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Homologous Recombination in Procaryotes

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INTRODUCTION

Although bacterial cells are, in general, genetically haploid, the genetic heterozygosity required for detecting homologous recombination may occur in one cell under a variety of circumstances. The cell may be transiently diploid due to the introduction of deoxyribonucleic acid (DNA) via mating (conjugation), bacteriophage infection (transduction), or the uptake of naked DNA (transformation). The DNA molecules available for recombination in these various circumstances may be circular or linear, double stranded or single stranded, or a mixture of these. As one might anticipate, the molecular mechanism of the recombination depends upon the form of these substrates.

Homologous recombination is thought to proceed by a series of enzymatically catalyzed reactions. This view, plus observations discussed below, led Clark (30) to postulate pathways of recombination analogous to pathways of small-molecule metabolism. I shall review our current knowledge of these recombinational pathways in *Escherichia coli* and phage λ. When possible, comparisons will be drawn with other bacteria. I shall discuss aspects of eucaryotic recombination that have played especially important roles in our thinking about recombination. I emphasize the means by which recombination-promoting genes and the corresponding enzymes have been discovered, since these considerations may be useful in elucidating additional pathways of recombination, in both procaryotes and eucaryotes.

Unitary Hypothesis of the Mechanism of Recombination

Multiple lines of evidence discussed in this review suggest a unitary hypothesis for one step of recombination by the known pathways: a 3′-OH end of single-stranded DNA (ssDNA) invades a second double-stranded DNA (dsDNA) molecule to form a joint molecule, which is processed into a complete recombinant (or two if the recombination is reciprocal). Variations of this hypothesis have been proposed by others (e.g., see references 181, 235, 238, and 247). Though it has yet to be rigorously demonstrated for any pathway of recombination, it may help to bring order out of the possibly confusing mass of data.

In this review, I describe mechanisms by which invasive 3′ ssDNA ends can be produced and mechanisms by which they synapse with a second dsDNA molecule and are processed into complete recombinants. The enzymes producing, synapsing, and destroying 3′ ssDNA ends depend upon which pathway of recombination is acting; the ability of these enzymes to act depends in turn on the nature of the substrate molecules available for recombination. I therefore discuss the substrate molecules in various modes of diploidy first, then the enzymes active in various pathways of recombination, and finally the mechanisms by which recombination may occur by these pathways, beginning with different substrates.

MODES OF GENETIC DIPLOIDY AND ACCOMPANYING DNA STRUCTURES

Transient Diploidy

Conjugation. The first noted genetic recombination in enteric bacteria resulted from the conjugation of *E. coli* K-12 cells mediated by the fertility factor F (136). The F factor can also be transferred to other enteric bacteria and promote recombination in them. Several other fertility factors have been discovered in gram-negative bacteria; certain factors can be transferred between enteric bacteria and nonenteric bacteria such as *Pseudomonas* spp. (100).

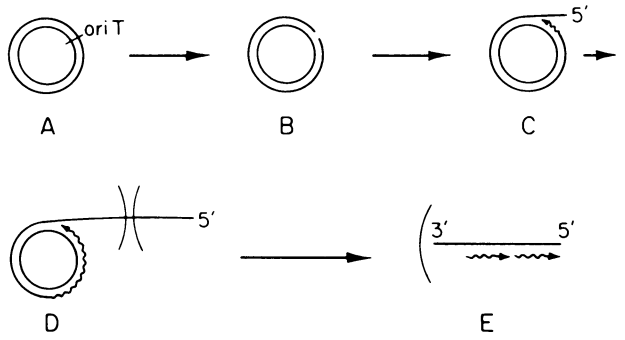

FIG. 1. F-factor-mediated DNA transfer in *E. coli*. The concentric circles in part A represent the circular dsDNA of the F factor or the Hfr chromosome. *oriT* is the origin of transfer DNA replication, which is nicked by F-specified proteins (B). DNA synthesis, represented by the wavy line in part C, displaces one DNA strand, whose 5′ end (perhaps bound to a "pilot protein") traverses the donor and recipient cell envelopes (arcs in D). In the recipient cell, complementary strand synthesis (wavy lines in E), perhaps initiated at numerous priming sites, converts the entering strand to dsDNA initially containing gaps, nicks, and a free 3′ end. This structure may recombine with the recipient chromosome (not shown) before the gaps and nicks are sealed.

The F factor of *E. coli* can exist as a 94.5-kilobase (kb) circular duplex DNA molecule replicating autonomously of the circular duplex chromosome of the cell (for reviews, see references 104, 295, and 296). During conjugation, the F factor is transferred by direct cell-cell contact at high frequency from cells containing it, designated F^+, to cells without it, designated F^-. Rarely, part of the chromosome of the F^+ cell is transferred to the F^- cell, where it either recombines with the F^- chromosome or is degraded (or perhaps simply diluted during growth, since it cannot replicate). In a population of F^+ cells, a small faction of the cells have the F factor integrated into the chromosome. These cells, designated Hfr (for high-frequency recombination), transfer their chromosome to F^- cells at high frequency in a linear order in one direction, starting from the locus of integration and extending to the point of (presumably random) breakage of the DNA during transfer.

The following events are thought to take place during transfer of chromosomal DNA from an Hfr donor (Fig. 1) (104, 295, 296). The F factor-specified proteins *traY* and *traZ* cut the donor DNA at a special site, *oriT*, in the F DNA. DNA synthesis initiated at the cut site displaces one strand of the Hfr chromosome, which is transferred, possibly through the F pilus, into the recipient cell as ssDNA. The 5′ end of that strand enters first and may have a "pilot protein" bound to it. DNA synthesis in the recipient cell converts at least part of the entering ssDNA into dsDNA. Because of the 5′ → 3′ direction of DNA synthesis, it is thought that complementary strand synthesis in the recipient is discontinuous, extending from numerous (presumably random) "priming" sites toward the entering end. Thus, the entering end may be converted into a flush duplex DNA end (with a protein bound to it), while the broken (trailing) end may remain as ssDNA with a 3′ end. Until the discontinuous fragments are joined, ssDNA gaps exist on the entering DNA. In conclusion, Hfr-introduced DNA has a mixed character, composed of ssDNA and dsDNA, with gaps and ends.

Transduction. Transduction is the transfer of parts of the chromosome of one bacterial cell (the donor) to another cell (the recipient) via phage particles. (For reviews, see references 169 and 172.) This phenomenon was first noted by Zinder and Lederberg (305) with cultures of *Salmonella typhimurium*, one of which was lysogenic for phage P22. Soon thereafter, a transducing phage (P1) was found for *E. coli* (140). Transducing phages have been discovered for many bacterial species, both gram-negative and gram-positive, but knowledge of the molecular events of transduction is extensive only for phages P1 and P22. (For reviews, see references 254 and 259).

The donor DNA is packaged as linear DNA into phagelike particles apparently as a consequence of the "headful" packaging mechanism of the phage, which proceeds as follows. During growth in the donor cell, phage DNA replication produces a concatemer, containing many phage DNA equivalents joined end to end. Packaging commences at a special DNA site (*pac*) and proceeds unidirectionally until the phage prohead structure is "full." Since this amount of DNA is greater than a phage equivalent, there are terminal redundancies (of about 2 to 10%). Packaging continues along the concatemer, using the newly created end as an initiation site. In this way, about 5 to 10 phages are packaged starting from one *pac* site on the concatemer.

Packaging of donor DNA may occur by either of two mechanisms. In the first, packaging is initiated at special sites resembling the phage *pac* site and occurring "accidentally" on the donor chromosome. Such a mechanism was dramatically demonstrated in *E. coli* cells into which the P1 *pac* site was inserted at a known location (the λ prophage containing a P1 *pac* "clone") (253). Growth of P1 in these cells produces a donor lysate with nearly 100 times the normal transducing titer for markers near, but to one side of, the *pac* site. Inversion of the *pac* site within the λ prophage yields increased transducing titer for markers on the other side. High stimulation is seen for markers up to five phage DNA lengths (about 10% of the bacterial chromosome) away from the inserted *pac* site. Lower stimulation is seen for an equivalent, additional distance. Since a single introduced *pac* site so dramatically increases the transducing titer, the wild-type chromosome evidently contains no fully active *pac* site. Perhaps it contains instead nucleotide sequences with partial activity.

A second mechanism for packaging of the donor chromosome may involve recombination between the phage DNA and accidental homologies on the donor chromosome. Such recombination would link the phage *pac* site to the donor chromosome. Vogel and Schmieger (277) suggested this possibility from their observation that high-frequency transduction of plasmids containing P22 DNA fragments depends upon homologous recombination functions in the donor cell. If this were the mechanism of packaging the bacterial chromosome, the first particle packaged would contain covalently linked phage and bacterial DNA. Schmieger (217) noted such chimeric DNA in P22 transducing particles, but most of the transducing particles contain no detectable phage DNA (48). P1 transducing particles also contain no detectable phage DNA (103). The first mechanism, packaging from accidental *pac* sites, would form particles containing only bacterial DNA, while the second mechanism, recombination between accidental homologies, would form primarily particles of the same type plus a few (perhaps undetectable) with both phage and bacterial DNA.

Although temperate phages are most commonly used for transduction, virulent phages can also be used provided they are properly mutated to avoid destruction of their host's chromosome. For example, mutants of *E. coli* phages T1 and T4 have been used for transduction (47, 298). In certain circumstances T4 may offer an advantage over P1 because of the ~50% greater DNA content of the T4 particles. Linkage of more widely separated markers may thus be detectable with T4 than with P1.

The bacterial DNA in P1 and P22 transducing particles is linear dsDNA. Upon injection into the recipient cell, most (~90%) of the DNA appears to become circular, with the ends held together apparently by a protein (214, 215). This DNA presumably gives rise to abortive transductants, which outnumber complete (integrated) transductants by about 10:1 (194). Since the donor DNA in abortive transductants is not replicated but is transcribed, these transductant colonies grow very slowly and contain only one cell capable of giving rise to a new colony. The remaining 10% of the injected DNA presumably remains as linear dsDNA until it either recombines with the recipient chromosome to produce complete transductants or is degraded. The protein circularizing the DNA of P1 abortive transductants appears to be phage encoded: certain P1 mutations increase the frequency of complete transductants about 10-fold, at the expense of abortive transductants (302). Perhaps the function of this protein is to protect newly injected phage DNA from nucleases in the host cell; the gene *2* protein of phage T4 protects phage DNA from the *E. coli* RecBCD enzyme (exonuclease V [ExoV]) (191).

In conclusion, transducing phage-introduced DNA appears to be mostly circular dsDNA and about 10% linear dsDNA.

Transformation. Transformation involves the transfer of naked DNA from one bacterial cell to another. It was the basis of the first noted recombination in bacteria, pneumococcal transformation (89). High-frequency transformation requires a complex cellular change, the development of "competence," and is limited to a few bacterial species (for reviews, see references 84, 243, and 256). Lower frequencies of transformation can be achieved in other species by treatment of the cells with ions such as Ca^{2+}. Among the enteric bacteria, transformation has been reported in *E. coli* (35, 167) and *S. typhimurium* (135) (for a review, see reference 94).

In species with "natural" competence, such as *Streptococcus pneumoniae*, *Bacillus subtilis*, and *Haemophilus influenzae*, linear dsDNA enters the cell, and one strand is degraded while the other is integrated into the recipient's chromosome. Circular dsDNA can also transform these cells, but it must be broken, either accidentally before entry or by cellular nucleases during entry. In other species, such as *E. coli*, the low frequency of the process has precluded studying it in detail. The behavior of linear and circular DNA suggests that both types enter the cell but that linear DNA is subject to degradation by cellular nucleases, especially RecBCD enzyme and exonuclease I (ExoI) (for a review, see reference 94).

Phage infection. Phages attach to the bacterial envelope and inject their DNA into the cell, where it is replicated and packaged into new phage particles which are released upon cell lysis or by secretion. During the intracellular growth phase, phages recombine with each other or with the host DNA, chromosome or plasmid. The resulting recombinant phages appear in the lysate. Alternatively, recombinant host DNA can be detected if the cell is not lysed, for example, if the phage is temperate, such as λ, or if the phage is mutationally inactivated for lytic growth.

Phage DNA can take a variety of forms. The DNA in the particles is linear dsDNA for such well-studied phages as λ, T4, T7, P1, P2, and P22. Upon injection, the DNAs of λ and P2 become circular by the annealing of their unique complementary ssDNA tails (12 and 19 nucleotides, respectively). P1 and P22 circularize by homologous recombination between their permuted redundant ends (about 2 to 10 kb) (254, 259). These circles replicate initially in the θ mode but then switch to the σ mode, with the regeneration of dsDNA ends. Packaging proceeds on the σ forms for λ, P1, and P22. P2, on the other hand, may not switch to the σ mode, since monomer circles are most efficiently packaged in cell extracts (202). T4 and T7 appear to remain linear throughout their infection cycle. T4 forms highly branched structures by recombination between the permuted redundant ends and internal homologous regions on separate molecules; these recombination-generated branches probably serve as replication forks (for a review, see reference 181). T7 forms linear concatemers presumably by recombination between its 160-base-pair (bp) terminal (but not permuted) redundancies.

In the ssDNA phages, such as M13 and φX174, the particle DNA is circular. Upon injection, complementary strand synthesis forms a dsDNA circle. A modified rolling-circle mode of replication produces progeny ssDNA circles from these dsDNA circles without the production of free DNA ends (for a review, see reference 127). A site-specific endonuclease (e.g., the φX174 *cisA* protein) nicks the dsDNA circle. DNA synthesis primed by the 3′ end displaces the 5′ end. The *cisA* protein remains bound to the displaced 5′ end and to the DNA polymerase. At the completion of the circular strand synthesis, the *cisA* protein joins the displaced 5′ and 3′ ends to generate the progeny ssDNA circle.

There is an interesting correlation of the presence of dsDNA ends and high-frequency recombination. Expressed as the average number of recombination events per 100 kb of DNA per infection cycle, the recombination rates of P2, φX174, λ, and T4 are about 0.002, 0.1, 2, and 15, respectively (for reviews, see references 45 and 288). P2 may have free DNA ends only transiently (during injection), λ has ends primarily late in the replicative stage, and T4 has ends throughout. φX174 may have ends only occasionally when the *cisA* protein accidently releases the DNA ends; there is a recombinational hotspot at the site nicked by the *cisA* protein (11). As discussed later (see sections, "Recombination Genes and Enzymes" and "Mechanisms of Recombination"), the requirements of DNA ends for recombination-promoting enzymes can account for this correlation.

Stable Diploidy

F′ factors. Excision of the F factor from an Hfr chromosome may occur by recombination at a point different from that which led to its integration. The excised factor may have gained a part of the bacterial chromosome adjacent to the site of integration; in this case the factor is designated F′ followed by the bacterial gene(s) incorporated, for example, F′*lac*. Transfer of the F′*lac* to an appropriately genetically marked F^- cell, for example, a *lacZ* mutant, produces a partially diploid (or merodiploid) strain that is genetically heterozygous. The *lacZ* mutation can be transferred by recombination from the chromosome to the F′*lac*, which in turn can be introduced into other cells to produce an endless variety of genetically heterozygous merodiploids.

At least some of the F (and F′) DNA molecules are dsDNA circles, as seen in the electron microscope (222). As noted in the preceding subsection, the *traY* and *-Z* proteins mediate cutting at *oriT* in preparation for DNA synthesis accompanying transfer. This cut was deduced by Everett and Willetts (58) to be a nick (i.e., a single-strand interruption). These authors analyzed the DNA in mature particles of a λ derivative containing *oriT*. About 10% of the packaged DNA contains a nick near *oriT* when the phage are grown in F *traY*$^+$*Z*$^+$ cells, but these nicks are undetectable when the phage are grown in F *traY* (or *-Z*) mutants. A more recent analysis of intracellular F DNA by the Southern (244) transfer-hybridization procedure has suggested, however, that some of the DNA may contain a double-strand break at *oriT* (R. Porter, personal communication). Since it is unlikely that broken dsDNA is packaged by λ, the DNA analyzed by Everett and Willetts (58) may have represented a minority of the intracellular F DNA. Thus, F and F′ DNA may contain significant amounts of both linear dsDNA and circular and nicked circular dsDNA.

