbcB* mutants [316]). This is a rather curious result since both mutations produce proteins which possess little, if any, exonucleolytic activity. Consequently, loss of this activity does not appear to be the basis for the phenotypic differences between *sbcB* and *xonA* alleles (479). In fact, the *sbcB* allele is reported to be equivalent to a deletion of the gene (611). This would seem to indicate that the *xonA* alleles must retain or possess some activity independent of the exonucleolytic function of exonuclease I.

Another observation corroborating the difference between *sbcB* and *xonA* alleles is that mutations which enhance the frequency of illegitimate recombination (measured as the frequency of rare deletions) map to *xonA*, whereas *sbcB* alleles do not show this phenotype (8). Illegitimate recombination is defined as recombination which is independent of the *rec* genes (for a review, see reference 7). The deletions occurred primarily at regions of microhomology, even though this phenotype does not require a functional *recA* gene. This phenotype of *xonA* alleles is the only one that can be observed in the absence of *recBC* mutations (8). *xonA* mutants, unlike *sbcB* mutants, do not require the *sbcC* mutation for suppression. The enhancement of deletions is dominant to the wild-type allele, implying that it is the presence of a function rather than an absence which results in this phenotype.

These observations raise the possibility that the protein product of the *xonA* gene, while being defective for exonucleolytic activity, either retains (479) or has acquired a second, undefined activity. This possibility was raised initially by Phillips et al. (479) to explain the differences between *sbcB* and *xonA* in the suppression of the *recBC* phenotype. They suggested that the *xonA* gene product, but not that of *sbcB*, interfered with the binding of proteins involved in the RecF recombination pathway, either through direct interaction or through competition for DNA binding sites. The latter seems unlikely because of the low abundance of exonuclease I (40 to 60 copies per cell) (479).

Recently, exonuclease I was shown to possess a DNA deoxyribophosphodiesterase activity that removes a 5′ deoxyribose phosphate from an apurinic/apyrimidinic (AP) site which has been incised by AP endonuclease (endonuclease IV) or AP lyase (535). How, or if, this activity relates to the differences between *sbcB* and *xonA* alleles in the suppression of the *recBC* phenotype or illegitimate recombination is not yet clear, but perhaps *xonA* mutants retain either this deoxyribophosphodi-

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esterase activity or the ability to negatively interfere with recombination proteins.

Biochemical Activities

Exonuclease I is a 53-kDa protein which has 3′ → 5′ exonucleolytic activity specific for ssDNA (334, 479). Digestion of ssDNA by exonuclease I produces 5′ mononucleotides but leaves the terminal dinucleotide intact (334). Hydrolysis is highly processive but cannot proceed if the 3′ terminus of the ssDNA molecule is phosphorylated (639). The mechanistic and catalytic properties of exonuclease I have been intensively studied, and most of these results are beyond the scope of this review (see references 51–53 for details). However, one result worth mentioning in view of the genetic data presented above is that exonuclease I appears to have two substrate-binding sites. One site serves as the catalytic site and extends from the 3′ terminus to nucleotide 7, while the second "anchor" site covers nucleotides 9 to 13 from the 3′ terminus. It was speculated that this second anchor site is most probably the reason that the exonucleolytic activity of exonuclease I is so processive (51–53). Given the two binding sites, it is possible to postulate how two classes of mutant alleles might arise, if mutations in one or the other site differentially affect the nucleolytic activity of exonuclease I.

Exonuclease I has the interesting property of interacting with two proteins that have clearly defined functions in recombination: the SSB and RecA proteins. Early density gradient centrifugation established that exonuclease I associates with SSB protein (437). In agreement, exonuclease I copurifies with SSB protein through several chromatographic steps (26, 27, 309a). Similarly, exonuclease I is found to contaminate RecA protein preparations (26, 27); this contamination seems to be more than fortuitous, since exonuclease I coelutes with RecA protein on at least six different chromatographic resins, remaining associated at salt concentrations as high as 1 M NaCl (327). Although ultimately separable (327), coelution under a variety of experimental conditions is suggestive evidence for protein-protein interactions. Despite these intriguing observations, no data have demonstrated that the association of exonuclease I with SSB and RecA proteins is either specific or functionally important.

SbcC AND SbcD PROTEINS

Biological Functions

The *sbcC* and *sbcD* genes were defined primarily as a mutations required in addition to the *sbcB* allele for suppression of the *rec* phenotype of strains carrying *recBC* mutations (352, 403a). The only other phenotype associated with mutations in these genes is the ability to propagate bacteriophage λ carrying long palindromic sequences (73, 195). This phenotype is seen even in the presence of wild-type copies of both the *recBC* and *sbcB* genes (195). The *sbcC* gene maps at approximately 9 min on the *E. coli* chromosome and produces a poorly expressed 118-kDa protein (461). It lies downstream from a gene encoding a 45-kDa protein. This gene was initially designated *orf-45* (461) but has been redesignated *sbcD* because its expression seems to be required for expression of the *sbcC* phenotype (195). In fact, the two genes overlap (i.e., the stop codon for the *sbcD* gene follows the start codon for *sbcC*) and appear to be transcriptionally and translationally coupled (461). A mutation in either gene is sufficient for both suppression of the *recBC* phenotype and maintenance of bacteriophage λ containing palindromic sequences (λ *pal*). Even though mutations in *sbcD* are sometimes polar on *sbcC*, supplying a wild-type copy of *sbcC* in *trans* still results in the ability to propagate λ *pal*. This suggests that the two proteins are involved in the same process and that they may be subunits of one enzyme (195).

Biochemical Activities

The SbcC protein possesses a putative nucleotide-binding fold (G-X_4-G-K-T) similar to other nucleotide-binding proteins and also has sequence homology with membrane-associated ATPases (195, 461). Neither the SbcC nor the SbcD protein possesses any of the sequence motifs (i.e., the so-called DEAD or DEAH boxes) associated with DNA or RNA helicases.

