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Interchangeable Parts of the *Escherichia coli* Recombination Machinery

Minireview

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In *Escherichia coli*, at least two groups of proteins, or "recombination machines," can operate independently on broken DNA to produce a 3′-terminated single-stranded DNA filament coated with RecA protein and ready for synapsis with intact homologous DNA. Recent analyses of mutants lacking one or more of the activities required for presynaptic filament formation by one recombination machine demonstrate that parts of the two normally separate machines can interchange to initiate homologous recombination.

Faithful repair of broken or damaged DNA occurs by homologous recombination. This process requires a series of enzymes, collectively forming a "recombination machine," that act on broken DNA. At least three broad classes of activities—helicases, nucleases, and synapsis proteins—constitute parts of this machine and can be provided either by one complex protein or by several separate proteins. In *Escherichia coli*, the recombination machine used depends on both the structure of the DNA (linear versus circular) and the genetic background of the cell. Like the ordered reactions that occur in biosynthesis and catabolism, the activities of these machines process the damaged DNA in a sequential fashion to produce a repaired or recombinant DNA molecule. These realizations led Clark (1971) to propose that two major pathways of recombination operate in *E. coli*. These pathways have been named for the gene product first found to be unique to each pathway. Subsequent work has revealed that the RecBCD and RecF pathways (or recombination machines) are largely independent. But recent analysis of an *E. coli* mutant that lacks RecBCD nuclease activity, normally required for that pathway of recombination, provides a striking example of how functional parts from these two recombination machines can be interchanged (Ivančić-Baće et al., 2003). This analysis also illustrates the common features of events required to produce a repaired or recombinant DNA molecule.

An apparently universal feature of homologous recombination, first demonstrated in *E. coli*, is the production of a 3′-terminated single strand (ss) of DNA coated with a synapsis protein such as RecA (Smith, 1989). In *E. coli* and presumably other organisms, production of this essential intermediate from a broken double-stranded (ds) DNA molecule requires the activity of a helicase to unwind the DNA and in many cases a nuclease to degrade the 5′-terminated strand, leaving a free 3′ ssDNA end (Figure 1). Single-strand DNA binding protein (SSB) has a high affinity for this tailed molecule and must be displaced for the end to be coated with RecA protein. These steps complete the presynaptic phase of recombination and DNA repair. A joint molecule is then formed by synapsis and strand exchange of the RecA-coated ssDNA with an intact homolog, and subsequent resolution of the joint molecule by either RuvABC or RecG (or both) yields the final products: repaired or, as detected following the interaction of homologous but genetically marked chromosomes, recombined DNA.

In *E. coli*, the RecBCD and RecF recombination machines use distinct sets of enzymes to produce a 3′-terminated ssDNA molecule coated with RecA protein (Figure 1). In the RecBCD pathway, all of the required functions reside in one machine: the helicase, nuclease, and RecA-loading activities are provided by RecBCD enzyme alone (for reviews see Kowalczykowski, 2000; Smith, 2001). In contrast, the RecF recombination pathway requires several separate enzymes to process the DNA into a presynaptic intermediate (for a review, see Lloyd and Low, 1996). RecQ unwinds the DNA, and RecJ, a 5′ to 3′ ssDNA exonuclease, frees a 3′-terminated end that is coated with SSB. RecF, RecO, and RecR, which may function as a complex, displace SSB and facilitate the production of a RecA coated-ssDNA filament. The RecBCD and RecF pathways thus use different proteins with parallel activities to achieve the same end. However, recent work, described below, demonstrates that under special circumstances parts of the RecBCD and RecF recombination machines can be used interchangeably to promote recombination.

RecBCD — a Single Protein Recombination Machine

The majority of recombination and dsDNA break repair in wild-type *E. coli* requires the RecBCD enzyme. This large (330 kDa) multisubunit enzyme is a highly processive helicase and ATP-dependent ds- and ssDNA exonuclease, which acts with high potency only on linear DNA substrates. The activities of RecBCD enzyme are regulated by Chi sites (5′-GCTGGTGG-3′), well-characterized hotspots of recombination in *E. coli*. The RecBCD-Chi interaction plays a central role in the production of a recombinogenic DNA molecule in the following steps (Figures 1 and 2). RecBCD enzyme binds to the blunt (or nearly blunt) end of dsDNA and initiates unwinding by the two helicase subunits within the holoenzyme: RecD, which is fast, and RecB, which is slower (A.F. Taylor and G.R.S., unpublished data). During unwinding, RecBCD's interaction with Chi results in the production of a 3′-terminated ssDNA end, onto which RecBCD enzyme loads RecA protein to yield the essential presynaptic intermediate (Anderson and Kowalczykowski, 1997).