Chromosomal duplications. A few bacterial genes, such as the ribosomal ribonucleic acid genes, are present in two or more copies in the wild-type chromosome (for a review, see reference 6). Other genes can be duplicated by first incorporating them into a specialized transducing phage, such as λ or φ80, and then integrating that phage into the chromosome of an appropriate recipient. The duplicate copies may be separated only by the phage DNA (~50 kb, if integration is at the gene locus) or by a large part of the chromosome (up to 2,000 kb, if integration is at the phage attachment site or elsewhere). In the second case, the duplicate copies may be in direct or inverted orientation. In principle, a single reciprocal recombination event between directly repeated copies deletes the intervening region, whereas that between inverted repeats inverts the intervening region. If the two copies are genetically marked, nonreciprocal recombination either converts one copy to the genetic state of the other or generates a recombinant state at one copy while leaving the other unchanged; the intervening region also remains unchanged.

The bacterial chromosome is, at least part and perhaps most or all of the time, a circular dsDNA molecule as seen in the electron microscope (22). Single-strand interruptions (nicks and gaps) must exist at least transiently at the sites of replication and repair. Occasional double-strand breaks in the bacterial chromosome have not been excluded, however. Transient double-strand breaks are created by DNA gyrase during its introduction of negative supercoils into the chromosome (19, 179). The ends of these breaks are presumably bound to DNA gyrase; it is not known whether recombination-promoting proteins have access to these ends. Nevertheless, rare single- and double-strand interruptions may be involved in recombination of the bacterial chromosome.

Plasmid-borne duplications. Plasmids are circular dsDNA molecules replicating autonomously of the chromosome. Most widely used in recombination studies are the small (5- to 10-kb) plasmids such as pBR322. Because of their multicopy nature (5 to 50 copies per cell), there is opportunity for interplasmidic recombination. A simple reciprocal event creates a dimer; subsequent recombination can create higher-order oligomers. Heterozygous dimers can be readily constructed outside the cell and introduced into the cell by transformation. As with chromosomal duplications, the duplicate copies of the genetically marked gene on the plasmid may be in direct or inverted orientation, and analogous reciprocal and nonreciprocal recombination events can occur. If the plasmid bears a part of the chromosome, there is opportunity for plasmid-by-chromosome or plasmid-by-F′

recombination, analogous to F′-by-chromosome recombination.

Plasmids exist primarily as supercoiled circular dsDNA in the cell, but as with the chromosome, transient nicks, gaps, and double-strand breaks are likely to exist during replication, repair, and supercoiling. Studies by Cohen and Clark (34) indicate that the usual θ (Cairns) form of replication can change to a linear form, perhaps σ (rolling circle), in the absence of RecBCD enzyme and ExoI. Since these enzymes act most efficiently on linear ssDNA or dsDNA (see next section), this result suggests that DNA ends do occur, perhaps at low frequency, on plasmids in wild-type cells. Thus, plasmids appear to be primarily circular dsDNA, with occasional linear forms.

RECOMBINATION GENES AND ENZYMES

Searches for mutant bacteria and phages altered in recombination have revealed many genes whose products are required for recombination. Identifying the enzymatic activities of these products is essential for an understanding of the molecular mechanism of recombination. But identification of these enzymes is not a straightforward task, since initially one does not know what activities to look for. This is a general problem in the study of recombination in both procaryotes and eucaryotes, because until recently there have not been reliable cell-free recombination systems. By contrast, cell-free replication systems have allowed the purification of gene products from wild-type extracts by their ability to complement mutant extracts (for a review, see reference 127). A cell-free recombination system from the yeast *Saccharomyces cerevisiae* promises to allow purification of the products of the many recombination-promoting genes identified in that organism (260, 262). In procaryotes, cell-free systems initially reported to promote recombination are difficult to reproduce reliably, possibly due to DNA destructive activities (120; R. Kolodner, personal communication), or they appear to promote recombination by artifactual mechanisms involving extensive degradation and annealing of ssDNA (24, 201, 213).

I shall review the means by which recombination-deficient mutants have been isolated and the circumstances that led to the discovery of the few known enzymatic activities of the corresponding genes. These considerations may be helpful in isolating recombination mutants in other organisms and in discovering the enzymatic activities of the many procaryotic and eucaryotic recombination genes whose activities are unknown.

The enzymatic activities of the few identified recombination gene products were discovered by one of two routes. First, known enzymes with a plausible role in recombination (for example, DNA polymerase I) were tested for a role in recombination by using mutants deficient in these enzymes. Second, known recombination-deficient mutants (for example, *E. coli recA*) were found to be deficient in activities not directly related to recombination; purification of the enzymatic activities from wild-type cells then revealed additional, recombination-related activities during biochemical characterization of the enzymes. Thus, phenotypes associated with recombination-deficient mutants, not the recombination phenotype itself, were crucial in discovering the biochemical activities of these recombination gene products.

Phenotypes quite generally associated with recombination deficiency, in eucaryotes as well as procaryotes, include (i) increased sensitivity to DNA-damaging agents such as X rays, ultraviolet light (UV), and alkylating agents, (ii) reduced viability, particularly of meiotic products of eucaryotes, and (iii) altered behavior of phages, especially of their mutants altered in recombination, repair, and replication. These associations appear to have multiple bases.

(i) Recombination is closely associated with recovery from DNA damage, partly because recombination between two DNA molecules damaged at different places can generate an undamaged molecule and partly because of overlaps in the enzymatically catalyzed reactions of repair and recombination. It is noteworthy that three recombination-promoting enzymes discussed later, RecA protein, RecBCD enzyme, and DNA polymerase I, have multiple enzymatic activities and participate in DNA repair as well as recombination.

(ii) The basis for reduced viability of recombination-deficient mutants is not clear, although in eucaryotes recombination-deficient mutants are generally meiotically lethal due to the apparent requirement for recombination to guide proper pairing and segregation of homologous chromosomes during meiosis (for a review, see reference 8). In procaryotes part of the reduced viability is probably due to the reduced ability to repair DNA damage and the consequent accumulation of unreplicable or lethally mutant chromosomes. In procaryotes the mechanism for the proper segregation of chromosomes into daughter cells is unknown, but it may involve recombination functions to separate interconnected DNA molecules into free monomers. Proper segregation of the *E. coli* P1 prophage, which replicates autonomously of the chromosome, depends upon the phage-encoded Cre recombination function acting at the *lox* site (for a review, see reference 254). P1 dimers, formed by homologous recombination between monomers or by replication, are separated into monomers by Cre; the monomers can then segregate into the daughter cells. Proper P1 prophage and CloDF13 plasmid segregation also depends upon the *E. coli* RecBCD enzyme (5, 93). It is unknown whether similar mechanisms aid chromosomal segregation.

(iii) Phages frequently utilize host functions for their own growth. For many phages, recombination plays an important role in their replication (for reviews, see references 181 and 236). It is thus not surprising that the growth of these phages, or that of their recombination-deficient mutants, is reduced or abolished in recombination-deficient bacterial hosts. Phage λ provides a good example (see subsection, "Recombination Genes and Enzymes of Phage λ").

The *recA* and *recBCD* functions of *E. coli* illustrate well how recombination-associated functions played an important role in identifying their enzymatic activities (Table 1).

RecA Protein

recA mutants of *E. coli* were first isolated by Clark and Margulies (31) by screening mutagenized colonies of an F^- culture for the inability to produce selectable recombinants after mating with an Hfr strain. Subsequent analysis showed that the *recA* mutants are highly pleiotropic. Unlike their wild-type parent, they are defective in transduction, highly sensitive to UV and X rays, nonmutable by UV, incapable of reactivating UV-inactivated phages, and non-UV inducible for phage λ lytic growth. The ability of UV (and other DNA-damaging agents) to induce multiple metabolic changes led to the SOS hypothesis, namely, that DNA damage induces the synthesis of enzymes needed for repair of the damages and that this induction requires RecA protein (91, 207, 299). This hypothesis also accounted for the massive synthesis of a protein, "protein X," after UV irradiation of wild-type, but not *recA* mutant, cells (92). Soon thereaf-

TABLE 1. *E. coli* genes affecting recombination and repair

Gene[a]	Position on chromosome (min)	Biochemical activity	Requirement for recombination[b]		Requirement for repair[c]		LexA controlled[d]
			WT	*recBC sbcB* (*C*)	WT	*recBC sbcB* (*C*)	
recA	58	RecA protein	+	+	+	+	+
recB	61	RecBCD enzyme subunit	+	−	+	−	−
recC	61	RecBCD enzyme subunit	+	−	+	−	−
recD	61	RecBCD enzyme subunit	−[e]	−	−	−	−
recE	30	ExoVIII	−	−[f]	−	−[f]	
recF	83	Unknown	−	+	+	+	−
recJ	63	Unknown	−	+	−	+	
recN	57	Unknown	−	+	−	+	+
recO	56	Unknown	−	+	+	+	
recQ	86	Unknown	−	+	−	+	+
ruv	41	Unknown	−	+	+	+	+
sbcA	30	Regulation of *recE*	−	−[g]	−	−[g]	
sbcB	44	ExoI	−	−[h]	−	−[h]	
sbcC	9	Unknown	−	−[h]	−	−[h]	
ssb	92	SSB	+		+		+
lexA	92	Repressor of the LexA SOS regulon	−	+[i]			+
polA	87	DNA PolI	+		+		
lig	52	DNA ligase	+		+		
gyrA	48	DNA gyrase subunit	+				
gyrB	83	DNA gyrase subunit	+				
uvrD	85	DNA helicase II	?[j]	?[j]	+		
topA	28	DNA TopoI		+	+		
dam	74	DNA adenine methylase	−[e]				
dcm	43	DNA cytosine methylase	−[e]				
mutH	61	Hemimethylated 5′ GATC 3′ binding protein	−[e]				
mutL	95	Unknown	−[e]				
mutS	59	DNA base mismatch binding protein	−[e]				
dut	82	Deoxyuridine triphosphatase	−[e]				

[a] The apparently missing *rec* genes are accounted for as follows. The single *recG* and *recH* alleles (257) appear to be a *recA* alleles (Clark, personal communication). *recI* and *recP* have not been assigned to any mutations. The single *recK* mutation (*rec-149*) (101) has not been studied further, except that it has been confirmed to differ from *recJ* (Clark, personal communication). *recL* (101) is synonymous with *uvrD*, which codes for DNA helicase II (168, 187, 230) (see subsection, "Other Recombination-Promoting Functions"). *recM* mutations (101) are weak alleles of *recJ* (Clark, personal communication).

[b] +, Gene function required for recombination in at least one mode of diploidy in wild-type cells (WT) or *recBC sbcB*(*C*) mutants; −, not required.

[c] +, Gene function required for efficient recovery from exposure to DNA-damaging agents, such as UV or mitomycin C, in wild-type cells (WT) or in *recBC sbcB*(*C*) mutants; −, not required.

[d] +, Gene is repressed by LexA or its expression is induced by exposure to DNA-damaging agents, such as UV or mitomycin C; −, not controlled by LexA or not induced by DNA-damaging agents.

[e] Recombination is altered; see subsections "RecBCD Enzyme and Its Interaction with Chi Sites" and "Correction of Mismatches in DNA."

[f] + in *recBC sbcA* mutants.

[g] Mutation in *sbcA* is required for recombination and repair proficiency in *recBC* mutants by the RecE pathway.

[h] Mutations in *sbcB* and *sbcC* are required for recombination and repair proficiency in *recBC* mutants by the RecF pathway.

[i] *lexA*(Ind$^-$) mutants in the *recBC sbcB*(*C*) background are recombination deficient.

[j] Uncertain; see subsection, "Other Recombination-Promoting Functions."

ter, the *recA* gene was cloned into phage λ (174), and protein X was identified as RecA protein (53, 90, 150, 173).

Finding a biochemical activity of RecA protein came from the study of one of the *recA*-associated phenotypes: the failure of λ prophages to be induced for lytic growth by UV or mitomycin C, two DNA-damaging agents. Roberts and Roberts (209) followed the fate of the λ cI repressor in *recA*$^+$ cells by radioactively labeling the cell proteins, extracting them before and after mitomycin C treatment, and fractionating by gel electrophoresis the proteins precipitated with antirepressor antibodies. Remarkably, the repressor was cleaved into two pieces at about the same time that phage lytic growth began (about 20 min after mitomycin C treatment). Cleavage occurred in wild-type cells but not in *recA* mutants. Similarily, extracts of wild-type but not *recA* mutant cells also cleaved exogenously added repressor (210). This cell-free activity allowed purification of the RecA protein, which was found to require adenosine 5′-triphosphate (ATP) and polynucleotide for stimulation of repressor cleavage. Ogawa et al. (188) found that the purified protein had adenosine triphosphatase activity dependent upon ssDNA.

Since the adenosine triphosphatase activity of RecA protein is dependent upon ssDNA, it seemed likely that the protein might promote reactions of DNA. This suspicion was quickly verified in several labs, which found that RecA protein promoted a variety of ATP-dependent interactions of homologous DNA molecules. These interactions, diagrammed in Fig. 2, include the annealing of complementary ssDNA, the formation of D loops between linear ssDNA and circular dsDNA, the conversion of linear dsDNA and circular ssDNA to linear ssDNA and circular dsDNA, and the formation of a single-stranded crossed connection (a "Holliday" junction) between two linear dsDNA molecules, at least one of which has a ssDNA tail.

Three features of the DNA substrates appear to be re-

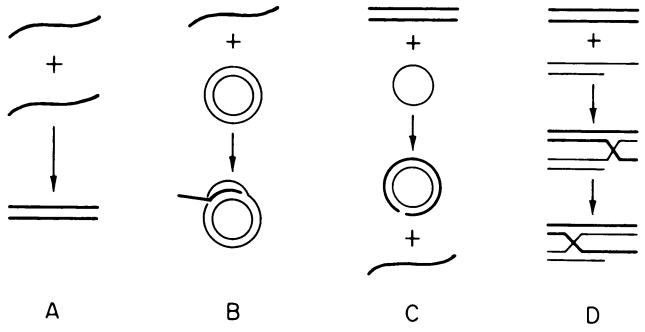

FIG. 2. Representative reactions of DNA promoted by RecA protein. Each line represents one strand of DNA. (A) Annealing of complementary ssDNA to form dsDNA. (B) D-loop formation from linear ssDNA and circular dsDNA. (C) Conversion of linear dsDNA and circular ssDNA into nicked circular dsDNA and linear ssDNA. (D) Reaction of two linear dsDNA molecules, one of which has an ssDNA tail. This tail displaces one strand of the fully dsDNA; the latter strand subsequently pairs with the complement of the tailed strand to form the cross-stranded structure designated the Holliday junction. Continued reciprocal strand transfer results in movement of the Holliday junction, designated branch migration. The reactions diagrammed here are facilitated by appropriate concentrations of SSB protein. (Modified from reference 236.)

quired for the formation of stable products in RecA protein-promoted reactions: extensive nucleotide sequence homology among the interacting DNAs, a region of ssDNA on at least one of the substrates, and a free DNA end on at least one of the substrates. (The last requirement is obviated when DNA topoisomerase I is included in the reactions.) Furthermore, all three features must be at homologous positions on the two substrates. These properties will be important in the discussion of mechanisms of recombination (see subsection, "Models of Recombination"). Two reviews discuss the enzymatic activities and structural features of RecA protein in detail; references to the extensive literature on RecA protein can be found in them (40, 204).