In strains that are $sbcC^+$, λ phage with long palindromic insertions are unstable unless the λ *gam* gene is functional (314). This may suggest that the λ Gam protein interacts with the SbcC protein in a fashion similar to that reported for the RecBCD enzyme. By analogy to the RecBCD enzyme model, Kulkarni and Stahl (314) proposed that, because the Gam protein interacts with and suppresses the exonucleolytic activity of RecBCD protein, the SbcC protein is also a nuclease. Recent studies have shown that the SbcC and SbcD proteins form a complex which possesses ATP-dependent dsDNA exonuclease activity (105a).

SSB PROTEIN

Deficiencies in the *ssb* gene adversely affect biochemical processes that involve the transient formation of ssDNA, such as replication, recombination, mutagenesis, transposition, repair, and the response to DNA damage (for a detailed review, see reference 430 and references therein). The product of the *ssb* gene is a helix-destabilizing protein more commonly known as SSB protein. The SSB protein has no known enzymatic activity but is distinguished by its preferential and cooperative binding to ssDNA. The roles of the SSB protein in the process of genetic recombination include the stimulation of RecA protein-promoted pairing and exchange of DNA strands, the trapping of ssDNA produced by DNA helicase activity, and the protection of ssDNA from nucleolytic degradation.

Biological Functions

The *ssb* locus was initially defined during a screen of temperature-sensitive mutants with defects in DNA replication. Extracts from cells carrying the *dnaM710* mutation failed to support the in vitro replication of G4 DNA at nonpermissive temperatures unless the reaction was supplemented with SSB protein (428). Designated *ssb-1*, this temperature-sensitive mutation maps to approximately 92 min on the *E. coli* chromosome (197). The identity of the *ssb* gene was verified when a mutation rendering sensitivity to radiation and alkylating agents, designated *exrB*, was found to be distinct from other radiation-sensitive mutations in this region, called *exrA* (*lexA*) (207, 208). This mutation, renamed *lexC113* (254), was also impaired in DNA synthesis and displayed defects similar to those of the *ssb-1* mutation (208). The notion that *lexC113* defined another *ssb* mutation was confirmed by demonstrating that the SSB protein isolated from *lexC113* cells was temperature sensitive (431), as was the protein isolated from *ssb-1* cells. A third *ssb* allele, *ssb-3*, is considerably more UV sensitive than the other two *ssb* mutations, with a sensitivity comparable to that of a Δ*recA* allele (546a).

In addition to defects in DNA replication, cells carrying the *ssb-1* mutation have an overall reduced recombination profi-

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ciency at nonpermissive temperatures, indicating that the SSB protein plays a role in genetic recombination. Recombination following P1-mediated transduction is fivefold lower in *ssb-1* strains (197), similar to the fourfold reduction in Hfr mating seen in *ssb-3* strains (546a). Additionally, the inversion of repeated DNA elements in λ phage, mediated by the RecBCD pathway, is decreased 10- to 20-fold in *ssb-1* strains (167). Finally, host-dependent recombination between *lacZ* regions in genetic crosses involving λ phage is decreased at least eightfold by the *ssb-1* mutation (203).

Biochemical Activities

The SSB protein has a single biochemical activity, the preferential and relatively nonspecific binding to ssDNA. SSB protein was initially purified on the basis of its tight binding to ssDNA-cellulose, analogous to that of the T4 gene *32* protein (567). The SSB polypeptide, as judged by SDS-PAGE, has an apparent molecular mass of approximately 18.5 kDa (688). The published DNA sequence predicts that the *ssb* gene product is composed of 177 amino acid residues with a calculated molecular weight of 18,843 (534). SSB protein binds a variety of nucleic acids including ss- and dsDNA, synthetic polynucleotides, and RNA. It displays a hierarchy in its equilibrium-binding affinities, preferring poly(dT) relative to native ssDNA and to other polynucleotides, while binding both dsDNA and RNA relatively weakly (438, 475, 530, 531, 688, 706; see references 301, 367, and 368). The strong dependence of SSB protein-ssDNA complex stability on monovalent-salt concentration (the apparent DNA-binding affinity decreases by approximately 10^6-fold when the NaCl concentration is raised 10-fold) suggests the presence of a major ionic component in binding (62, 475). By virtue of its preferential binding to ssDNA, the SSB protein destabilizes duplex DNA; hence, it has been referred to as both a helix-destabilizing protein (368, 559) and a DNA-unwinding protein (435–437, 567, 688). At physiological salt and protein concentrations, the SSB protein exists as a tetramer, each subunit having a DNA-binding site (312, 371, 435, 475, 688, 705). Studies of the mutant SSB-1 protein support these conclusions, since the single change (His-55 to Tyr) in this mutant decreases both the stability of the tetrameric structure and its ssDNA-binding affinity (63, 64, 705).

The binding of the SSB protein tetramer to single-stranded nucleic acids has a number of interesting characteristics (for reviews, see references 367 and 368). The site size of the SSB protein tetramer varies between 35 and 65 nucleotides depending on solution conditions, particularly the type and concentration of added salts; these different site sizes reflect distinct DNA-binding modes (i.e., the number of subunits within the tetramer interacting with the nucleic acid) (59, 60, 65, 371, 688). Electron microscopy studies indicate that these distinct binding modes correspond to SSB protein-ssDNA complexes with different morphologies (88, 211). The larger site size (SSB_{65}) is favored at higher salt concentrations (100 to 200 mM NaCl or >12 mM $MgCl_2$) and lower binding densities (59, 371). The SSB_{65} mode produces a beaded periodic appearance, with the apparent contour length of the ssDNA being compacted by 75%. At higher protein-to-ssDNA ratios, the SSB_{35} mode predominates. This mode has a smoother appearance and produces a 60% reduction in the ssDNA contour length. The beaded nature of the SSB_{65} complex is due to the existence of dimers of SSB protein tetramers (octamers) (88). Consequently, the differences in the apparent contour length of the ssDNA within these complexes correspond to the extent of ssDNA wrapping around the various structures of bound SSB protein (octamers or tetramers) (65, 312).