The 3′-tailed substrate can be produced by RecBCD enzyme in two different ways. The reaction of purified RecBCD enzyme with a DNA substrate containing a Chi site is dependent upon the concentrations of ATP and Mg^{+2} (Figure 2; for reviews, see Kowalczykowski, 2000; Smith, 2001). When the concentration of ATP exceeds that of Mg^{+2} so that most of the Mg^{+2} is chelated by ATP (Figure 2, left), RecBCD makes a single-stranded endonucleolytic cut a few nucleotides to the 3′ side of Chi. Continued unwinding of the DNA leaves a 3′-

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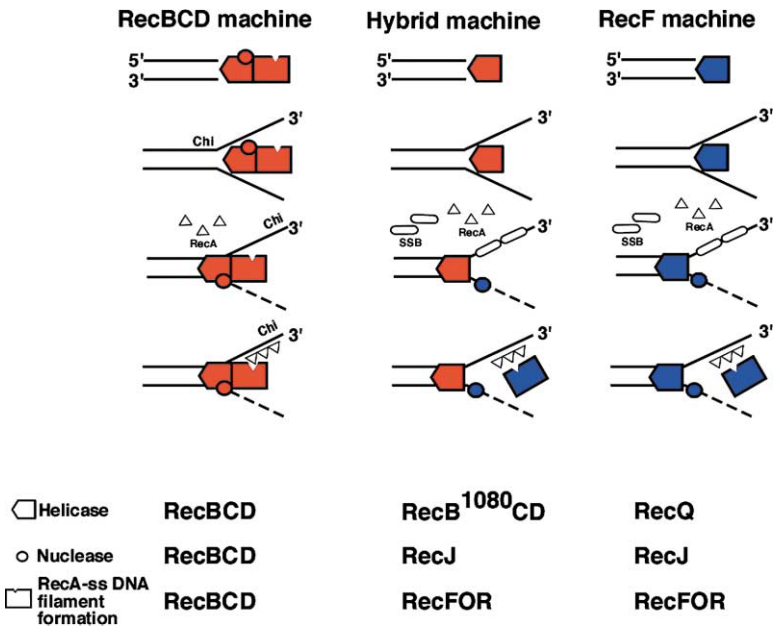

Figure 1. Interchangeability of Recombination Activities from the RecBCD and RecF Recombination Machines

Recombination activities (helicase, nuclease, and ssDNA-RecA filament formation) are shown interacting with dsDNA (black lines) containing a Chi sequence (5′-GCTGGTGG-3′) for the RecBCD (represented by red components), RecF pathway (blue components), and hybrid (blue and red components) recombination machines in *E. coli*. The three illustrated parts of RecBCD enzyme do not correspond to individual polypeptides. The intermediates produced by RecBCD enzyme are a composite of the intermediates in the two distinct reactions detailed in Figure 2. The hybrid recombination machine operates with RecB^{1080}CD (nuclease-deficient) enzyme and contains parts of the RecBCD and RecF pathway machines. Nucleolytic degradation is indicated by a dashed line. RecA-ssDNA filament formation is shown following loading of RecA (triangles) by RecBCD enzyme or displacement of SSB (ovals) by RecF, RecO, and RecR. Other hybrid machines are not shown and use combinations of the activities listed in Table 2 (see text).

terminated ssDNA tail with Chi near the end. When the concentration of Mg^{+2} exceeds that of ATP (Figure 2, right), RecBCD degrades the strand with a 3′ terminus at which it initiates unwinding. Degradation of this strand by the 3′ to 5′ exonuclease is reduced when the enzyme reaches Chi, but the complementary strand is cut near Chi. Unwinding and continued activity of the 5′ to 3′ exonuclease results in degradation of the non-Chi-containing strand. Which of these reactions more nearly reflects that inside the cell is currently unclear, but a product common to both conditions is a 3′-terminated ssDNA tail with Chi. RecBCD enzyme loads RecA protein onto this Chi tail, completing the formation of a presynaptic filament.