The extent of nucleotide sequence homology required for efficient recombination appears to be about 40 to 50 bp. This estimate comes from studies with both purified RecA protein and intact cells. Gonda and Radding (83) found that molecules with as little as 151 bp of terminal homology were efficiently synapsed by RecA protein, while those with 30 bp were not. Four groups have estimated that recombination in *E. coli* requires a minimum of about 40 to 50 bp. These groups studied recombination between phage T4 or λ and derivatives of plasmid pBR322, containing inserted DNA fragments with overlapping homologies of different lengths. The inserts contained fragments of the T4 *rII* region (234), the human insulin gene (282), a mouse immunoglobulin gene (223), or λ (115). In the last three studies the recombination events were catalyzed primarily by the *E. coli* RecBCD pathway, which requires RecA protein (see subsection, "Recombinational Pathways" for a description of this pathway), while in the first study they were catalyzed by T4-encoded functions, including the RecA proteinlike UvsX protein (303).

The requirement for ssDNA in RecA protein-promoted reactions is directly related to the mechanism by which RecA protein catalyzes synapsis between homologous DNA molecules. RecA protein polymerizes on ssDNA at a density of one RecA protein monomer per three to five nucleotides. The coated ssDNA then repeatedly binds to dsDNA and dissociates until regions of extensive (>~50 bp) homology synapse. If a DNA end is available, the ssDNA wraps around its complement, forming hybrid DNA (hDNA), while the other strand of the dsDNA is displaced. The strand transfer reaction continues, with the elongation of the hDNA. If both molecules are dsDNA and linear and one has an ssDNA tail (Fig. 2D), a second strand transfer reaction can be initiated, with the formation of a cross-stranded structure. This structure is commonly called a Holliday junction, proposed by Holliday (99) to be an intermediate in recombination.

The strand transfer reaction of RecA protein has a preferred polarity with respect to the DNA substrates. Two types of reactions have indicated this polarity. The reaction between a linear dsDNA and a circular ssDNA (Fig. 2C) forms stable hDNA only if the 3′ end on the dsDNA is homologous with, and complementary to, the circular ssDNA (39, 110, 283). Similarly, in the presence of SSB protein, the reaction between a linear ssDNA and a circular dsDNA (Fig. 2B) forms stable hDNA only if the 3′ end on the ssDNA is homologous with the circular dsDNA (122). Although there are alternative ways to define the polarity of the strand transfer reaction, his view states that RecA protein requires a free 3′ homologous end on either the ssDNA or the dsDNA molecule for stable strand transfer to occur (122).

RecA-like proteins appear to be widely distributed in bacteria. DNA clones complementing *E. coli recA* mutants have been isolated from *Salmonella typhimurium* (197), *Proteus mirabilis* (284), *Shigella flexneri*, *Proteus vulgaris*, *Erwinia carotovora* (113), *Vibrio cholorae* (81), *Pseudomonas aeruginosa* (189), *Rhizobium meliloti* (13), and *Neisseria gonorrhoeae* (125). The RecE protein of *B. subtilis* is similar to the *E. coli* RecA protein (157). A requirement for these proteins in recombination is shown in some cases by the recombination deficiency of mutants lacking the proteins. The proteins appear to be remarkably conserved throughout these species: they have similar molecular weights (about 40,000) and in some cases have been demonstrated to induce the SOS response when cloned into *E. coli recA* mutants. These common features of RecA protein support the view that the mechanisms of recombination are similar in diverse bacteria.

RecBCD Enzyme and Its Interaction with Chi Sites

***recBCD* genes and multiple activities of the RecBCD enzyme.** In their screening for recombination-deficient mutants of *E. coli*, Clark and Margulies (31) also found *recB* and *recC* mutants. Similar mutants were found by screening for X-ray sensitivity (102); some of the X-ray-sensitive mutants were found to be recombination deficient (52). (*recD* mutants are discussed later in this section.) Although *recB* and *recC* mutants have indistinguishable phenotypes, they are distinguishable from *recA* mutants by several criteria. (i) *recB* and *recC* mutations map close to each other between *thyA* and *argA* (51), whereas *recA* mutations map about 3 min away (293). *recB* and *recC* mutations form two well-behaved complementation groups (294), while *recA* mutations form a single, third group (293). (Since *recB* and *recC* [single and double] null mutants have indistinguishable phenotypes, "*recBC*" mutants is used to indicate *recB*, *recC*, or *recB recC* mutants.) (ii) *recBC* mutants have a higher residual level of recombination (10^{-1} to 10^{-3} relative to wild type) than *recA* mutants ($<10^{-4}$). The degree of residual recombination in *recBC* mutants depends upon the type of cross examined (see next section). (iii) *recBC* mutants are less

sensitive to UV and X rays than *recA* mutants are (52). (iv) *recBC* mutants manifest the "cautious degradation" phenotype, while *recA* mutants manifest the "reckless degradation" phenotype (292): when the DNAs of these mutant cells are labeled and the cells are then irradiated with UV, about 75% of the DNA of *recA* mutants is rendered acid soluble after about 2 h, while only about 5% of the DNA of *recBC* mutants becomes soluble.

This last behavior suggested that the *recB* and *recC* genes might control the synthesis of a single nuclease. As expected from this view, *recA recB* (or *recC*) double mutants manifest the cautious degradation phenotype. The view was confirmed by Buttin and Wright (21), who found that extracts of *recBC* mutant cells contained about five times less nuclease activity on linear dsDNA than extracts of wild-type cells. Surprisingly, the nuclease activity required ATP even though the reaction is exergonic. Other laboratories made similar reports at about the same time (9, 190). An endonuclease activity of the enzyme, active on circular ssDNA, but not circular dsDNA, was found by Goldmark and Linn (82). The enzyme was designated ExoV (300) or frequently RecBC enzyme. As discussed later in this section, the discovery (3) of a third gene, *recD*, coding for part of the enzyme led to the designation RecBCD enzyme, which I use in this review.

ATP-dependent deoxyribonucleases with properties similar to those of the *E. coli* RecBCD enzyme have been found in a variety of bacteria, both gram negative and gram positive (for a review, see reference 268). The first reported was from *Micrococcus luteus* (275). Early studies on the mechanism of the enzyme's action were conducted concurrently by H. O. Smith and his colleagues, working with the enzyme from *H. influenzae*, and by S. Linn and his colleagues, working with the *E. coli* enzyme; the two enzymes are similar.

In addition to nuclease and adenosine triphosphatase activities, the enzyme also has a DNA unwinding activity. Brief reaction of the enzyme with linear dsDNA produces ssDNA several thousand nucleotides long, as well as dsDNA with long ssDNA tails (69, 165). These reaction intermediates, detected in the electron microscope or by sucrose gradient centrifugation, are converted to oligonucleotides (about 5 nucleotides long) by the nuclease activities of the enzyme. The inhibition of the nuclease, but not unwinding, activities of the enzyme by Ca^{2+} ions (211) greatly facilitated further characterization of the unwinding reaction. After brief (~30-s) reaction and fixation of the enzyme on the DNA with glutaraldehyde, two DNA forms are seen in the electron microscope (264, 269): loop tails and twin loops (Fig. 3). Both the distance of the loops from the initial DNA end and the size of the loops increase at a constant rate. These observations led to a model (see Fig. 3 for details) in which loop tails are percursors to twin loops. This supposition is supported by the observation that increasing concentrations of ssDNA-binding protein (SSB) in the reaction mixture lead to increasing frequencies of loop tails at the expense of twin loops (265). Only linear dsDNA molecules with flush or nearly flush ends are unwound by the enzyme: circular dsDNA molecules, whether supercoiled, nicked, or containing gaps ranging from 10 to 774 nucleotides, or linear molecules with ssDNA tails of >~30 nucleotides are not detectably unwound (266). This result is in accord with the ability of the enzyme to bind rapidly and tightly to linear dsDNA but only feebly to circular DNA (290). The nuclease activity may, however, convert the gapped and linear molecules into unwindable substrates. The ssDNA produced by the RecBCD enzyme unwinding may be the primary substrate for RecA protein-promoted synapsis (see subsection, "Models of Recombination").

Although our understanding of the enzyme's action is still not complete, the following (admittedly simplified) view may be useful in summarizing its activities. In this view, the enzyme binds to the ends of dsDNA and unwinds it (Fig. 3). During this unwinding, it makes occasional nicks, producing long ssDNA fragments and dsDNA with ssDNA tails. After exiting the DNA molecule, the enzyme (or more likely a second enzyme molecule) digests the ssDNA with its ssDNA exonuclease activity, while another enzyme molecule unwinds the remaining dsDNA (after digesting any ssDNA tails). In this view the ssDNA endonuclease activity, which is much weaker than the approximately equally active ssDNA and dsDNA exonuclease activities, is an aberrant manifestation of the nicking-during-unwinding activity. Furthermore, the dsDNA exonuclease activity is a combination of nicking-during-unwinding plus ssDNA exonuclease activities.

The multiple activities of RecBCD enzyme have made it difficult to determine the mechanistic role(s) that the enzyme plays in recombination. Special classes of mutants altered in one or another aspect of the enzyme have, however, helped to determine the roles of its various activities.

The *recD* gene was revealed by the study of a special class of mutants lacking the RecBCD enzyme nuclease activity but retaining recombination proficiency, resistance to DNA-damaging agents, and high cell viability (3, 26). By genetic analyses, these mutations fall into two classes, now designated *recD* and *recC*‡. These two classes respectively lack the activities of the α and β subunits which had been obtained from RecBCD enzyme by dissociation in high salt and separation by column chromatography (147). Further analysis showed that the *recD* gene encodes the ~60-kilodalton (kDa) polypeptide of the α subunit, while the *recB* and *recC* genes encode the ~130- and ~120-kDa polypeptides of the β subunit (3). From the nucleotide sequence of the *thyA-argA* region (62–64), calculated molecular weights of the three polypeptides are 133,193 (RecB), 128,860 (RecC), and 66,973 (RecD). The nuclease-deficient recombination-proficient phenotype is conferred by null mutations in *recD* (such as nonsense, deletion, and insertion [14]) and by special point mutations in *recC*. This result suggests that the RecD polypeptide, and its proper interaction with the RecC polypeptide, is required for the nuclease activity.

The recombination proficiency of *recD* (and *recC*‡) mutants is not completely understood. It was hypothesized that these mutants retain the DNA unwinding activity of RecBCD enzyme, since they are inducible for SOS functions by nalidixic acid (27), but the unwinding activity has not been directly detected in repeated attempts (A. F. Taylor, personal communication). The *recD* and *recC*‡ mutants may lack both nuclease and unwinding activities; some as yet unidentified activity of RecBCD enzyme maintained in these mutants may be responsible for the recombination proficiency.

An additional phenotype of *recD* and *recC*‡ mutants implies that the wild-type RecBCD enzyme inhibits recombination (in the absence of Chi-stimulating sites described in the next section). Recombination between close markers in λ vegetative crosses is 5- to 10-fold more frequent in *recD* and *recC*‡ mutants than in *recBCD*$^+$ cells. This hyper-Rec phenotype is recessive to wild type, which indicates that the wild-type enzyme inhibits recombination (26). This inhibitory activity may be the nuclease activity, since in each case

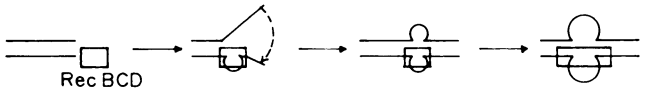

FIG. 3. Model for unwinding of DNA by RecBCD enzyme. The enzyme (rectangle) binds to a DNA end and brings one strand into its "front" and lets the strand out its "back" with the formation of a growing ssDNA loop. The other strand is displaced as an ssDNA tail which subsequently anneals to its complement, forming dsDNA and a second loop complementary to the first. As the enzyme continues unidirectionally along the DNA, the loops move and grow in size. (Modified from reference 264.)

examined inactivation of the nuclease relieves the inhibition. Since *recBC* null mutants are recombination deficient, the wild-type enzyme must both promote and inhibit recombination. Thus, the production of recombinants by RecBCD enzyme is governed by the balance between its two actions. This outcome obviously complicates the issue of the recombination proficiency of the *recD* and *recC‡* mutants.

Interaction of RecBCD enzyme with Chi sites. Chi sites enhance homologous recombination near their location on the chromosome. Their genetic properties and interaction with RecBCD enzyme are only briefly reviewed here (for more extensive reviews, see references 237, 242, and 245; G. R. Smith, *in* K. B. Low, ed., *The Recombination of Genetic Material*, in press).

Chi sites were first noted as mutations enhancing the growth of λ *red gam* mutants (97), whose DNA packaging depends upon recombination to form dimers (248) (see below for a discussion of λ mutants and the replication and recombination of λ). Although wild-type λ contains no fully active Chi site, mutations creating Chi occur spontaneously at four identified loci in λ (246). On the other hand, the *E. coli* chromosome contains an estimated 10^3 Chi sites, corresponding to one per 5 kb on the average (60, 166). In each case analyzed, the active Chi site contains the nucleotide sequence 5′ G-C-T-G-G-T-G-G 3′, and mutations creating or inactivating chi occur within that octamer (239). Recombination enhancement (Chi activity) is maximal at the Chi site and diminishes to the left of Chi approximately exponentially (by a factor of about 2 each 2.5 kb); enhancement to the right of Chi is less than that to the left (54, 133, 246, 251). Inversion of a Chi site in λ largely inactivates the site (60). This result implies that Chi interacts with another site in λ; this second site is *cos*, the site on the replicative form of λ which is cut at the initiation of packaging (117). Chi can also be activated by an intracellular restriction enzyme (252). These double-strand cuts allow RecBCD enzyme to enter the λ chromosome, as demonstrated by the following evidence.

The first suggestion of an interaction of Chi sites with RecBCD enzyme came from the observations that Chi stimulates the RecBCD pathway of recombination, but not the RecE, RecF, or λ Red pathways (77, 250). (See subsection, "Recombinational Pathways" for a discussion of these pathways.) More direct evidence came from the study of special *recB* and *recC* mutants, designated TexA and RecC*, with reduced or undetectable Chi activity, though they are still largely proficient for recombination by the RecBCD pathway (164, 219). The Tex mutants were isolated as mutants with enhanced frequency of excision of transposon Tn*10*; the TexA mutations were mapped and complemented as *recB* and *recC* mutations and were subsequently observed to have reduced Chi activity. The RecC* mutants were isolated from a *recC* missense mutant as pseudorevertants that had regained resistance to a DNA-damaging agent and recombination proficiency, but had not regained Chi activity. The pseudoreverting mutations map and complement as *recC* mutations, suggesting that the RecC polypeptide (or perhaps the intact RecBCD enzyme) directly interacts with Chi. The nuclease activity of RecBCD enzyme is required for Chi activity, since the *RecD* and *RecC‡* mutants discussed earlier in this section have no detectable chi activity, although they are recombination proficient (26).

As predicted from these observations, purified RecBCD enzyme cuts DNA containing Chi more efficiently than non-Chi DNA (198). Cutting occurs on one strand, that containing the Chi sequence 5′ G-C-T-G-G-T-G-G 3′, a few nucleotides to its 3′ side (263). Cutting occurs only during DNA unwinding and only as the enzyme is unwinding from right to left (as Chi is written here) (263). This cutting is an essential part of Chi's stimulation of recombination: mutations (TexA, RecC*, RecC‡, and RecD) that reduce or abolish Chi genetic activity correspondingly reduce or abolish Chi cutting (198). Similarly, single-base-pair mutations in the Chi site coordinately reduce or abolish Chi cutting and Chi genetic activity (28, 29). The oriented entry of RecBCD enzyme into the right end of λ DNA (due to blockage of the left end by λ prohead) coupled with the enzyme's orientation dependence of cutting at Chi can account for the orientation dependence of Chi's stimulation of recombination in λ (119, 263). The ssDNA tail extending from Chi produced by RecBCD enzyme as it unwinds DNA is proposed as an efficient substrate for D-loop formation by the RecA and SSB proteins (see subsection, "Models of Recombination").