Electron microscopy (88, 211, 530, 567), among a number of other techniques (438, 567, 688), demonstrates that the binding of the SSB protein to ssDNA is cooperative (i.e., the binding of SSB protein to ssDNA adjacent to an already bound tetramer is favored relative to an isolated binding event). However, the binding of the SSB protein to ssDNA displays two different types of cooperativity (372). "Unlimited" nearest-neighbor cooperativity, which may be transiently stabilized under conditions which favor the SSB_{35}-binding mode, enhances the formation of long clusters of protein as a result of interactions on either side of bound SSB protein tetramers. "Limited" cooperativity, which is seen for the SSB_{65}-binding mode, does not result in contiguous clusters of bound protein and is best described by a model in which the interaction of SSB tetramers is limited to the formation of octamers (61).

The ssDNA-binding activity of the SSB protein contributes, albeit in an auxiliary manner, to the activities of both the RecA and RecBCD proteins. SSB protein stimulates the rate of RecA protein-promoted joint molecule formation and subsequent exchange of DNA strands (71, 110, 112, 114, 115, 414, 454, 559). The amount of SSB protein required for optimal stimulation of RecA protein-dependent activities is dependent on the ssDNA concentration (114, 261, 307, 414, 443, 647). These, and other observations, are most consistent with the view that the binding of the RecA and SSB proteins to ssDNA is competitive, with the ATP-bound state of the RecA protein having both a high affinity for ssDNA and the ability to displace the SSB protein from ssDNA (306, 330, 647). Stimulation of RecA protein activity by SSB protein during presynapsis corresponds to effects at the DNA level and is not due to any specific protein-protein interactions (306, 430, 454). Native ssDNA contains secondary structure, which normally prevents formation of the contiguous RecA protein-ATP-ssDNA ternary complex that is essential for homologous pairing. SSB protein binds to these regions and is subsequently displaced by RecA protein, allowing the formation of a continuous filament. This effect of SSB protein is manifest as a stimulation of the ssDNA-dependent ATP hydrolysis activity of RecA protein since the amount of RecA protein that is bound to the ssDNA is increased (307, 443, 454). Other observations support a role for SSB protein in presynaptic filament formation. First, under conditions which destabilize secondary structure (e.g., low Mg^{2+} concentrations) (647) or which enhance the association of RecA protein with ssDNA (e.g., low Mg^{2+} concentrations in the presence of volume-occupying agents) (331), the requirement for SSB protein is alleviated. Second, many single-stranded DNA-binding proteins, such as the T4 gene *32* protein, the SSB protein encoded by the F factor, the β protein of λ phage, and an SSB protein (yRP-A) from *Saccharomyces cerevisiae*, can substitute for the *E. coli* protein in the stimulation of presynaptic filament and joint-molecule formation (85, 161, 226, 307, 453, 559). Finally, RecA protein-promoted homologous pairing between gapped circular and linear dsDNA does not require SSB protein unless the ssDNA gap is greater than 300 nucleotides (104, 341, 696).

In addition to its presynaptic role, SSB protein plays a role in the postsynaptic phase of DNA strand exchange. Reactions involving circular ssDNA and linear dsDNA occasionally exhibit the formation of homologously paired networks containing many DNA molecules (85). These networks are the consequence of RecA protein-dependent reinvasion of dsDNA by the DNA strand displaced from joint molecules. Formation of these homologously paired DNA networks is suppressed by either SSB or gene *32* proteins because they bind to and

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sequester the displaced DNA strand (85). Beyond the simple sequestration of the displaced strand and resultant inhibition of network formation, SSB protein has a more direct postsynaptic role. Under conditions where SSB protein is not required for presynapsis, the rate of RecA protein-promoted joint-molecule formation is still augmented by the presence of SSB protein (332). The amount of SSB protein required for maximal stimulation is directly proportional to the input dsDNA concentration (rather than to the ssDNA concentration, as in the case of presynapsis). These results suggest that SSB protein further enhances DNA strand exchange through the binding and stabilization of the displaced DNA strand (332). In agreement, the symmetric four-strand exchange reaction (Fig. 4C), which does not produce a displaced strand, neither requires nor is stimulated by SSB protein (104, 341, 696).

The notion that RecA and SSB proteins compete for ssDNA-binding sites provides a consistent explanation for the behavior of mutant RecA proteins in vivo (see the section on RecA protein, above). The ability of mutant RecA proteins to compete with SSB protein in vitro correlates with the phenotypes of *recA* mutations (302). The *recA430* and *recA142* alleles display partially and completely defective recombination phenotypes, respectively. Biochemical characterization of the RecA430 and RecA142 proteins reveals that their ability to compete with the SSB protein for limited ssDNA-binding sites is deficient. The RecA430 protein displays a reduced ability to displace SSB protein from ssDNA, consistent with its partial recombination defect (423). The RecA142 protein cannot compete with SSB protein under any conditions and thus is displaced from ssDNA by SSB protein; consequently, all activities of RecA142 protein are inhibited in the presence of the SSB protein (304, 308). The behavior of a class of *recA* mutants that displays constitutive SOS induction (*recA441*, *recA730*) and/or enhanced recombination activity (*recA441*, *recA730*, and *recA803*) relative to the wild-type *recA* allele (202, 273, 386, 390, 711) supports this correlation (302). Each of these mutant proteins competes with SSB protein for ssDNA-binding sites more effectively than the wild-type protein; consequently, the mutant proteins display more efficient joint molecule formation and LexA repressor cleavage activity under suboptimal reaction conditions (329, 330, 389, 390). The *recA803* mutation partially alleviates the UV sensitivity of *recF* mutations and results in cells that can overcome the requirement for RecF protein in recombinational repair. This result indicates that the activities of the RecA803 protein are altered to compensate for the loss of functions normally intrinsic to the RecF protein in vivo (390). The RecA803 protein also has an increased ability to compete with the SSB protein for ssDNA binding, suggesting that RecF protein may either assist the RecA protein in this competition or displace SSB protein from ssDNA (389). The validity of this hypothesis is discussed in the section on RecF, RecO, and RecR proteins, above.

Another potential function for SSB protein in homologous recombination is the binding to and transient stabilization of ssDNA produced by the action of DNA helicases or strand-specific nucleases. The mechanisms illustrated in Fig. 2 and 3 propose that initiation of recombination requires formation of ssDNA. The preferential binding of ssDNA by the SSB protein should maintain or protect the ssDNA until it can be utilized by RecA protein for joint molecule formation. Biochemical studies confirm that the SSB protein can indeed efficiently bind to and "trap" ssDNA tails produced by the helicase activity of the RecBCD enzyme (514, 624, 625, 633) (see the section on RecBCD enzyme, above). In the absence of an ssDNA-binding protein, the complementary single strands of DNA reanneal behind the RecBCD helicase, forming twin loops; the presence of an ssDNA-binding protein, such as SSB protein or the T4 gene *32* protein, prevents reannealing, thereby enhancing the formation of ssDNA tails in the form of loop-tail structures (624, 625, 633). In support of this role, an in vitro recombination reaction demonstrating homology-dependent DNA heteroduplex formation and requiring the concerted action of RecBCD and RecA proteins is stimulated by SSB protein (141, 510). The RecA protein can also trap ssDNA, but it is less effective than SSB protein because of its relatively low rate of association with ssDNA (see the section on RecA protein, above); these observations offer an additional explanation for the requirement of SSB protein in recombination processes both in vivo and in vitro.

A final function for SSB protein is the protection of ssDNA from degradation by nucleases. Strains deficient in *ssb* function exhibit a high rate of DNA degradation following UV irradiation (22, 335, 681, 700, 701). Biochemical studies on SSB protein have also demonstrated that this protein can protect ssDNA from degradation by a variety of nucleases (385, 429, 436, 437). For example, the nonspecific nuclease activity of the RecBCD enzyme yields ssDNA fragments which range in size from oligomers to thousands of nucleotides; this nonspecific nuclease activity of the RecBCD enzyme is inhibited by SSB protein (385). Thus, both by trapping ssDNA and by protecting it from further degradation by nucleases, the ssDNA-binding activity of the SSB protein contributes to the maintenance of ssDNA needed for joint molecule formation.

DNA POLYMERASE I

DNA polymerase I is a DNA repair polymerase which is required for normal levels of viability and growth. In addition to its $5' \rightarrow 3'$ polymerization function, DNA polymerase I has a $3' \rightarrow 5'$ exonuclease proofreading function as well as $5' \rightarrow 3'$ exonuclease activity required for excision repair, nick translation, and the degradation of RNA primers so that the RNA-DNA hybrid can be made into fully duplex DNA. It is encoded by the *polA* gene, which is located at 87 min on the *E. coli* chromosome between *metE* and *rha* (269). This gene encodes a protein of 928 amino acids (M_r 103,000) (257). Under wild-type conditions, there are approximately 400 molecules of DNA polymerase I per cell (299).

Biological Functions

Several genetic observations suggested a role for DNA polymerase I in processes such as UV repair and cell growth. A mutant with an amber mutation of *polA* (*polA1*) which displays reduced ($\sim 10^{-2}$) levels of polymerase activity but has essentially wild-type levels of $5' \rightarrow 3'$ exonuclease activity (333) shows sensitivity to UV and gamma irradiation and to DNA-damaging agents, although recombination is only slightly affected (129, 212). It was postulated that the polymerase function was required for the repair of ssDNA gaps in damaged DNA. A mutant with a similar phenotype was also identified in a screen for recombination-deficient mutants (590). To confirm the observation that *polA recA* or *polA recBC* double mutants could not be constructed (213), a temperature-sensitive allele of *polA* (*polA12*) with a preferentially sensitized $5' \rightarrow 3'$ exonuclease activity was isolated; in combination with mutations in either *recA*, *recB*, or *recC*, the double-mutant strain was inviable at 42°C (439).

At least partial activity of DNA polymerase I is required for inversions between homologous DNA sequences (167). This was tested by infecting *recBC*$^+$ cells that were either *polA*$^+$, *polA1*, or *polA480ex* (a temperature-sensitive allele possessing

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DNA polymerase, but not 5′ → 3′ exonuclease, activity at the restrictive temperature [294, 654]) with λ phage which were defective for phage recombination function (*red gam*). In the presence of host recombination function, an invertible DNA segment in the phage is flipped into the opposite orientation, with an accompanying change in phenotype. In the *polA1* background, inversion was significantly reduced (10- to 70-fold) (167). Cells which possessed DNA polymerase activity but not 5′ → 3′ exonuclease activity (i.e., the *polA480ex* strain at 41°C) had no effect on the inversion frequency. These experiments suggested that limited DNA synthesis, similar to that observed during DNA repair, was required for recombination and that 5′ → 3′ exonuclease activity was not required. In a similar fashion, the effect of polymerase I activity on conjugal recombination was examined; a DNA polymerase I mutant (*polA107*) which was defective in polymerase activity but not 5′ → 3′ exonuclease activity had reduced levels (10- to 20-fold) of recombination (729).