All RecBCD enzyme-promoted recombination may require Chi or Chi-like sites. Wild-type λ lacks Chi and recombines at about 20% the rate of λ with one Chi site (133). This residual recombination can be quantitatively accounted for by the partial activity of sequences in λ differing from Chi at a single base pair (28, 29). If it were possible to remove all of these Chi-like sequences from λ, this phage would be predicted not to recombine by the RecBCD pathway.

Recombination in the Absence of RecBCD Enzyme: *sbcA*, *-B*, and *-C* Suppressors

The higher residual levels of recombination following conjugation in *recBC* null mutants than in *recA* mutants suggested that *E. coli* has at least one other low-level pathway of recombination dependent upon RecA protein but independent of RecBCD enzyme. This view was substantiated by the isolation of mutations increasing the activity of these pathways and thereby restoring recombination proficiency to *recBC* mutants. These suppressor mutations, favorite objects for geneticists' study, are designated *sbc* (for suppressor of *recBC*) at loci *A*, *B*, etc.

***sbcA*.** Clark and his colleagues isolated *sbcA* and *sbcB* mutants as mitomycin C-resistant pseudorevertants of a *recB21 recC22* double mutant (so that true revertants would be unlikely). The first group, *sbcA*, has regained dsDNA exonuclease activity, as measured in cell-free extracts, but this activity is ATP independent (10). The suppression of the recombination deficiency in *recBC* mutants by the *sbcA* locus was also found in a separate line of experiments. Certain F^- *recBC* mutants are proficient for recombination when mated with certain Hfr strains (160, 161): the special Hfr strains are those that inject *sbcA* as an early marker, and the special F^- *recBC* mutants are those that cannot give rise to the *sbcA* mutants. These initially perplexing observations

are now understood as follows. Certain *E. coli* K-12 strains, those that do give rise to *sbcA* mutants, contain a cryptic prophage designated rac (for recombination activation) that can be turned on to produce recombination proficiency either by mutation (*sbcA*) or by zygotic induction upon entry into an F^- strain lacking the rac prophage. The rac prophage resembles the λ prophage in gene organization and shares limited nucleotide sequence homology with λ (111). In fact, the rac prophage had been detected in a third line of experiments: very rare pseudorevertants of λ *red gam* mutants arise during growth in certain *E. coli* hosts (those containing rac) but not in others (85). These λ pseudorevertants have incorporated part of the rac prophage and produce an ATP-independent nuclease during lytic growth. (See below for a further description.)

The rac-encoded nuclease, designated exonuclease VIII (ExoVIII), resembles the λ exonuclease in its mode of action (78, 108, 109, 130). ExoVIII digests one strand of dsDNA, starting at its 5′ end, to 5′-mononucleotides. The enzyme has a marked preference for dsDNA ends, digesting nicked or gapped DNA only slowly. As a result of its digestion, long ssDNA molecules are produced, either free or attached to dsDNA. In the latter case the ssDNA tail has a free 3′ end. ExoVIII differs from λ exonuclease in molecular weight: the former is composed of four (or perhaps three) identical ~140-kDa polypeptides (108), while the latter contains two 24-kDa polypeptides (148). ExoVIII and λ exonuclease, like RecBCD enzyme, produce 3′ ends from linear dsDNA; the former enzymes accomplish this by exonucleolytic degradation, while RecBCD enzyme does so by DNA unwinding.

The rac prophage gene coding for ExoVIII, *recE*, was identified by mutations that return *recBC sbcA* mutants to recombination deficiency (79). *recE* is located near *sbcA* (152), which evidently controls *recE* expression. The molecular nature of the *sbcA* mutations is unclear, however. *sbcA* mutations do not simply inactivate the rac prophage repressor, since they do not lead to excision of rac while zygotic induction of rac does (57). DNA hybridization analyses have revealed a variety of structural alterations, apparent point mutations, deletions, and inversions, associated with *sbcA* mutations (111, 112, 297). Some of the *sbcA* mutations may fuse *recE* to another gene, or they may activate *recE* by altering transcriptional and translational control sites.

sbcB. A second group of suppressors of *recBC*, designated *sbcB*, was isolated from a strain lacking the rac prophage (129). Like *sbcA* mutants, they were isolated on the basis of a recombination-associated phenotype, mitomycin C resistance. Unlike *sbcA* mutants, which express a new nuclease activity, *sbcB* mutants are deficient in a nuclease activity, ExoI. ExoI digests ssDNA from the 3′ end, releasing 5′-mononucleotides (139). Clark (30) hypothesized that ExoI destroys a DNA structure that can be converted in *recBC sbcB* mutants (i.e., $ExoV^-$ $ExoI^-$) to a complete recombinant. ExoI-deficient mutants, designated *xon*, were also isolated by screening mutagenized clones with a rapid nuclease assay (301). *xon* and *sbcB* are thought to be allelic, since both sets of mutations inactivate ExoI. Some *xon* mutants have the *sbcB* recombination-proficient phenotype, while others, however, do not; but both *sbcB* and *xon* mutations suppress, to varying degrees, the UV sensitivity of *recBC* mutants. Possibly, *xon* mutants that do not suppress the recombination deficiency retain low levels of destructive ExoI. In agreement with this view, deletion of the *xon* gene, by the phage P2 site-specific recombination event termed eduction, results in the *sbcB* phenotype (270). Alternatively, the recombination-proficient strains might have contained unsuspected *sbcC* mutations, described next.

sbcC. Subsequent studies showed that *sbcB* mutations alone are insufficient to restore recombination proficiency to *recBC* mutants; mutations at another locus, *sbcC*, are also required (154). *recBC sbcB* mutant cultures usually contain *sbcC* mutants, presumably because suppression of the partial inviability of *recBC* mutants requires both *sbcB* and *sbcC*. Thus, previous studies with "*recBC sbcB*" mutants probably used *recBC sbcBC* mutants. [In this review, the designation *sbcB(C)* is used for mutants likely to contain both mutations.] The *sbcC* gene maps near *lac*, distant from other known genes influencing recombination (154), but the biochemical function of the *sbcC* product is unknown.

Functions Required for Recombination in *sbc*-Suppressed Strains: RecE, -F, -J, -N, -O, and -Q and Ruv

To determine the gene functions required for recombination in *recBC sbcA* [or *sbcB(C)*] mutants, recombination-deficient derivatives of these strains were obtained. These derivatives are defective in one or another of the genes listed here. Two of the genes (*recQ* and *ruv*) were identified initially in thymineless death-resistant or UV-sensitive mutants, which were then tested for recombination proficiency. In only one case (*recE*) is the enzymatic function known.

recE. As mentioned in the discussion of *sbcA*, *recE* is the gene coding for ExoVIII. *recE* mutants were obtained by screening mutagenized clones of a *recBC sbcA* mutant for failure to recombine with an Hfr strain to produce prototrophs (79). The mutations map near *sbcA* and result in undetectable levels of ExoVIII.

recF. *recF* mutants were obtained from a *recBC sbcB(C)* strain by screening as for *recE* mutants (101). The mutations map between *dnaN* and *gyrB* (208). Nucleotide sequence analysis of this region revealed an open reading frame of 357 amino acids, overlapping *dnaN* by one nucleotide and ending 28 nucleotides before the *gyrB* coding region (16). This open reading frame is very likely the *recF* gene since Tn*3* insertions into it produce the RecF phenotype. By standard cloning and expression analysis, no polypeptide has been detected from this open reading frame, but fusion of it to strong promoters (λ p_L and p_R) results in low-level expression of a 40-kDa polypeptide. The reason for the limited expression is not clear.

To date, no activity of the RecF function has been identified. The location of *recF* between *dnaN* and *gyrB* raises the possibility that its function is to control the expression of one or both of these genes or to interact with their products. *dnaN* codes for the β subunit of DNA polymerase III (20), while *gyrB* codes for a subunit of DNA gyrase (76). A hint about the RecF function may come from special *recA* mutations, designated *srfA*, that suppress all *recF* mutations tested (278, 279). Since the suppression is not allele specific, these *recA* mutations presumably bypass the normal RecF function. In accord with this view, one of the mutations, *recA803*, which changes Ilv-37 to Met−37 in the RecA protein, is dominant (A. J. Clark, personal communication). RecF function might modify RecA protein activity in either strand transfer or regulation of gene expression (see subsection, "Regulation of Expression of Recombination Genes").

recJ. Mutations in *recJ* were obtained in the search that produced *recF* mutants (101). The mutations are located near *serA* (159), and the *recJ* gene product is a polypeptide of about 53 kDa (159a). The activity of the gene product is unknown.

recN. Lloyd et al. (156) isolated a set of Mu d(*lac bla*) insertion mutations that resulted in increased β-galactosidase levels after exposure to DNA-damaging agents and that rendered a *recBC sbcB(C)* mutant recombination deficient. One of these mutations defined a new gene, *recN*, located near *tyrA* (195). Additional *recN* mutations were isolated by Kolodner et al. (121) by screening mutants with Tn*5* insertions cotransducible with *tyrA* for recombination deficiency in Hfr crosses and for UV and mitomycin C sensitivity. The *recN* gene has been cloned (195), and its product is a 60-kDa polypeptide (cited in reference 196), but the activity is unknown.

recO. The *recO* gene is defined by one mutation, a Tn*5* insertion, isolated in the screen for *recN*::Tn*5* mutations (121). The gene order is *recO tyrA recN*, but there is no information on the *recO* gene product.

recQ. The *recQ* gene is defined by one mutation, obtained by enriching a nitrosoguanidine-treated culture for mutants resistant to thymineless death (184). In a *recBC sbcB(C)* background, the *recQ* mutation lowers recombination proficiency about 100-fold in Hfr crosses. The *recQ* gene is located between *corA* and *metE* and its nucleotide sequence has been determined (105); the deduced polypeptide has a molecular weight of 68,350. The relation between the two phenotypes of the *recQ* mutation, resistance to thymineless death and recombination deficiency, is unclear.

ruv. *ruv* mutants were first isolated on the basis of their UV sensitivity (192). The *ruv* gene was later shown to be inducible by DNA-damaging agents (226). Lloyd et al. (153) found that *ruv* mutants are recombination deficient in Hfr crosses in *recBC sbcB(C)* backgrounds. The *ruv* gene has been cloned (227), but the nature of its product has not been reported.

Recombination Genes and Enzymes of Phage λ

The ability of phage λ to recombine in *recA* mutants of *E. coli* (18) suggested that λ encodes its own homologous recombination functions. This suggestion was confirmed by the finding of λ mutants deficient in recombination (when growing in *E. coli recA* mutants). Franklin (68) noted that φ80-λ hybrids with deletions in the central genome region were recombination deficient. Echols and Gingery (50) and Signer and Weil (233) screened for recombination-deficient (Red^-) mutants of λ, utilizing the ability of λ Red^+ infecting phages to "rescue" wild-type alleles from a prophage by recombination. The *red* mutations mapped in the central genome region and seemingly formed three complementation groups with complex interactions (225). Biochemical analyses, however, showed that the *red* mutations inactivated either λ exonuclease (λ exo) or β-protein or both. The complex genetic interactions presumably result from the close physical association of the two proteins.

λ exo had previously been detected in extracts of λ-infected cells and purified (126, 148, 203). The gene for λ exo was inferred to be in the p_L (leftward) operon since a mutation (t_{11}) inactivating p_R did not abolish its synthesis (203). Since the *red* mutations were in the leftward operon region, these mutants were tested and found in some cases (*exo* mutants) to lack λ exo (225). λ β protein was initially detected as an antigenic species that copurified with λ exo but which could be separated from it (206). Testing the *red* mutants revealed that some lacked the β protein (225).

λ exo digests dsDNA from the 5′ ends, releasing 5′-mononucleotides and generating long ssDNA tails with 3′ ends extending from a dsDNA interior (148) (Fig. 4). Eventually, the enzyme produces ssDNA half the length of the initial substrate, and this ssDNA is very slowly hydrolyzed. The enzyme will not efficiently initiate at a nick or gap, but it will initiate at a branch point (Fig. 4). In the latter case, part of one strand is digested until the 3′-end tail has been assimilated, producing an unbranched nicked dsDNA molecule; digestion then stops (25). In summary, λ exo requires either a dsDNA end or a branch point for efficient action.

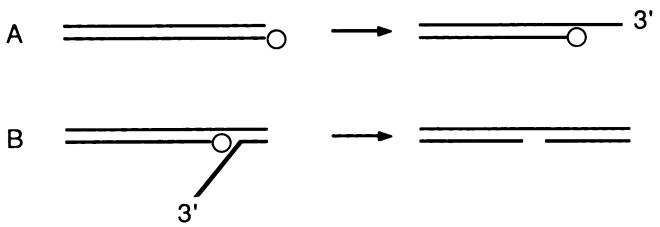

FIG. 4. Action of λ exonuclease on DNA. (A) Exonucleolytic digestion of one strand, from the 5′ end, produces dsDNA with a 3′ ssDNA tail and, eventually, ssDNA. (B) Digestion of one strand from its 5′ end at a branch point proceeds until the ssDNA tail has been assimilated. This reaction may be aided by the λ β protein. (Modified from reference 25.)

λ β protein, like RecA protein, promotes the annealing of complementary ssDNA (Fig. 2a) (116, 183). But β protein differs significantly from RecA protein: the β protein does not utilize ATP and does not promote strand transfer reactions, such as those in Fig. 2b to d. By complexing with λ exo, β protein increases the affinity of λ exo for DNA (205). The complex complementation pattern of *exo* and *bet* mutants suggests that the two proteins are intimately associated in the cell. These observations imply that the two proteins act in concert, perhaps as suggested in Fig. 4.

As in numerous organisms, recombination and replication are closely associated in λ. In brief, the replication cycle of λ proceeds as follows. (See references 70 and 236 for more complete descriptions.) Following injection, λ DNA becomes circular by annealing of the cohesive end sites (*cos*) and is initially replicated in the θ mode. Later, replication changes to the σ mode, provided the host RecBCD enzyme is inactive so that its nuclease activity does not destroy the nascent linear ends on these molecules. The λ *gam* gene product largely inactivates this nuclease activity; λ gam^+ phage can thus replicate in the σ mode, whereas λ *gam* mutants (in $recBCD^+$ hosts) are restricted to the θ mode. Since monomeric λ cannot be efficiently packaged, the monomeric circles produced by θ replication must recombine to produce packageable dimeric λ DNA. This recombination can be promoted by either the λ Red pathway or the *E. coli* RecBCD pathway; the latter is dependent upon RecA protein (see subsection, "Recombinational Pathways"). λ *red gam* double mutants produce very few progeny phage in *recA* mutant hosts, since they are locked in θ replication by active RecBCD enzyme, yet cannot recombine by either the Red or the RecBCD pathway. Pseudorevertants of λ *red gam* mutants able to grow on *E. coli recA* mutant hosts have incorporated the *recE* gene, coding for the recombination-promoting enzyme ExoVIII, as mentioned in the discussion of *sbcA*.

An additional association derives from the stimulation of replication by recombination and vice versa. Recombinational invasion of one duplex DNA molecule by the end of another is postulated to generate a replication fork (for a review, see reference 235). Replication from this fork may in turn be required to produce a complete recombinant (247,

249). A similar situation occurs in phage T4 (for a review, see reference 181). For further discussion of these associations, see the subsection, "Models of Recombination."

Other Recombination-Promoting Functions

Due to the close connections among DNA replication, repair, and recombination, some gene products initially studied for their involvement in replication or repair were subsequently found to be required for recombination.

SSB protein. The *E. coli* SSB protein was isolated on the basis of its ability to bind to ssDNA (232). The procedure was analogous to that for the isolation of the phage T4 gene *32* product (1) required for T4 replication and recombination (56, 273). The *E. coli* SSB protein is also required for replication in intact cells (88) and cell-free extracts (177). The subunit molecular weight of the protein is 19,500. SSB is a tetramer in solution but may function primarily as a higher-order oligomer or polymer due to its highly cooperative binding to ssDNA (for a review, see reference 128). By binding to ssDNA, the protein favors the unwinding of dsDNA; under certain conditions it can also promote the reassociation of ssDNA into dsDNA, presumably by removing kinetic blocks due to intrastrand base-paired regions ("hairpins") in the ssDNA. SSB protein facilitates most of the RecA-promoted reactions discussed earlier; one basis for this facilitation appears to be the coating of excess ssDNA with SSB protein, thereby allowing RecA to concentrate on the locus of strand exchange (for a review, see reference 40).

The first *E. coli ssb* mutant was isolated as an X-ray-sensitive but recombination-proficient mutant (87) and was designated *lexC113* because its phenotype was similar to that of *lexA* mutants (see next subsection). Genetic mapping showed that *lexC113* was not in *lexA* (107). The second *ssb* mutant (*ssb-1*) was isolated as a mutant temperature sensitive for DNA replication (177). Both mutations alter SSB protein, and *lexC113* was redesignated *ssb-113* (177).

Glassberg et al. (80) found that the *ssb-1* mutant had, at intermediate temperature, a fivefold reduced proficiency for recombination following P1-mediated transduction. In these crosses linkage of two markers (*purD* and *metA*) was increased from 40% (in ssb^+) to 75% (in *ssb-1*); this result is expected if recombination in the *purD-metA* interval is decreased relative to that outside this interval. At high temperature, the *ssb-1* mutation reduces by about 15-fold the frequency of inversion of an inverted repeat in phage λ, when the inversion occurs by the RecBCD pathway (54).

DNA PolI. DNA polymerase I (PolI) has both DNA-polymerizing and 5′→3′ and 3′→5′ exonuclease activities in an 109-kDa polypeptide (for a review, see reference 138). Mutants defective in the polymerase activity are viable, but radiation sensitive (43), while mutants temperature sensitive in the 5′→3′ exonuclease activity are inviable (at high temperature) (124).

Involvement of PolI in recombination was first noted by Konrad (123), who found that polymerase-deficient mutants promote recombination between duplications on the *E. coli* chromosome at elevated frequency. This hyper-Rec phenotype is thought to result from the recombinogenicity of DNA lesions (nicks, gaps, and double-strand breaks) that are more slowly repaired in certain mutants, such as *polA*, than in wild type. This phenotype provided the basis for isolating, and surveying for, hyper-Rec mutants (123) (see below).

By contrast, *polA* mutants deficient in the polymerase activity have a reduced recombination proficiency in Hfr crosses (304) and in the λ inversion assay (54); *polA* mutants deficient in the 5′→3′ exonuclease activity are as proficient as wild-type cells. Presumably, the polymerase activity of PolI is required to fill gaps remaining after strand exchanges have been completed.

The hyper-Rec phenotype of *polA* mutants, and *lig* mutants described next, in one mode of diploidy and their hypo-Rec phenotype in another presumably reflect a difference in the rate-limiting step of recombination in the two modes. For recombination of chromosomal duplications, production of DNA ends may be the limiting step, whereas for recombination following conjugation or during λ vegetative growth ends may be so abundant that another step is rate limiting. In the latter case, PolI deficiency reduces recombination. This view is concordant with the circularity of DNA available for recombination of chromosomal duplications and its linearity in conjugation and λ vegetative growth.

DNA ligase. DNA ligase of *E. coli* seals nicks in dsDNA at which 5′-PO_4 and 3′-OH ends are adjacent in a nicotinamide adenine dinucleotide-dependent reaction (for a review, see reference 137). The enzyme is composed of a single 75-kDa polypeptide. DNA ligases are widely distributed in bacteria, both gram negative and gram positive, and are encoded by some phages, such as T4.

E. coli mutants deficient in DNA ligase were isolated by screening mutagenized clones for reduced activity (86). A *lig* mutant temperature sensitive for growth demonstrated the essential nature of the enzyme (86). With respect to recombination, *lig* mutants parallel the *polA* polymerase-deficient mutants just described: *lig* mutants are hyper-Rec for recombination of a duplication on the *E. coli* chromosome (123) but are hypo-Rec for conjugation (304) and inversion in λ (54). Presumably, DNA ligase is required to seal the nicks left after strand exchange, but when ligase is present at a reduced level, recombinogenic lesions persist. A similar dichotomous behavior of ligase-deficient mutants (*cdc9*) of the yeast *Saccharomyces cerevisiae* has been noted; by one test they are hypo-Rec (59) and by another they are hyper-Rec (71).

DNA gyrase. DNA gyrase was discovered as a factor required for λ integrase-promoted site-specific recombination in cell-free extracts (75). The λ integration requirement is for a negatively supercoiled DNA substrate, which DNA gyrase forms from relaxed circular DNA in an ATP-dependent reaction (for a review, see reference 73). The enzyme is composed of two polypeptides, one of about 105 kDa coded by *gyrA* (formerly *nal*) and another of about 95 kDa coded by *gyrB* (formerly *cou*). The enzyme is inhibited by the antibiotics nalidixic acid (Nal) and coumermycin (Cou), and mutants resistant to these drugs are altered in the respective genes (74, 76).

A requirement for DNA gyrase in homologous recombination was first noted by Hays and Boehmer (96), who studied recombination between direct repeats in λ; the recombination event produces phages with either one copy or three copies of the repeat (or both phage types if the event is reciprocal). Production of the single-copy phage is reduced about fivefold by coumermycin in gyr^+ cells, but is not significantly reduced in a *gyrB* (Cou^r) mutant. This recombination event, measured after infection of *E. coli* λ lysogens with UV-irradiated λ duplication phage, is RecBCD independent, but RecA dependent.

DNA gyrase is also required in a RecBCD-dependent recombination event, the inversion in λ mentioned previously (54). Coumermycin and nalidixic acid separately reduce the inversion frequency 10- to 50-fold in gyr^+ cells, but

coumermycin is without significant effect in *gyrB* (Cou^r) cells. Ennis et al. (54) hypothesize that DNA gyrase is required to form a negatively supercoiled dsDNA recipient for an ssDNA invasion promoted by RecA and SSB proteins (see subsection, "Models of Recombination").

DNA TopoI. DNA topoisomerase I (TopoI) was discovered as an enzyme that converts negatively supercoiled DNA to the relaxed state (281). The enzyme contains one subunit of 110 kDa. Mutants, designated *topA*, lacking the enzyme activity were independently and nearly simultaneously found by assaying mutagenized clones of *E. coli* (255) and *S. typhimurium* (274). *S. typhimurium supX* mutants are pleiotropically altered in gene expression, growth, and DNA repair (193), and they lack TopoI (274).

topA mutants in a *recBC sbcB(C)* background have reduced proficiency for plasmid-by-plasmid recombination (121); an effect of *topA* mutations on other homologous recombination events has not, to my knowledge, been reported. TopoI allows RecA protein to form stably interwound hybrid DNA molecules when the substrates are topologically blocked, e.g., when both are circular or when one has nucleotide sequence heterologies at the ends (41). Thus, TopoI may aid recombination of circular molecules, as noted above, or the pairing of internal regions of linear molecules. For example, TopoI may allow RecA and SSB proteins to anneal a D loop with a gap in a homologous DNA molecule, thereby completing formation of a Holliday junction (see Fig. 7).

DNA helicase II. Helicase II unwinds dsDNA provided there is an adjacent ssDNA region in which the protein can initiate binding (for a review, see reference 72). Unlike the RecBCD enzyme, which unwinds DNA catalytically, DNA helicase II unwinds DNA stoichiometrically: one protein monomer of about 75 kDa is required for each 5 bp unwound. The protein binds to the strand with 5′→3′ polarity in the direction of unwinding, which proceeds at about 1,000 bp/s. Unwinding requires ATP hydrolysis, which can be induced by ssDNA binding to the enzyme.

The gene coding for DNA helicase II is *uvrD* (168, 187, 230). Mutations in this gene were obtained in a variety of ways and were previously called *recL*, *uvrD*, *uvrE*, or *mutU*. The single *recL* mutant was obtained as a derivative of a *recBC sbcB(C)* mutant deficient in recombination following conjugation (101), but the recombination deficiency may have been due to additional mutations frequently arising in the *recBC sbcB(C) recL* strains (Clark, personal communication). *uvrD* and *-E* mutants were obtained as UV-sensitive, hypermutable mutants (229). *mutU* mutants were obtained as hypermutable strains (38). The role of DNA helicase II in recombination is uncertain, since there are reports of *uvrD* mutants being hyper-Rec (61), hypo-Rec (101, 304), or unaffected (304) for recombination following conjugation. By unwinding DNA starting from an end or gap, DNA helicase II may produce a free 3′-ended tail that could be a substrate for RecA-promoted synapsis.

Additional hyper-Rec mutants. To develop a rapid screen for hyper-Rec mutants, Konrad (123) constructed an *E. coli* strain with one copy of the *lac* operon at its normal locus and another copy in phage φ80 integrated at its prophage attachment site. Nonoverlapping deletions in each copy rendered the strain Lac$^-$, but recombination between the duplicate copies could produce Lac$^+$ cells at low frequency. On indicator plates, such as MacConkey agar, the strain produces white (Lac$^-$) colonies with a few red (Lac$^+$) papillae. Konrad screened colonies from a mutagenized culture and found a large number of mutants producing a significantly higher-than-average number of papillae per colony. Further analysis of seven mutants revealed *pol*, *lig*, *dam*, and *dut* mutants among the hyper-Rec mutants. *pol* and *lig* mutants have been discussed above. *dam* mutants are deficient in DNA adenine methylase (171). Failure to properly correct DNA mismatches in *dam* mutants (see subsection, "Correction of Mismatches in Heteroduplex DNA") may result in elevated frequencies of nicks and gaps in the DNA and elevated recombination frequencies, as in *pol* and *lig* mutants (see also reference 170). *dut* mutants (also called *dnaS* or *sof*) are deficient in deoxyuridine triphosphatase, an enzyme that converts deoxyuridine 5′-triphosphate to deoxyuridine 5′-monophosphate (276). In the absence of deoxyuridine triphosphatase, deoxyuridine 5′-triphosphate levels rise and the nucleotide is aberrantly incorporated into DNA. U is removed from DNA by *N*-uracil glycosylase; a single-strand gap is formed by exonuclease III to remove the baseless sugar (267). These gaps, before their filling by PolI, are presumably the recombinogenic structures present at increased levels in *dut* mutants.

Feinstein and Low (61) isolated *E. coli* mutants producing increased frequencies of recombinants following Hfr-mediated conjugation. Their hyper-Rec mutants proved to be *mutS* and *mutL* mutants, whose products are involved in mismatch correction. Other previously isolated mutants (*mutH* and *uvrD*) altered in mismatch correction were also hyper-Rec in this test. One explanation for this hyper-Rec phenotype is the failure to correct mismatches, a process that can destroy potential recombinants. (See subsection, "Correction of Mismatches in Heteroduplex DNA" for further discussion.)

In summary, the hyper-Rec phenotypes in these cases appear to result from the increased recombinogenicity of the substrates or from the decreased destruction of recombination intermediates.

Regulation of Expression of Recombination Genes

As discussed previously, RecA protein (protein X) synthesis is induced by DNA-damaging agents (92). This and subsequent findings elucidated the LexA regulon, a set of genes repressed by LexA and whose products are required for DNA repair (among other things) (for a review, see reference 151). DNA damage leads to the formation of an inducing signal (possibly ssDNA) that activates RecA protein to promote autocleavage of the LexA repressor (149). The genes of the LexA regulon, including *recA* and *lexA* themselves, are thus induced and repair is effected. The removal of the inducing signal thereby leads to recovery of LexA-mediated repression of the regulon.

Several types of mutations in *lexA* and *recA* affect the regulation of the LexA regulon. Mutations in *lexA* inactivating the LexA repressor result in constitutive, high-level expression of the many genes in the regulon; these mutations are designated *lexA*(Def). Mutations changing LexA protein such that it is not cleaved in the presence of activated RecA protein result in lack of induction by DNA-damaging agents, and the genes are expressed at a low level; these mutations are designated *lexA*(Ind$^-$). Mutations in *recA* rendering the RecA protein activated even in the absence of DNA-damaging agents phenotypically mimic *lexA*(Def) mutations and were formerly designated *tif*. Finally, mutations rendering RecA protein nonactivatable mimic *lexA*(Ind$^-$); these mutants, formerly designated *lexB*, are noninducible for SOS functions but are recombination proficient. *recA* null mutants are recombination deficient and their LexA regulon is noninducible.

In addition to the *recA* gene, the *recN*, *recQ*, and *ruv* genes are repressed by *lexA* and are induced by DNA-damaging agents (105, 156, 226). These four gene products are required for recombination in *recBC sbcB*(*C*) mutants (see above). The *recF* gene, whose product is also required for recombination in *recBC sbcB*(*C*) mutants, does not appear to be controlled by *lexA* (quoted in reference 159); other recombination genes have not, to my knowledge, been tested. Since *recN* gene expression is strongly reduced in *lexA*(Ind$^-$) mutants (156), it is not surprising that *recBC sbcB*(*C*) *lexA*(Ind$^-$) mutants are recombination deficient (158).

The finding that four recombination genes (*recA*, *recN*, *recQ*, and *ruv*) are under LexA control led to the hypothesis that recombination proficiency in *recBC sbcB*(*C*) mutants might be inducible, either by the entry of exogenous DNA or by agents such as UV that stimulate recombination (158, 196). The hypothesis was strengthened by the finding that *recN* expression is increased by *sbcB* mutations (196). This finding suggests an alternative explanation for the recombination proficiency of *recBC sbcB*(*C*) mutants: the absence of ExoI, encoded by *sbcB*, might result in elevated expression of the LexA regulon and hence a sufficiently high level of RecA, RecN, RecQ, and Ruv proteins (and possibly others) for recombination to occur. This cannot be the sole explanation for *sbcB*(*C*) suppression, however, since *recBC lexA*(Def) mutants are recombination deficient (158). On the other hand, there may be some inducible genes not under LexA control and whose products are needed for recombination in *recBC* mutants. Thus, the hypothesis that recombination proficiency in *recBC sbcB*(*C*) mutants is inducible remains attractive but unproven.

In addition to *lexA* regulating certain *rec* genes, some *rec* genes themselves play a role in regulating *lexA*. (i) As noted above, *recA* is repressed by LexA repressor, and activated RecA protein promotes LexA repressor cleavage. (ii) RecBCD enzyme is required for induction of the LexA regulon by certain DNA-damaging agents such as nalidixic acid, but not by others such as UV (92, 175). It has been hypothesized that the DNA unwinding activity of RecBCD enzyme produces the ssDNA inducing signal that activates RecA protein, since *recC*‡ and *recD* mutants (nuclease deficient, recombination proficient) are inducible by nalidixic acid (27) and by receipt of UV-damaged DNA from an F′ donor (7). But failure to find DNA unwinding activity in the *recC*‡ and *recD* mutants renders this hypothesis uncertain (see above). (iii) RecF function is required for induction of the LexA regulon by certain agents such as UV, but not by others such as nalidixic acid (4, 175). To my knowledge, other *rec* genes have not been tested in a similar way. The complementary actions of RecBCD enzyme and RecF function suggest that there are two (or more) pathways for producing RecA-activating signal from different DNA-damaging agents. These inducing pathways may be closely related to the two recombination pathways discussed next.