These data were originally interpreted in the context of a model whereby RecBCD enzyme degraded DNA after encountering χ until a region of homology was reached (167). It was proposed that DNA polymerase I was required to fill in the region of degraded DNA. Although this model appeared to be supported by the finding that limited DNA synthesis in phage which have undergone recombination is located to the region of the exchange (566), current in vitro biochemical studies concerning the effect of χ on the dsDNA exonuclease activity of RecBCD enzyme (see the section on RecBCD enzyme, above) do not readily accommodate this model. Thus, it is currently believed that the role of DNA polymerase I is to fill in gaps created by partial degradation of the DNA during recombination and repair (577).

Biochemical Activities

DNA polymerase I has been extensively characterized (299), but only studies relevant to its involvement in recombination are mentioned here. In vitro studies suggest that DNA polymerase I has a role in the repair of cross-links by a recombinational mechanism that involves participation of both nucleolytic and polymerization activities of DNA polymerase I (571). The 5′ → 3′ exonuclease activity of DNA polymerase I is used to degrade the DNA from a nick introduced on the furan side of a psoralen cross-link. This degradation exposes a ssDNA gap which can be bound by RecA protein to initiate recombinational repair across the region of the cross-linked DNA. Following DNA strand exchange and excision of the cross-linked oligonucleotide, the polymerase activity of this protein is required to repair any gaps which remain.

DNA polymerase I can act on D-loop structures created by the invasion of supercoiled DNA by ssDNA fragments (346). Provided that the fragments have a free 3′-hydroxyl group at their terminus, DNA polymerase I can synthetically extend this primer to a length that is limited by the extent of negative supercoiling in the D-loop structure. In addition to DNA synthesis, DNA polymerase I can endonucleolytically cleave the supercoiled DNA molecule that is a participant of the D-loop structure, provided both that the invading ssDNA fragment has a 3′-hydroxyl group and that DNA synthesis is occurring. Both of these activities pertain to the fate of D-loop structures illustrated in Fig. 2: the 3′ end of ssDNA fragments is the preferred substrate for DNA strand invasion promoted by RecA protein (see the section on RecA protein, above), and extension of this invasive 3′-hydroxyl group by DNA synthesis may serve to stabilize joint molecules. Alternatively, the aforementioned endonuclease activity of DNA polymerase I may provide the DNA strand nick that is needed to convert paranemic joint molecules to plectonemic species.

An unusual activity of DNA polymerase I that may be related to its D-loop-cleaving ability is its ability to cleave bifurcated DNA substrates (383). The enzyme will cleave an ssDNA arm at the base where it joins a region of dsDNA. This ssDNA arm can be up to 200 nucleotides in length but must possess a free 5′ end. Cleavage is dependent on the structure of the DNA substrate rather than its sequence and is enhanced by the presence of a primer DNA that is complementary to the template strand (i.e., the 3′-terminal arm). In fact, the typical preferred substrate has the appearance of the 5′-tailed molecules depicted in the next-to-last line of Fig. 3, raising the possibility that DNA polymerase I is involved not only in the repair of gaps in recombinant product molecules but perhaps in the trimming of 5′ tails as well. The relationship of this recently described endonuclease activity of DNA polymerase I to its D-loop cleavage activity reported earlier is unclear.

Finally, biochemical studies suggested that a possible complex exists between DNA polymerase I and, at minimum, the RecB subunit of RecBCD enzyme. This complex was first noted on the basis of ATP-dependent stimulation of polymerase activity from crude *E. coli* extracts that was reduced in *recB* and *recC* mutants (223, 545). More recently, this stimulation of polymerase activity was found to be *recB* but not *recC* dependent (614); this observation is consistent with the fact that the RecB subunit is both an ATPase and a weak DNA helicase (44) (see the section on RecBCD enzyme, above). The putative role of an association between DNA polymerase I and RecBCD enzyme remains a mystery. It is plausible that DNA polymerase I acts at the 3′-hydroxyl group of the invading strand to catalyze synthesis, and perhaps strand displacement, in the opposite orientation from which strand invasion occurs. When such DNA synthesis is necessary, it is possible that the supposed association between RecBCD enzyme and DNA polymerase I targets the synthetic enzyme to the site of the invading end; however, there are no in vitro data to support this presumption.

DNA TOPOISOMERASES

DNA topoisomerases catalyze both the introduction and the relaxation of superhelicity in DNA through the transient cleavage and re-formation of phosphodiester bonds. The type I topoisomerases relax superhelical DNA by nicking one strand of a duplex molecule, resulting in a change of the linking number by units of 1. The type II topoisomerases catalyze the ATP-dependent introduction of negative supercoiling by breaking both strands within duplex DNA, resulting in a change of the linking number by units of 2. This section will focus on the role of *E. coli* DNA topoisomerases I and II in recombination; for a general review of DNA topoisomerases and their roles in vivo, see references 145 and 668–671.

DNA Topoisomerase I

DNA topoisomerase I (ω protein) is a single polypeptide of about 100 kDa that catalyzes the removal of negative supercoils, leading to relaxation of supercoiled DNA (666). Relaxation is accomplished by breakage of one strand of the DNA helix, which is then passed through its complementary strand and religated (274).