MECHANISMS OF RECOMBINATION

Recombinational Pathways

Based upon his studies of *recBC* and *sbc* mutants, Clark (30) proposed the concept of recombinational pathways, analogous to pathways of small-molecule metabolism, such as those of amino acid biosynthesis. Because our knowledge of these recombinational pathways is still incomplete, their definition is not as sharp as those of small-molecule metabolism. For the latter, we know in most cases the substrates, the products, the enzymes, and the corresponding genes for each of the individual reactions in the pathway. For the recombinational pathways, we know the end product (recombinant DNA), but we often do not know whether both reciprocal recombinant types are produced or whether one or both parental types emerge in addition. The starting substrates are known (parental DNA), but we often do not know its exact form: whether there are nicks, or gaps, or ends, for example. The pathways have been named for the first discovered gene product unique to that pathway. Additional required gene products have been found, but others are likely still unknown. In some cases, we know the enzymes specified by these genes, but even here our knowledge may be incomplete, due to the multifunctional nature of some of the enzymes (e.g., RecA protein and RecBCD enzyme). Finally, the possibilities that recombinational pathways overlap (by sharing common steps) or branch (by permitting alternate outcomes of initial events) lend additional complexity. In spite of these uncertainties, the concept of recombinational pathways has been a useful way of summarizing our knowledge of diverse recombination events.

According to Clark's proposal (30), the RecBCD pathway is predominant in wild-type cells; the RecE pathway, in *recBC sbcA* cells; and the RecF pathway, in *recBC sbcB* cells. (The RecBCD pathway was initially named the RecBC pathway [30] but has been renamed to reflect the renaming of the enzyme [3].) These identifications were based upon the conjugational and transductional recombination proficiency of wild-type, *recBC sbcA*, and *recBC sbcB*(*C*) cells and upon the recombination deficiency of certain mutants. Since *recBC* mutants (in *sbcABC*$^+$ backgrounds) are recombination deficient, the *recBCD* gene product is required for recombination in wild-type cells and gives its name to the RecBCD pathway. This pathway does not require the *recE* or *recF* gene products, since these mutants (in *recBC*$^+$ backgrounds) are recombination proficient. Similarly, *recE* mutants (in *recBC sbcA* backgrounds) and *recF* mutants [in *recBC sbcB*(*C*) backgrounds] are recombination deficient, and *recE* and *recF* give their names to their respective pathways.

Clark (30) recognized that these pathways are not entirely independent, since all three require the *recA* gene product. He therefore proposed that the pathways overlap, sharing the RecA protein-catalyzed step. The strand transfer activity of RecA protein fits well with this proposal (if one assumes that RecA protein is the only protein in the cell with this activity). The ability of the RecBCD enzyme and ExoVIII to produce ssDNA with 3′-OH ends as substrate for RecA protein also fits well with the proposal that these two enzymes catalyze steps, in the RecBCD and RecE pathways, prior to the RecA protein-catalyzed step. Furthermore, the inhibitory action of ExoI in the RecF pathway is accounted for by its ability to destroy ssDNA with 3′-OH ends; presumably, ExoI acts before RecA protein (or competes with it). It should be noted, however, that none of these observations directly demonstrate the sequential order of the proposed steps. The possibility that certain recombination functions act at both early and late steps, as discussed later for RecBCD enzyme (see subsection, "Models of Recombination"), holds potential additional complexity. I return later to the question of the determination of the sequential order of recombination steps.

While these proposals are, in my opinion, still valid frameworks for understanding recombination in *E. coli*, they

require modification to account for subsequent findings. Gillen et al. (79) reported that the *recF* gene product is required for conjugational recombination in *recBC sbcA* strains; therefore, RecF is apparently required for both the RecE and RecF pathways. Subsequent studies have shown that *recJ*, *recN*, and *ruv* are also required for both pathways (159). Thus, the RecE and RecF pathways appear to overlap extensively; they may, in fact, differ only by the initial step(s). To my knowledge, the *recF*, *-J*, *-N*, *-O*, and *-Q* and *ruv* gene products are required only by the RecE and RecF pathways and not by the RecBCD pathway. Therefore, the RecBCD pathway may overlap the RecE and RecF pathways only by the RecA protein-catalyzed step. (SSB protein, PolI, DNA ligase, and DNA gyrase, functions required by the RecBCD pathway, have not, to my knowledge, been tested for roles in the RecE and RecF pathways.)

An additional complexity arose when it was found that the requirement by a certain recombinational pathway for a particular gene product depends upon the mode of diploidy during which the recombination occurs. Gillen and Clark (77) found that λ-by-λ recombination (both Red$^-$) in *recBC sbcA* strains does not require RecA protein, although that in *recBC sbcB(C)* strains does. Similarly, Fishel et al. (66) and Laban and Cohen (131) reported that plasmid-by-plasmid recombination in *recBC sbcA* strains does not require RecA protein. Perhaps the RecE pathway does not require RecA protein when both substrate molecules in the cross can replicate: both of the λ's and both of the plasmids do replicate, but only the recipient chromosome in conjugations and transductions does. λ-by-λ recombination by the λ Red pathway requires RecA protein when λ replication is blocked, but not when it is allowed (248) (see below for further discussion). In general, the requirement for a particular gene product may depend upon the structure of the substrates available; in other words, different substrates may follow different pathways.

Sequential Order of Recombination Gene Functions

The concept of a pathway of recombination implies the sequential action of recombination gene functions. In small-molecule metabolism, such as amino acid biosynthesis, the sequential order of gene functions was first indicated by cross-feeding studies: mutants blocked late in a pathway accumulate intermediates that diffuse out of the cell, enter mutants blocked at earlier steps, and are converted in these cells to the final product, thus supporting their growth. The inability of the macromolecular intermediates of recombination to move readily from one cell to another precludes this type of study. Further elucidation of small-molecule metabolism came from studies with cell-free extracts capable of synthesizing the metabolite: suspected intermediates isolated from one mutant extract can be converted to the metabolite by another mutant extract only if it is blocked at a step before that in the first extract. As noted earlier, cell-free extracts (of *Saccharomyces cerevisiae*) competent for recombination have only recently been developed (260, 262). Extracts of this type, whether from yeasts or bacteria, should be exceptionally useful in determining the order of gene functions and in determining the structure of the intermediates in recombination.

In the absence of recombinationally competent extracts, three other approaches have been used to deduce the order of gene functions. The first is a theoretical consideration based upon the enzymatic activities of known recombination functions and their plausible roles in proposed models of recombination. For example, RecA protein strand transfer activity requires ssDNA in at least part of one of the substrate DNA molecules. Therefore, a function producing ssDNA, such as a nuclease digesting one strand or a DNA unwinding enzyme, presumably precedes RecA protein action. ExoVIII and RecBCD enzyme are plausible candidates for this function. The possibility that these enzymes act at a different (or additional) step of recombination precludes a firm conclusion, however.

A second approach was developed by Birge and Low (15) based upon the possibility that ribonucleic acid polymerase could synthesize a wild-type messenger ribonucleic acid, and thus give rise to active protein, from a recombinational intermediate. They mated an *E. coli lacZ118* Hfr strain and a *lacZ36* F$^-$ strain and assayed the culture for β-galactosidase about 2 h later. Abundant enzyme was found with wild-type and *recB* or *recC* mutant F$^-$ strains, but not with *recA* mutant F$^-$ strains. They concluded that formation of the transcribable recombination product requires RecA protein but not RecBCD enzyme. If this product is an intermediate in recombination that is converted by RecBCD enzyme into an inheritable recombinant DNA molecule, then RecA protein must act before RecBCD enzyme. As noted later in this section, this conclusion can be challenged.

In an extension of this analysis, Porter et al. (200) found that in other circumstances (in λ *lac*-by-F'*lac* crosses) RecBCD enzyme is required for the production of the transcribable product. They suggested that the early requirement for RecBCD enzyme depends upon the nature of the substrates: for example, ssDNA entering in the Hfr crosses may obviate a requirement for RecBCD enzyme unwinding activity, which might be necessary with the parental dsDNA in λ *lac*-by-F'*lac* crosses. Porter and his colleagues noted that λ *lac*-by-F'*lac* crosses produced about 50 times more β-galactosidase than λ *lac* by chromosomal *lac*, even though the numbers of F'*lac* and chromosomal *lac* copies per cell are nearly equal. They found that the *oriT* site on the F'*lac* and RecBCD enzyme were necessary for the high level of β-galactosidase synthesis (221). These observations led them to propose that RecBCD enzyme enters the F'*lac* at the *oriT* site, which may be, as noted earlier, a double-strand break. This hypothesis fits well with the model of RecBCD enzyme-promoted recombination described later.

Although the transcribable DNA product revealed by these studies is clearly related to recombination (it requires heterozygous parental DNA, RecA protein, and in some circumstances RecBCD enzyme), it has not been established that this product is an intermediate in recombination. To my knowledge, there is no evidence that the transcribable product is not a side product of recombination. Recent evidence forces a reevaluation of this issue. Lloyd et al. (155) reported that in Hfr crosses with *sbcBC*$^+$ recipients formation of the transcribable DNA product requires either RecBCD enzyme or the *recF*, *recJ*, and *recO* functions; formation of complete recombinants (i.e., Lac$^+$ colony-forming units) requires RecBCD enzyme but not the *recF*, *recJ*, or *recO* functions. Evidently the transcribable product formed in the absence of RecBCD enzyme is not formed by the mechanism that forms complete recombinants. Furthermore, in the absence of *recF*, *recJ*, and *recO* functions, RecBCD enzyme appears to act early (before formation of the transcribable product), while in the presence of *recF*, *recJ*, and *recO* functions, RecBCD enzyme appears to act late.

A scheme that accounts for these findings is the following:

parental DNA $\underrightarrow{\text{A, BCD}}$ X $\underrightarrow{\text{BCD}}$ recombinant DNA

parenteral DNA $\underrightarrow{\text{A, F, J, O}}$ X $\underrightarrow{\text{?}}$ recombinant DNA

In this scheme, X is the transcribable DNA product (or is converted into it) and may be the same in the two pathways shown. Notice that RecBCD enzyme acts both early and late, but appeared in the original studies (15) to act only late because of the alternative *recA*, *-F*, *-J*, *-O*-dependent formation of X. This scheme helps to rationalize the apparent late action of RecBCD enzyme in Hfr-by-F$^-$ crosses (15) but early action in λ-by-F′ crosses (200). A model incorporating both early and late actions of RecBCD enzyme is discussed later.

A third approach to deducing the order of gene functions could use the assay for hybrid DNA formation developed by Lichten and Fox (143), described in the next section. These authors (142) found that hDNA formation requires RecA protein in λ Red$^-$-by-λ Red$^-$ crosses. Infection of other mutant hosts could sort the recombination functions into two groups: early functions required for hDNA formation and late functions not required. As with the β-galactosidase assay just discussed, it would be necessary to demonstrate that the hDNA measured is a true intermediate.

hDNA Formation during Recombination

hDNA, that with one strand from each parent, is a hallmark of break-join recombination. hDNA encompassing a site of genetic difference between the parents is called heteroduplex DNA. Both are designated hDNA, and the terms are used interchangeably except in cases of ambiguity. The existence of hDNA and its association with recombination has been determined both genetically (by the segregation of both genetic types from haploid progeny, such as phage or meiotic products) and physically (by the detection of DNA with density intermediate between that of normal [light] and isotopically labeled [heavy] DNA of the parents). The more frequent occurrence of hDNA among recombinants than in the total population or, alternatively, the more frequent occurrence of recombinants among progeny with hDNA than in the total population demonstrates the association of the two. Since its first detection in phage T2 (98), hDNA has been assumed to be an intermediate in recombination (e.g., see reference 141) and was a central feature of even the earliest break-join recombination models (e.g., see references 99, 114, and 287). The ability of RecA protein to form hDNA fortifies the role of hDNA in recombination dependent upon RecA protein.

Genetic detection of hDNA is simplest in phage (and fungal spores) that contain only one copy of dsDNA for a particular locus. Phage with hDNA for a marker influencing plaque morphology thus produce plaques with a mottled appearance, some parts appearing like plaques of one parental type and other parts appearing like the other type. In phage λ, $c\text{I}^+$ (turbid plaques) and $c\text{I}^-$ (clear plaques) are most conveniently used. Alternatively, phage from individual plaques can be analyzed in subsequent platings for traits of both alleles of, e.g., an amber mutation in an essential gene.

In organisms with two or more copies of dsDNA for a particular locus, genetic detection of hDNA is more complicated. In phages T2 and T4, the terminal redundancies in the packaged DNA molecules, if heterozygous, also can produce mottled plaques. But these can be distinguished from hDNA by the increased frequency of the hDNA types in conditions blocking DNA synthesis (e.g., treatment with fluorodeoxyuridine) (220), since replication destroys hDNA. In bacteria, which generally contain several copies of the chromosome, hDNA is more difficult to detect genetically.

hDNA was first detected physically as DNA of intermediate density ("heavy-light") produced by recombination between DNA containing heavy isotopes (of C and N) and that containing ordinary (light) isotopes (176). Bromodeoxyuridine-containing DNA has also been used as a source of "heavy" DNA, but excision-repair activities on this DNA can produce confusion. Heavy-light DNA is produced during recombination of λ in the lytic cycle (176) of *E. coli* following conjugation (228), of *S. typhimurium* following P22-mediated transduction (49), and of *Streptococcus pneumoniae*, *B. subtilis*, and *Haemophilus influenzae* following transformation (17, 67, 186).

More recently, hDNA has been physically detected based upon the differential electrophoretic mobilities of an hDNA restriction fragment and the corresponding parental homoduplex DNA fragments. Lichten and Fox (142) prepared artificial hDNA from two λ DNAs, one wild type and the other containing a 0.6-kb deletion. A restriction fragment of this hDNA migrates about halfway between the corresponding wild-type and deletion restriction fragments (2.4 and 1.8 kb, respectively). This feature, plus the ability of the hDNA fragment to stick to nitrocellulose in the absence of denaturation in the normal Southern (244) transfer-hybridization procedure, enabled Lichten and Fox (142) to detect the hDNA fragment in λ DNA extracted from *E. coli* mixedly infected with λ. The markedly reduced levels of the hDNA fragment in DNA from singly infected cells (but mixed before extraction) and in DNA from recombination-deficient cells argue that the hDNA is an intermediate in recombination. This technique may allow the detection of hDNA, and its detailed analysis, in a variety of organisms.

Correction of Mismatches in DNA

Base-pair mismatches may arise by errors in DNA replication; many (perhaps all) organisms can remove these mismatches by directed excision and resynthesis of the preferred strand and thereby lower the mutation rate. Mismatches also occur during recombination whenever hDNA encompasses a site of genetic difference between the parental DNA molecules, and these mismatches are also subject to correction. Such correction can either produce genetic exchanges in addition to those generating the hDNA or destroy them, depending upon the direction and extent of the correction. These alternatives are explained in Fig. 5, which illustrates how mismatch correction can have profound effects on the production of recombinant types (for a review, see reference 33).