The structural gene encoding DNA topoisomerase I, *topA*, is located at 28 min on the *E. coli* chromosome (642). Mutants defective in topoisomerase I activity display slow growth, reduced transposition and transcription, increased sensitivity

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to UV and methyl methanesulfonate, and high superhelicity of plasmid DNA (489, 602; for reviews, see references 145 and 667). In addition, *topA* mutants display reduced levels of intramolecular plasmid recombination (1,000-fold reduction) (182, 288) and reduced expression of the *recA* gene (653). Suppressor mutations of *topA* have been mapped to the loci encoding DNA gyrase and topoisomerase IV activities (219, 266, 472). Topoisomerase I can complement slow-growth defects of *top3* mutations in *Saccharomyces cerevisiae* (665). This result, along with a high sequence identity (40%) between the *topA* and *TOP3* gene products (665), suggests that these two enzymes have related functions and may affect recombination similarly.

In vitro, topoisomerase I facilitates the RecA protein-dependent formation of homologously paired, plectonemically wound joint molecules between two topologically constrained substrates (i.e., circular ssDNA and supercoiled DNA) (35, 120, 717); otherwise, RecA protein normally requires a free DNA end to form stable plectonemic joint molecules (125). This finding demonstrates that RecA protein does not recognize a free DNA end specifically but that the requirement for a free end is due to topological restraints (120). On the basis of this result, a likely role for topoisomerase I in genetic recombination is to stabilize the pairing of DNA molecules at internal homologous sites.

DNA Topoisomerase II

DNA topoisomerase II is a two-subunit enzyme commonly referred to as DNA gyrase. It was identified as an activity that stimulated in vitro integrative recombination of bacteriophage λ (192). The genes encoding the subunits of DNA gyrase were defined from studies of the DNA replication inhibitors nalidixic acid and novobiocin. The *nalA* (*gyrA*) gene confers resistance to nalidixic acid, while the *couB* (*gyrB*) gene is the target of coumermycin A_1; these genes map to 48 and 83 min on the *E. coli* chromosome, respectively (191, 193, 220, 609).

DNA gyrase is the only bacterial activity which catalyzes negative supercoiling in DNA. In the presence of specific gyrase inhibitors or gyrase mutants, titratable supercoils are lost from both plasmids (363, 433) and the chromosome (147, 601, 660). DNA gyrase has been implicated in a variety of processes, each of which must contend with the topological constraints of DNA or require the free energy of supercoiled DNA (for reviews, see references 145, 668, and 669 and references therein). Several in vivo studies demonstrate a requirement for DNA gyrase activity, and therefore presumably DNA supercoiling, in genetic recombination; the pleiotropic consequences of DNA gyrase inhibition, however, may result in secondary regulatory aberrations.

Transfection assays involving *E. coli* spheroplasts demonstrated that *recA*-dependent joint molecule formation between φX174 ssDNA fragments and covalently closed circular DNA occur at higher frequencies (20- to 100-fold) when the duplex DNA is supercoiled (235). In cell extracts, inhibitors of DNA gyrase partially repress (50 to 80%) resolution of a tetrameric plasmid molecule into smaller circular products, suggesting that supercoiled DNA is the preferred substrate for this intramolecular recombination reaction (287). Antagonists of either DNA gyrase protein subunit (coumermycin and, to a lesser extent, oxolinic acid) decrease both *recA*- and *recB*-dependent repair and *recA*-dependent *recB*-independent recombination of tandem repeats in UV-irradiated λ phage (221). Similarly, coumermycin or nalidixic acid treatment inhibits the inversion of tandem repeats in λ which occurs via the RecBCD pathway of homologous recombination (167).

The two protein subunits, gyrase A (M_r 97,000) and gyrase B (M_r 90,000) (191, 193, 609), assemble as a 400-kDa tetramer with A_2B_2 quaternary structure (434). The ATP-dependent introduction of negative supercoils by DNA gyrase is achieved through concerted breakage, passage through the break, and reunion of both strands of a duplex DNA molecule. Two lines of evidence implicate the gyrase A subunit in the transient DNA breakage-rejoining activities of gyrase (444, 648): phosphotyrosine covalent linkages are formed between the gyrase A subunit and the 5′ termini at the DNA duplex break, and specific antagonists of the gyrase A subunit, such as nalidixic acid, interfere with the DNA-rejoining step. The facts that coumermycin A_1 is a competitive inhibitor of the DNA-dependent ATPase activity of gyrase B and that heat or urea treatment of this subunit greatly enhances a latent DNA-independent ATPase activity indicate that ATP binding and hydrolysis are intrinsic to the gyrase B subunit (410, 608).

Biochemical studies of model homologous recombination reactions demonstrated the need for negative supercoiling in all reactions involving covalently closed dsDNA substrates. The RecA protein-promoted formation of joint molecules between covalently closed dsDNA and linear ssDNA requires that the recipient duplex be negatively supercoiled (141, 292, 413, 510, 559). The resultant plectonemically paired joint molecules require superhelicity for their continued stability in the absence of RecA protein. Although RecA protein can promote the homologous pairing of intact and gapped homologous duplexes (71, 119), topological constraints limit the extent of DNA strand exchange. However, in the presence of DNA gyrase, covalently closed heteroduplex DNA containing one strand from each parental molecule can be detected; both RecA protein and DNA gyrase are required, but a type I topoisomerase cannot substitute for DNA gyrase (69). Finally, homologous pairing between regions of duplex DNA involving gapped DNA with heterologous regions of ssDNA and covalently closed circular dsDNA also requires negative supercoiling (81, 104). The resultant paranemically paired joint molecules also require negative supercoiling for their stability. Thus, as might be expected, DNA gyrase should be essential for all processes involving covalently closed DNA as the recipient of a DNA pairing or DNA strand invasion reaction.