Mismatches in hDNA were postulated by Holliday (99) to be the basis of two recombination phenomena in fungi: gene conversion and map expansion. (i) Gene conversion is the localized non-Mendelian segregation of a genetic marker during meiosis and, if there is a flanking marker showing Mendelian (i.e., 2:2) segregation, conversion produces one recombinant type but not its reciprocal. Holliday (99) explained gene conversion by the gain of one copy of the genetic marker at the expense of the loss of its allele by the excision of one strand of hDNA and resynthesis to the homoduplex state. (ii) Considering markers in the order *a–b–c*, map expansion is the occurrence of recombinants for the largest interval (*a–c*) more frequently than the sum of recombinants for the two smaller intervals (*a–b* plus *b–c*). The phenomenon is observed only when two of the markers

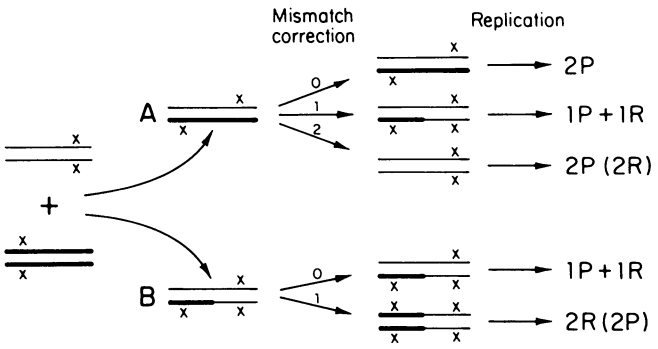

FIG. 5. Consequences of mismatch correction on recombination. The two parental dsDNA molecules (far left) represented by light and heavy paired lines contain genetic markers (x's). Two types of hybrid DNA are considered: (A) neither DNA strand broken and rejoined between the markers, with the formation of two mismatches; and (B) one DNA strand broken and rejoined between the markers, with the formation of one mismatch. The consequences of no, one, or two mismatch corrections on these hDNAs are diagrammed; only one direction of correction is diagrammed at this stage. Replication of these molecules results in the number of parental (P) or recombinant (R) dsDNA molecules indicated. In parentheses are shown the outcomes if certain mismatch corrections had been in the directions opposite to those diagrammed.

are close to each other. Fincham and Holliday (65) explained map expansion by the destruction of potential recombinants for the smaller intervals by the frequent correction of both markers on one strand (cocorrection) when the markers are close (*a–b* or *b–c*) but not so frequently when the markers are farther apart (*a–c*) (Fig. 5).

In *Streptococcus pneumoniae* transformation, mismatch correction of hDNA to the recipient type was postulated by Ephrussi-Taylor and Gray (55) to be the basis for the low transformation frequency of certain markers (designated low efficiency) relative to other markers (high efficiency) in the same gene. They hypothesized that hDNA was formed at the same frequency in both cases but that the donor strand of low-efficiency markers was excised and replaced with the recipient allele. *Streptococcus pneumoniae hex* mutants are transformed at high frequency by both low- and high-efficiency markers, and it was hypothesized that the *hex* mutants are defective in the mismatch correction (132). This hypothesis is well supported by further studies (for a review, see reference 33).

In phage λ vegetative crosses, mismatch correction was hypothesized to be a cause of negative interference (291). Considering markers in the order *a–b–c*, negative interference is the occurrence of *a–b–c* double-exchange recombinants (i.e., recombinants with a genetic exchange in each interval) more frequently than the product of the frequencies of *a–b* and *b–c* recombinants. In other words, the double exchange occurs more frequently than expected from two independent events. Amati and Meselson (2) found negative interference values of about 3 for distant λ markers (i.e., when *a* and *c* are >~5 map units apart) but interference values rising to about 100 for close markers (i.e., when *a* and *c* are <~1 map unit apart). They inferred that genetic exchanges in λ occur in clusters, so that if a recombinant has a genetic exchange in one small interval (say *a–b*), then it is more likely than a phage picked at random to have a genetic exchange in a neighboring small interval (*b–c*). Amati and Meselson (2) found that triple and quadruple genetic exchanges, as well as doubles, occurred at higher frequencies than predicted from independent events. The clustered genetic exchanges responsible for negative interference could arise by independent mismatch correction of markers included in a single hDNA region (Fig. 5).

These are at least two mechanisms of mismatch correction that can act on λ DNA during its recombination in *E. coli*; presumably they can act on *E. coli* DNA as well. The first mechanism excises long (>~1-kb) sections of one DNA strand and is directed in its choice of strand by the state of methylation of the DNA. This methyl-directed correction of mismatches appears to be the major mechanism for removing errors of replication in *E. coli*, but it may also have significant effects on recombination. This process was hypothesized by Wagner and Meselson (280) from the behavior of artificially prepared heteroduplexes on λ DNA introduced into *E. coli* by transfection. The mechanism of the correction has been studied with both intact cells and cell-free extracts (for reviews, see references 33 and 180a and M. Meselson, *in* K. B. Low, ed., *The Recombination of Genetic Material*, in press).

Mutants lacking methyl-directed mismatch correction were first obtained as hypermutable strains, and their lesions defined the *mutH*, *mutL*, *mutS*, *uvrD* (formerly *mutU*), and *dam* genes. The *dam* gene product methylates the adenine in the sequence 5′ G-A-T-C 3′ (171). The *mutS* gene product binds to base-pair mismatches (258). The *mutH* gene product appears to recognize hemimethylated 5′ G-A-T-C 3′ (i.e., that with a methylated adenine on one strand but nonmethylated adenine on the other) and to nick the nonmethylated strand (P. Modrich, personal communication). DNA excision and resynthesis, possibly extending from the hemimethylated 5′ G-A-T-C 3′ to the mismatch (or possibly in the reverse direction), accompany correction of the base-pair mismatch (162, 163). The *uvrD* gene product, DNA helicase II, may participate in the excision and resynthesis. Extensive excision and resynthesis can thus correct two (or more) mismatches, occurring within about a gene's length of each other, to the same parental genotype (Fig. 5).

Methyl-directed mismatch correction should not accompany recombination involving fully methylated parental DNA unless there is DNA synthesis involved in the exchange reactions. In phage λ, in which the effects of mismatch correction on recombination in *E. coli* have been most thoroughly studied, replication outstrips *dam* protein-directed methylation, resulting in frequent hemimethylated sites. Such sites provide the opportunity for methyl-directed correction of mismatches in hDNA.

A second mechanism of mismatch correction excises very short (<5-nucleotide) sections of one DNA strand and is directed by mismatches in sequences related to 5′ C-C-$^{A}_{T}$-G-G 3′ (145, 146). The second C in this sequence is methylated by the *dcm* gene product (95). Spontaneous deamination of the methylated C creates T, and the function of this correction mechanism may be to convert the T back to C (144). This mechanism evidently is not 100% efficient, however, since this C is a hotspot for transition mutations creating the amber codon 5′ U-A-G 3′ (37). As a consequence of this hotspot, amber mutations may frequently be in nucleotide sequences subject to very short patch repair. Recombination studies with such mutations may therefore give a "marker effect," that is, a higher frequency of recombinants with a reference marker than observed with another mutation at the same or nearby site. Such anomalies in mapping λ *c*I mutations led Lieb (144) to the discovery of very short patch repair, which may also be the basis of mapping anomolies in F′*lac*-by-chromosomal *lac* crosses

(36) and in Hfr *lac*-by-chromosomal *lac* crosses (185), as well as the basis of the negative interference in λ crosses observed by Amati and Meselson (2) since two of the mutations they used (*P3* and *P80*) are in 5′ C-C-$^{A}_{T}$-G-G 3′ sequences (for a discussion, see reference 207a).

Resolution of Recombination Intermediates

DNA structures in which one or two single strands of DNA connect two dsDNA molecules have been widely proposed as intermediates in recombination. For example, Holliday (99) proposed the structure in Fig. 2d containing two crossed strands. Enzymes capable of cleaving such structures into two separate dsDNA molecules have therefore been sought.

Mizuuchi et al. (180) found that endonuclease VII coded by T4 gene *49* can cleave Holliday junctions by cutting the crossed strands at homologous positions. This corresponds to "diagonal" cutting across the junction when it is drawn as a cruciform (Fig. 6). Cleavage occurs with approximately equal frequency along the two diagonals, which would result in equal frequencies of formation of molecules with recombinant or parental configuration of DNA distant from the junction (see Fig. 7). The enzyme can cleave other DNA structures, such as Y-shaped molecules and hairpins (106). This activity may be important in converting replicational and recombinational intermediates into unbranched dsDNA molecules suitable for packaging into mature phage particles. That endonuclease VII is involved in recombination was shown by the lower frequency of recombinants among the mature particles in a gene *49* mutant infection than in a wild-type infection (178, 182).

Holliday junction-resolving enzymes have been reported from other organisms. deMassy et al. (44) found that an endonuclease of phage T7, coded by gene *3*, can also cleave Holliday junctions as well as Y-shaped molecules. A similar activity from the yeast *Saccharomyces cerevisiae* has been reported (261, 285). To date, no such activity from bacteria has been directly demonstrated.

The suggestion that RecBCD enzyme acts late in recombination (see above) led to the hypothesis that RecBCD enzyme cleaves Holliday junctions (for an example, see reference 60). This hypothesis is consistent with the observation that a derivative of phage λ (Red$^-$ Gam$^-$) containing a large inverted repeat (a palindrome) cannot form plaques on wild-type *E. coli* but can form plaques on *E. coli recBC sbcB(C)* mutants (134). The authors proposed that RecBCD enzyme and ExoI block λ palindrome growth by cleaving the cruciform structure in a manner reflecting their normal cleavage of Holliday junctions. Such an activity seems unrelated to the known ssDNA exonuclease activity of ExoI (139). Although T4 endonuclease VII readily cleaves supercoiled, circular palindromic DNA (i.e., that in the cruciform structure), RecBCD enzyme does not detectably do so (K. Mizuuchi, M. O'Dea, and M. Gellert, personal communication). The possibility that RecBCD enzyme cleaves Holliday junctions during unwinding (after entering a dsDNA end) or that it cleaves only Holliday junctions that it makes (see Fig. 7) has not been excluded, however. Thus, the hypothesis that RecBCD enzyme cleaves Holliday junctions remains attractive but not directly demonstrated.

Models of Recombination

In the Introduction, I mentioned the hypothesis that the invasion of dsDNA by the 3′-OH ends of ssDNA is an important step in various mechanisms of recombination. The models below describe alternative mechanisms for producing the 3′-OH ssDNA invasive ends and alternative mechanisms for processing the joint molecules into recombinants. These alternatives depend upon the different activities of the enzymes that function in different pathways of recombination. The ability of these pathways to function appears to depend in turn on the nature of the substrates available for recombination in different modes of diploidy.

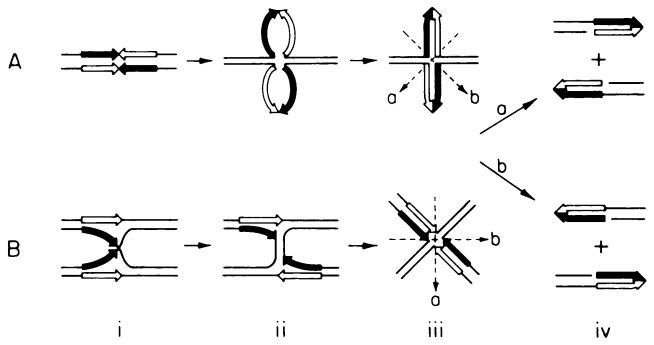

FIG. 6. Relation of cruciform structures and Holliday junctions and their cleavage into recombinant products. Each line represents one DNA strand. (A) A linear dsDNA contains a palindrome represented by the arrows: the sequences of the heavy and light arrows are complementary. Upon unwinding, the complementary sequences on each strand anneal to form a cruciform. (B) Two linear dsDNA molecules are connected by reciprocally exchanged strands, forming a Holliday junction (Fig. 2D). A 180° rotation of the lower branch relative to the upper forms the structure in panel B, part (ii). Bending of the four arms creates a cruciform (B, part iii). The connecting point of the crossed strands is a phosphodiester bond; all base pairs can remain intact (231). Thus, the length of this connection is exaggerated in panel B, parts i and ii, for clarity; the relation in panel B, part iii is more nearly accurate. Cleavage (arrows a and b) of two DNA strands of like polarity in cruciform structure A produces the structures in part iv. Similar products result from cleavages of the Holliday structure (not shown).

Recombination promoted by RecBCD enzyme and Chi sites. In the model in Fig. 7, RecBCD enzyme binds to a dsDNA end (step A) and unwinds the DNA with the production of, first, a loop-tail structure (step B) and, second, a twin-loop structure (step C), as described earlier (Fig. 3 and see above). When the enzyme encounters a properly oriented Chi site, it cuts the strand containing 5′ G-C-T-G-G-T-G-G 3′, converting one loop into two ssDNA tails (step D). Continued unwinding elongates the 3′-OH tail (containing Chi near its end), and rewinding shortens the 5′ tail (step E). Unwinding and rewinding continue until the enzyme releases the 5′ tail, resulting in the collapse of the second loop (step F). At this point, the enzyme has produced a long (perhaps several kilobases) 3′-OH ssDNA tail extending from a dsDNA molecule containing a gap. Invasion of the 3′-OH tail into the second dsDNA parental molecule (supercoiled by DNA gyrase) is catalyzed by RecA and SSB proteins and produces a D loop (step G). The displaced strand, aided by RecA and SSB proteins, pairs with the ssDNA gap in the first parental DNA; this reaction may require either TopoI or the cutting of the D loop, possibly by RecBCD enzyme. The hDNA in the resultant cross-stranded structure, the Holliday junction (step H), may be elongated by the continued unwinding by RecBCD enzyme and strand transfer by RecA and SSB proteins. Cutting of the Holliday junction, perhaps by RecBCD enzyme, and exchange of DNA ends form either

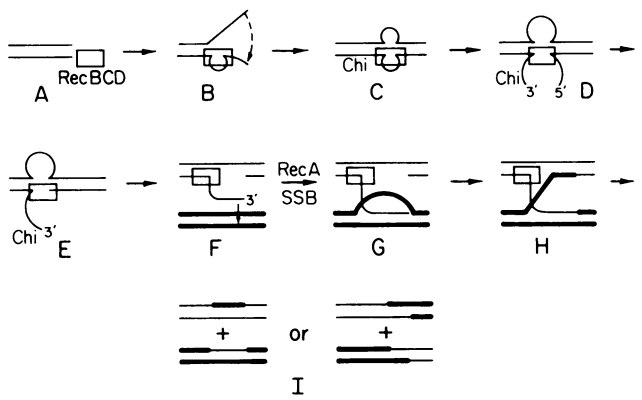

FIG. 7. Model for recombination promoted by RecBCD enzyme and Chi sites. Steps A to C are the unwinding and rewinding of DNA by RecBCD enzyme (rectangular box) described in the legend to Fig. 3. Cutting of one DNA strand near the Chi sequence (D) generates an ssDNA tail, which is elongated by continued unwinding by RecBCD enzyme (E). Aided by RecA and SSB proteins (F), the tail invades homologous dsDNA of the second parent (heavy lines), which may be circular and supercoiled by DNA gyrase (not shown). The displaced strand, a D loop (G), anneals with the gap in the first parental DNA to form a Holliday junction (H). The Holliday junction is cleaved to form either of two pairs of recombinant molecules (I) containing hybrid DNA flanked by parental (left) or recombinant (right) DNA configurations (Fig. 6). Gaps and nicks remaining after cleavage of the Holliday junction are filled by DNA PolI and sealed by DNA ligase (not shown). (Modified from reference 241.)

of two pairs of recombinant molecules (step I): resolution in the "horizontal" direction forms recombinants with hDNA flanked by parental DNA (single-strand insertions; step I, left), whereas resolution in the "vertical" direction forms recombinants with hDNA flanked by recombinant DNA (step I, right) (also Fig. 6). After cutting of the Holliday junction, any remaining gaps are filled by DNA PolI, and nicks are sealed by DNA ligase.