DNA HELICASES

DNA helicases catalyze the unwinding of duplex nucleic acids; this localized disruption of hydrogen bonds between complementary base pairs is dependent on the hydrolysis of an NTP cofactor. The fundamental role that DNA helicases play in the processes of DNA replication and repair is recognized; however, with the exception of the RecBCD enzyme, their involvement in generalized genetic recombination is not completely understood (for reviews, see references 4, 189, 190, 365, 366, 407, and 409). Possible roles for a helicase in genetic recombination (Fig. 2 and 3) include both the creation of ssDNA for use by RecA and RecT proteins in homologous pairing reactions (141, 510, 515, 679) and the helicase-dependent extension of DNA heteroduplex (281, 644). Helicases may also participate destructively by unwinding of homologously paired DNA molecules (70, 280, 441a) and by promoting branch migration in a reverse direction that regenerates the parental substrate molecules (361, 644, 699a). The helicases described below exclude those discussed more fully elsewhere (see the sections on RecBCD enzyme, RecG protein, RecQ protein, and RuvAB proteins, above).

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DNA Helicase I (*traI*)

Helicase I is a 180-kDa protein that is encoded by the *traI* gene located on the F plasmid (1, 3, 5). During the process of bacterial conjugation, the product of the *traI* gene is required for the transfer of the F plasmid from donor to recipient cells (247, 703). Through processive DNA unwinding in a 5′ → 3′ direction, helicase I facilitates the transfer of ssDNA from the F plasmid into the recipient cell (313, 319). Beyond this important function in conjugal DNA transfer, there is no evidence for a direct role in recombination, and hence helicase I will not be discussed further here.

DNA Helicase II (*recL*, *uvrD*, *uvrE*, *mutU*)

Helicase II is encoded by the *uvrD* gene, located at approximately 84 min on the *E. coli* chromosome (15, 227, 315, 401, 463, 464). Mutations in *uvrD* were isolated independently and have been variously designated as *recL*, *uvrE*, or *mutU* alleles (238, 318, 466, 564, 565, 573). Cells defective in helicase II show increased mutation frequencies (16, 108, 318, 564, 573, 655a, 685), sensitivity to UV irradiation and other DNA-damaging agents (15, 16, 401, 564, 565, 573, 655a, 685), and some loss in viability (420a). Although noted primarily for the defective DNA repair phenotype, some *uvrD* mutants also exhibit elevated frequencies of conjugational recombination (170, 685, 729), intragenic recombination involving F′ × F⁻ and Hfr × F⁻ crosses (16, 170, 349), and λ phage × prophage crosses (239), whereas other mutations and a deletion of the *uvrD* gene confer a slight defect in conjugal recombination (238, 420a). Although the role of helicase II in recombination is unknown, the former result suggests that helicase II functions as an "antirecombinase"; through the unwinding of paired DNA regions, it could disrupt recombination intermediates and inhibit the recombination process (70, 441a). In contrast, the behavior of the deletion mutant and, in particular, the notable deficiency of *uvrD helD* double-deletion mutants in conjugational and transductional recombination in *recBC sbcBC* strains argues an essential role for these helicases when RecBCD enzyme function is lost (420a).

DNA helicase II, with a monomeric size of 82 kDa (174), catalyzes DNA unwinding by an unusual mechanism (2, 313, 408). Unwinding requires a stoichiometric complex between the protein and the DNA, with the number of base pairs unwound being directly dependent on the concentration of helicase II in the reaction (313, 408). At low ratios of protein to nucleotide (0.1 helicase II monomer per nucleotide), a region of ssDNA is required to initiate unwinding (2, 313, 408, 525); a 3′-ended ssDNA tail is preferred (194, 405, 525). At higher concentrations of helicase II (greater than approximately 0.6 helicase II monomer per nucleotide), both linear dsDNA substrates with blunt ends and circular nicked duplex molecules are unwound, with initiation occurring at a DNA end or nick (524, 525). SSB protein can increase the extent of DNA unwound but inhibits unwinding at high concentration (408). It is noteworthy that helicase II can unwind DNA-RNA hybrid molecules at a rate 10 times greater than that of an equivalent dsDNA molecule (406). The mechanism of unwinding of these hybrid molecules may be catalytic and processive rather than stoichiometric (407a), suggesting that DNA-RNA hybrid molecules may be the physiological substrate for helicase II action. Although helicase II has been extensively characterized biochemically, a specific role in recombination has yet to be determined. Analogous to the effects that the phage T4 Dda helicase exhibits on DNA strand transfer promoted by the T4 UvsX protein (280, 281), helicase II may also render both "recombinase" and "antirecombinase" activities on reactions promoted by the RecA protein (70, 441a).

DNA Helicase III

DNA helicase III was discovered in a search for additional helicases in a *rep* strain (122, 189, 721, 722), but the gene encoding this activity has not been identified. The purified enzyme appears to consist of single polypeptide of 20 kDa, but sedimentation analysis suggests that the enzyme may function as a dimer (189, 721). Helicase III can unwind partially duplex DNA molecules (approximately 500 bp long) (721) with a 5′ → 3′ polarity (722). The unwinding reaction is stimulated by SSB protein at low helicase III concentrations but not at higher helicase III concentrations (722). This result suggests that SSB protein may aid unwinding by binding to the displaced strand. The specific mechanism of unwinding has yet to be determined. Due to the lack of both biochemical information and mutational analysis, the role of helicase III in genetic recombination is unknown.

DNA Helicase IV (*helD*)

Helicase IV is encoded by the *helD* gene, which maps at 22 min on the *E. coli* chromosome (713). This 75-kDa protein has limited unwinding capabilities; it fully unwinds short dsDNA regions (71 bp) with 3′ ssDNA tails, presumably in the 3′ → 5′ direction, yet unwinding is substantially reduced with larger substrates (<20% of a 343-bp dsDNA substrate is displaced) (712). The amount of DNA unwound when the smaller substrate is used increases in a linear fashion with increasing enzyme concentration, but this effect is not seen with the larger dsDNA substrate (712). SSB protein stimulates unwinding of the longer substrate about fourfold (407a). Thus, the enzyme may be acting stoichiometrically on short regions of dsDNA and may require accessory proteins to unwind longer regions of DNA.