This model of recombination is based upon the activities of the enzymes, the genetic properties of Chi, and the behavior of special *recBCD* mutants, as discussed previously (see above). In λ Red$^-$ Gam$^-$ vegetative crosses, in which Chi action has been most thoroughly studied, RecBCD enzyme gains access to the DNA primarily at the right end after the λ *cos* site is cut by λ terminase proteins to initiate packaging; presumably, binding of the λ prohead to the left end blocks RecBCD enzyme entry there (117, 118). RecBCD enzyme therefore travels from right to left on λ DNA and generates a long (several kilobases) invasive 3′-OH ssDNA tail to the left of Chi. The leftward action of Chi and its stimulation over a long distance are thus accounted for. Since RecBCD enzyme cuts at Chi only as it is unwinding DNA from right to left (with respect to the Chi sequence as it is found in λ with an active Chi site) (263), the orientation dependence of Chi is accounted for. Inversion of the *cos* site allows RecBCD enzyme to enter the left end of λ and thereby activate an inverted Chi (117).

Formation of the D loop (step G) is catalyzed by RecA and SSB proteins (for a review, see reference 40). This reaction is favored when the invading ssDNA has a free 3′-OH end homologous to the dsDNA (122) and when the dsDNA is negatively supercoiled (224). These properties are in accord with the strand polarities of Chi and RecBCD enzyme just discussed and with the requirement for DNA gyrase for RecBCD enzyme-promoted recombination (54). Rosenberg (212) has presented genetic evidence that the strand polarity of "patch"-type λ recombinants (Fig. 7I, left) is opposite to that predicted by the model in Fig. 7 and the strand cut by RecBCD enzyme near Chi. She found that the strand polarity of the recombinants was the same with and without Chi. The genetic factor responsible for the strand polarity bias has not been reported. Further investigations are needed to resolve the seeming contradiction between the strand polarity formed inside the cell and the strand polarity of RecBCD enzyme cutting outside the cell.

The initial reactions (steps A to G) in the model in Fig. 7 have been demonstrated with purified components (RecBCD enzyme, RecA protein, and SSB protein), but they remain to be coupled to produce synapsed molecules from dsDNA substrates. Wiegand et al. (289) and DasGupta et al. (42) reported that RecBCD enzyme can cleave D loops, but at least one other enzyme in the cell has that activity too, since extracts of *recBC* null mutants catalyze the reaction. With linear dsDNA substrates (one of which contains an ssDNA end), RecA protein can form Holliday junctions and move them slowly (~4 bp/s) along the molecules (39). RecBCD enzyme has been hypothesized to resolve Holliday junctions (60), but as discussed above this resolution reaction has not been directly demonstrated. Finally, filling of gaps by DNA PolI and sealing of nicks by DNA ligase are well-established reactions.

In summary, the model in Fig. 7 is supported by extensive genetic and enzymatic evidence, but the final steps (H and I) remain to be directly demonstrated, as do coupling all of the reactions in a coordinated fashion with purified components.

Although this model of recombination stemmed primarily from studies on λ vegetative crosses, it can account for recombination in other modes of diploidy in which the RecBCD pathway is active. These appear to be those situations in which there is a free dsDNA end available to allow RecBCD enzyme to enter. In Hfr crosses, RecBCD enzyme may enter either end (or both ends) of the entering DNA once it has been converted from ssDNA to dsDNA. In transduction, the enzyme may enter the linear dsDNA (which presumably produces the complete transductants) but not the circular dsDNA (which presumably produces the abortive transductants). In both Hfr crosses and P1-mediated transductions, Chi increases the frequency of exchange in the interval containing Chi (or the interval to Chi's left) in a *recB*-dependent fashion (46). In crosses with an F′, RecBCD enzyme may enter F *oriT* directly (if it contains a dsDNA break) or create a dsDNA break from the ssDNA break and then enter. In crosses involving λ phage infecting a lysogen, the λ DNA may circularize before RecBCD enzyme enters it. These proposals account for the high recombination frequency (and the RecBCD enzyme dependence) in λ *lac*-by-F′*lac* crosses and the low-frequency RecBCD enzyme independence in λ *lac*-by-chromosomal *lac* crosses (200). The RecBCD enzyme independence of recombination between or within plasmids or the chromosome is consistent with these molecules being primarily (or exclusively) circular. These molecules recombine primarily by the RecE and RecF pathways.

The model in Fig. 7 may apply to recombination in bacteria other than *E. coli*. RecBCD enzyme is widely distributed in bacteria, both gram-negative and gram-positive (268). The Chi sequence appears to be the activating signal for the enzymes from enteric bacteria. Chi is active in λ vegetative crosses in *S. typhimurium* (240). Extracts of most enteric bacteria surveyed, from the families *Enterobac-*

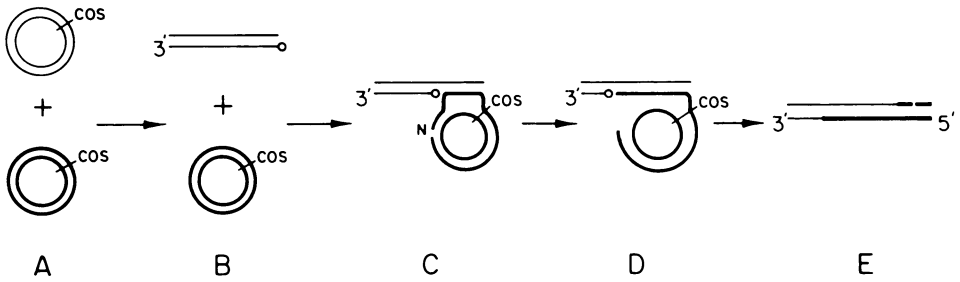

FIG. 8. Model for λ Red-promoted recombination. (A) Two monomeric circular λ DNA molecules. One is cut by λ terminase proteins at the cohesive end site (*cos*) to produce a linear molecule. (B) λ exo (small open circle) binds to the right end of the cut DNA and digests the 5′ end strand, producing a 3′ ssDNA tail; the left end is presumably blocked by the λ prohead. (C) The 3′ tail anneals with one strand of the circle. This reaction may occur concomitantly with strand digestion (Fig. 4) and may be aided by β protein complexed with λ exo. Strand transfer ceases when a nick (N) in the circle is encountered. (D) The dimeric structure is packaged from the left end of the linear part to *cos* on the circular part, producing the linear recombinant (E). Repair DNA synthesis (dashed line) using the invaded circular DNA as a template may be required to restore an intact *cos* site. Note that the minor material contribution (heavy solid line in part E) has its 5′ end at the right. When replication is permitted, DNA ends may arise at positions other than *cos*, allowing recombination to initiate throughout the genome. See text for discussion. (Modified from reference 247.)

teriaceae and *Vibrionaceae*, contain a Chi-cutting activity with properties similar to that of the *E. coli* RecBCD enzyme (218). *recBCD*-like genes have been cloned from *S. typhimurium*, *Shigella dysenteriae*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Proteus mirabilis* and introduced into *E. coli* with a *recBCD* deletion; Chi is active in these hybrid cells (240; N. McKittrick and G. Smith, unpublished data).

In contrast, Chi does not appear to be the activating signal for the enzymes from other bacteria. Purified RecBCD enzyme from *H. influenzae* and extracts of *Pseudomonas aeruginosa* do not contain detectable Chi-cutting activity (218). When introduced into *E. coli* with a *recBCD* deletion, DNA clones from *P. aeruginosa* and *Pseudomonas putida* confer ATP-dependent nuclease activity and recombination proficiency but no detectable Chi activity in λ vegetative crosses (McKittrick and Smith, unpublished data). These results raise the possibility that nonenteric bacteria use a signal other than the Chi sequence for recombination. With this proviso, the mechanism in Fig. 7 may occur in many bacteria, since the requisite enzymes are widely distributed.

Recombination promoted by λ Red functions. The identified proteins required for recombination by the λ Red pathway are λ exo and β protein. In addition, Red-promoted recombination is strongly influenced by concomitant DNA replication (249). Without replication, recombination occurs almost exclusively near the *cos* site of λ and is strongly enhanced by RecA protein (248). With replication, recombination occurs with nearly uniform frequency across the λ genome and is independent of RecA protein.

As in the RecBCD pathway, DNA ends play an important role in the Red pathway (for a review, see reference 245). dsDNA ends are presumably entry sites for λ exo. In the absence of DNA replication these ends are formed primarily or exclusively at *cos* by the λ terminase protein, which cuts λ intracellular circular molecules into packaged linear molecules (247). DNA replication in the late σ mode presumably leads to DNA ends uniformly distributed across the genome. ssDNA with free 3′ ends may arise either by λ exo digestion from dsDNA ends (Fig. 4A) or by leading-strand DNA replication being primed at a site distant from the 3′ end. In summary, potentially invasive 3′ ssDNA ends may arise at *cos* (in the absence of replication) or throughout the genome (in the presence of replication).

In the model shown in Fig. 8, the 3′ ssDNA end invades a circular dsDNA molecule, forming a D loop. This reaction might be promoted by β protein complexed with λ exo (see subsection, "Recombination Genes and Enzymes of Phage λ"). Alternatively, D-loop formation may be promoted by RecA protein. Resolution of this structure into a recombinant may occur when an accidental nick in the invaded molecule is encountered (Fig. 8C) or when an unknown enzyme nicks the strand of the invaded molecule to which the invading strand is annealed. Exchange of DNA ends at the nick and ligation forms a duplex, with hDNA extending from this point to the initial 3′ invading end. Local (repair) DNA synthesis may be primed by this end; such synthesis would use the invaded DNA as template. Thus, the hDNA region would become flanked by homoduplex DNA of opposite parentage. If the invading 3′ end had been partially degraded, such repair synthesis would be required to restore the *cos* site for packaging.

This model accounts for the following facts about λ recombination occurring in the absence of DNA replication. The λ parent making the lesser material contribution contributes the strand with the 5′ end at the right end of the genome (286). The model accounts for this finding by λ exo digesting the 5′ end of the DNA from the invading parent. Recombination occurs almost exclusively at *cos* or at the site of intracellular cutting by *Eco*RI endonuclease; these sites are presumably entry sites for λ exo (247, 248, 271, 272). In the absence of RecA protein, recombination is almost exclusively at the right end, presumably because the λ prohead bound to the left end prevents the entry of λ exo there (247). When one parent contains a cuttable (wild-type) *cos* and the other parent contains a noncuttable (mutant) *cos*, recombinants genetically exchanged for markers (*R*, *S*) near the right end inherit the noncuttable *cos* almost exclusively (247). The model accounts for this finding by the repair synthesis, using the invaded (uncut) parent as template (Fig. 8E). The model also accounts for the nonreciprocality of λ Red-promoted recombination (which was observed in crosses permissive for replication) (216). Since reciprocal strand exchange is not postulated in the model, reciprocal recombinants are not predicted.

In the presence of replication, λ Red-promoted exchanges are nearly uniformly distributed across the genome (reviewed in reference 23). The model in Fig. 8 would account

for this finding if the ends of σ molecules are uniformly distributed across the genome. Electron microscopy of σ molecules revealed that about half of the ends are at or near the origin of θ replication (*ori*) and about half are distributed across the genome, but it is not known whether this latter half had been broken during or after extraction from the cell (12). Thus, the generation of recombination-promoting ends by replication remains an attractive but not rigorously demonstrated possibility.

The strong enhancement of recombination by RecA protein in the absence of DNA replication (especially at the left end of λ) is not accounted for. In other words, λ Red-promoted recombination requires, or is strongly stimulated by, either RecA protein or replication. Perhaps RecA protein promotes a type of strand invasion that β protein can accomplish only with the aid of replication.

Recombination promoted by the RecE and RecF pathways. Of the many gene functions needed for these pathways, enzymatic activities are known for only three: *recA*, *sbcB*, and *recE*. This limited knowledge precludes a detailed hypothesis for recombination by these pathways. RecA protein presumably acts to pair homologous ssDNA and dsDNA. The other two known activities, ExoI (*sbcB*) and ExoVIII (*recE*), presumably impinge upon the formation of the 3′-OH ssDNA substrate for RecA protein. Clark (30) proposed that in *recBC* mutants a potentially recombinogenic substrate is produced by an unknown mechanism but quickly destroyed by ExoI; mutational inactivation of ExoI by *sbcB* mutations (coupled with an *sbcC* mutation) thus leads to recombination proficiency by the RecF pathway. The specificity of ExoI for 3′-OH ssDNA ends is in accord with the proposal that such ends are the primary substrate for RecA protein. Activation of expression of *recE* by *sbcA* mutations yields ExoVIII, which produces 3′-OH ssDNA ends and recombination proficiency by the RecE pathway. The specificities of RecA protein, ExoI, and ExoVIII are consistent with the hypothesis that 3′-OH ssDNA ends are essential intermediates in the RecE and RecF pathways.

The source of the hypothesized 3′-OH ssDNA ends in the RecE and RecF pathways seem clear in some modes of diploidy but not in others. ExoVIII produces 3′-OH ssDNA ends, but only from linear dsDNA; circular molecules, whether supercoiled, nicked, or gapped, are not efficient substrates (109). This linear dsDNA substrate requirement is the same as that of RecBCD enzyme, and ExoVIII may act on the same set of substrates as does RecBCD enzyme: the RecE pathway can act at high frequency in all cases in which the RecBCD pathway can act. ExoVIII presumably produces 3′-OH ssDNA ends (and recombination) near the initial dsDNA end, just as RecBCD enzyme produces 3′-OH ssDNA ends (and recombination) near the Chi site. In addition, the RecE and RecF pathways, unlike the RecBCD pathway, can promote recombination between molecules that are primarily circular: plasmid by plasmid (66, 131) and λ *lac* by chromosomal *lac* (199). The low frequency of this recombination may stem from the low frequency of dsDNA breaks in these molecules. The source of these hypothesized dsDNA breaks is not known, nor is it known why the RecE pathway but not the RecBCD pathway can act on them (if they are produced).

In conjugation, 3′-OH ends may remain after complementary DNA strand synthesis. Presumably they are formed in cells at low frequency (and destroyed by ExoI), but at high frequency by RecBCD enzyme and ExoVIII (but not destroyed by the relatively feeble ExoI). Formation of the 3′-OH ssDNA ends may depend upon one or more of the *rec* genes functioning in the RecF pathway but not in the RecBCD pathway (*recF*, *recJ*, *recN*, *recO*, *recQ*, and *ruv*).

Since RecA protein is required for recombination by the RecE and RecF pathways in most modes of diploidy, RecA protein presumably acts in these pathways as it does in the RecBCD pathways. Other proteins known to function in the RecBCD pathway (SSB protein, DNA gyrase, DNA PolI, and DNA ligase) may also function in the RecE and RecF pathways, but to my knowledge this has not been established. Determining these requirements and elucidating the biochemical activities of the *recF*, *recJ*, *recN*, *recO*, *recQ*, and *ruv* gene products will allow formulation of more explicit models of recombination by the RecE and RecF pathways.

FUTURE DIRECTIONS

Studies of recombination in procaryotes have provided detailed information about many of the enzymes promoting recombination and general knowledge about the nature of the substrates and products. This information has led to explicit models of recombination. These models cannot yet be accepted as established mechanisms of recombination because of gaps in our knowledge of recombination. The activities of several recombination functions, particularly those of the RecE and RecF pathways of *E. coli*, are unknown. It is likely that additional gene products remain to be discovered for the RecE and RecF pathways, and perhaps for the RecBCD pathway as well. The nature of the substrates and products inside the cell needs to be more precisely determined. Techniques such as Southern hybridization not available to investigators of Hfr conjugation, for example, should prove fruitful in this regard. Whether recombination proceeds in other bacteria as it does in *E. coli* remains to be established by comparative studies of the enzymes and behavior of mutants in diverse species. Finally, it is essential to aChieve homologous recombination outside the cell, using either crude extracts or purified enzymes, to verify that all essential components are accounted for and that these components can coordinately produce recombinant DNA from parental DNA. Such systems will also be useful in determining the structures of the DNA intermediates in recombination. Procaryotes are likely to continue to play a leading role in advancing our knowledge of the mechanism of homologous recombination.

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