Cell lacking helicase IV activity (Δ*helD*) are viable, show no increased sensitivity to UV irradiation, and display a slightly increased resistance to methyl methanesulfonate; however, overproduction of even small amounts of helicase IV is lethal (420a). In comparison, Δ*uvrD* strains are profoundly UV and methyl methanesulfonate sensitive, and these deficiencies are not substantially altered by introduction of a *helD* deletion. Interestingly, however, the double deletions (*helD uvrD*) result in a substantial loss of recombination proficiency (420a). The effects are most prominent in a *recBC sbcBC* strain, in which deletion of both helicase genes reduces conjugational and transductional recombination by 100- to 200-fold; in a *recBC sbcA* strain, the reduction is more modest (20-fold), and in an otherwise wild-type strain, the reduction is only 3-fold. Either of the single mutations confers a much smaller effect: the *helD* deletion alone shows 2- and 6-fold reductions and a 1.5-fold increase in recombination efficiency in *recBC sbcBC*, *recBC sbcA*, and wild-type strains, respectively, whereas the *uvrD* deletion shows 3-, 6-, and 3-fold decreases, respectively (420a). These results argue that although helicase IV and UvrD proteins have little effect on recombination in wild-type cells, together they play a rather important role in the RecF pathway. Whether that role is in initiation of recombination or DNA heteroduplex extension is not yet known, but genetic analysis shows that these two helicases must share these responsibilities with the RecQ, RecG, and RuvAB helicases. Finally, helicase IV can also rescue cells containing the normally lethal (at nonpermissive temperatures) double mutations, *uvrD*(Ts) and *rep3* (407a). Temperature-resistant pseudorevertants of these double mutations map to *helD*, implying

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that helicase IV can substitute for an essential function provided by the helicase II or Rep proteins.

Rep Protein

Rep protein helicase activity is essential for the replication of several phages, specifically the φX174, M13, fd, and P2 phages (67, 99, 130; for reviews, see references 299 and 622). The *rep* gene is located at 85 min on the *E. coli* chromosome and encodes a 68-kDa protein (67, 165, 550). In the presence of phage-encoded replication initiation proteins (e.g., φX174 CisA protein), Rep protein can catalytically unwind dsDNA in the 3' → 5' direction and stimulates replication of the phage chromosome ≈20-fold (13, 14, 148, 164, 165, 549, 723). The Rep protein alone catalyzes limited unwinding of short (<150-bp) duplex regions of DNA which contain a 3'-end ssDNA tail (74, 148, 369, 587, 723).

The physiological role of the Rep protein is unknown. *rep* mutants are viable but have increased numbers of replication forks which move more slowly (99, 131, 321). Double mutations in the *rep* and *uvrD* genes cause decreased viability relative to the single mutants (623, 685). Recombination levels are decreased two- to threefold for both conjugal and intragenic recombination events in cells defective for the Rep protein (729).

CONCLUDING REMARKS

The enzymes involved in genetic recombination speak for themselves. The last decade has seen these participants become more articulate each year. These enzymes encompass a wide array of unique and interesting biochemical activities. Discoveries include proteins that are capable of recognizing and pairing homologous DNA sequences, unwinding DNA and simultaneously scanning for recombination enhancing sequences, responding to regulatory switches that alter enzymatic activity, recognizing and pushing against DNA crossover structures, and selective binding and cleavage of the particular structure presented by the Holliday junction. It is clear that most of the pieces needed to reconstruct the puzzle of genetic recombination are now at hand. It simply remains to reconstruct the puzzle. This will require a concerted effort of biochemical and genetic analysis both to uncover additional activities and to determine the physiological relevance of those activities. Development of in vitro recombination reactions of increasing complexity in terms of protein components should ultimately permit reconstitution of the recombination process. Knowledge of the detailed working of this process will bring satisfaction not only to those interested in fundamental biological processes, such as genetic recombination, but also to those wishing to use recombination and its enzymes for practical purposes. Presently, the most evident applications are gene targeting and gene replacement methods. As these recombination enzymes and reactions come under increasing control and understanding in the laboratory, it is more likely that they can be manipulated to serve the interests of the experimentalist. For example, RecA protein can now be used to target homologous sequences in large genomes (172, 253a, 552); after identification of the target sequence, the next step would be to see if recombination enzymes can be used to target gene replacement in organisms in which this has proven difficult (310a). Similarly, the studies with *E. coli* will serve as a guide for studies of recombination in eukaryotic organisms (see reference 206a for a recent review). The existence of both double-strand break and DNA-reannealing mechanisms of recombination in several eukaryotic organisms is well established (638). Furthermore, the question whether enzymatic activities in eukaryotes similar to those found in prokaryotes are responsible for mediating these recombination events has at least partial answers. Double-strand breaks in *Saccharomyces cerevisiae* are processed to produce resected DNA with 3'-ssDNA overhangs similar to those depicted in Fig. 2 (609a, 610); the manner by which these ends are created (i.e., by the action of a solitary nuclease or by the combination of nucleases and helicases) remains unknown. The mechanism of homologous pairing and DNA strand exchange derived from the studies of *E. coli* RecA protein may have universal applicability, since functional and/or structural homologs have been found in organisms as diverse as bacteriophage T4, all eubacteria, yeasts, and many vertebrates including chicken, mouse, and human (225a, 306a). Hot spots for recombination also exist in eukaryotes (578a); what makes these sites "hot" remains a burning topic of inquiry. The number of other similarities and, perhaps more interesting, differences remains to be determined. Without doubt, the knowledge derived from the studies of *E. coli* will guide such studies and will also serve as a paradigm for recombination mechanisms in the ensuing years.

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