One of the essential functions of the RecBCD recombination machine, the nuclease activity, can be inactivated by mutation of either *recB* (Yu et al., 1998) or *recD* (Amundsen et al., 1986). The C terminus of RecB contains a motif of acidic amino acids similar to that found in some restriction enzymes and thought, in RecBCD enzyme, to bind Mg^{+2} to form the nuclease active site. The *recB1080* mutation changes one of these acidic amino acids, substituting alanine for aspartic acid at amino acid 1080. Although this mutation eliminates nuclease activity, it does not affect DNA unwinding (Yu et al., 1998; A.F. Taylor and G.R.S., unpublished data). In addition, the mutant enzyme can load RecA, but, curiously, only if RecD is removed (Amundsen et al., 2000; Anderson et al., 1999); thus, a *recB1080* Δ*recD* strain is recombination-proficient, whereas *recB1080 recD*$^{+}$ is recombination-deficient (Amundsen et al., 2000).

These observations indicate that RecA-loading by RecBCD enzyme is required for recombination and that the RecD subunit can inhibit RecA-loading (Amundsen et al., 2000; Anderson et al., 1999). Other studies have indicated that wild-type RecBCD enzyme is changed at Chi, perhaps by loss of RecD inhibition, to allow it to load RecA (Anderson and Kowalczykowski, 1997; Taylor and Smith, 1992). In *recB1080* mutants, recombination is blocked by the loss of nuclease activity and by the inhibition of RecA-loading activity. The role of the RecD subunit in nuclease activity is less clear, but mutations in *recD* eliminate nuclease and Chi hotspot activities while leaving the cell recombination-proficient. The RecBC(D^{-}) enzyme maintains helicase activity and, as a result of the loss of RecD inhibition, loads RecA-protein on DNA ends even in the absence of a Chi site (Churchill et al., 1999).

RecF Pathway—a Multi-Protein Recombination Machine

In *E. coli* cells with the appropriate genetic background and lacking RecBCD enzyme, the activity of a second recombination machine can be observed. The DNA repair- and recombination-deficient phenotype of *recBC* mutants is suppressed in cells mutant in both *sbcB*, which encodes the 3′ to 5′ ssDNA exonuclease I, and *sbcCD*, which encodes the complex dsDNA exonuclease SbcCD (Lloyd and Low, 1996). Recombinants are produced because DNA substrates or intermediates for the RecF recombination machine are preserved in the absence of these inhibitory nucleases. As noted previously, the combined activities of several proteins—RecQ, RecJ, RecF, RecO, and RecR—process the DNA substrates: DNA is unwound by RecQ protein, and the 5′ end is digested by RecJ, leaving 3′-tailed ssDNA coated with SSB (Figure 1). By a mechanism not fully understood, the combined activities of RecF, RecO, and RecR replace SSB with RecA and complete presynaptic filament formation. It is known, however, that purified RecF, RecO, and RecR proteins can form a complex (Hegde et al., 1996; Umezu et al., 1993), that RecO protein binds to SSB-coated ssDNA (Kantake et al., 2002), and that RecOR complexes prevent RecA dissociation from ssDNA (Shan et al., 1997). Although details of this mechanism of RecA-ssDNA filament formation remain to be established, it is clear that the RecF machine requires the activities of several separate proteins to initiate recombination.

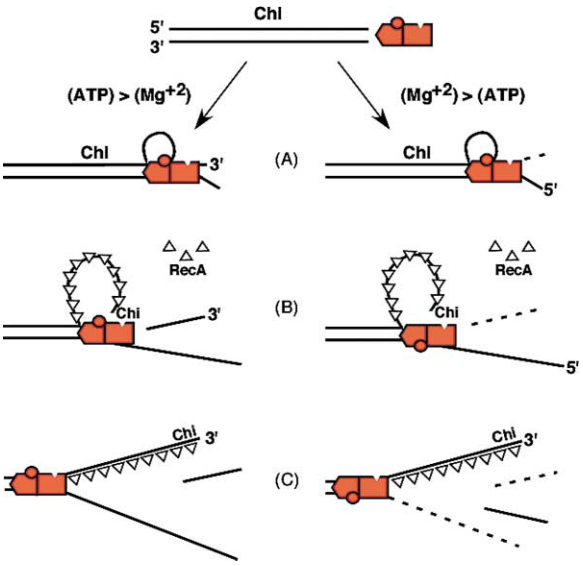

Figure 2. Reaction of RecBCD Enzyme with DNA Containing a Chi Sequence under Reaction Conditions Differing in the Relative Concentrations of ATP and Mg^{+2}

RecBCD and DNA are represented as in Figure 1; movement of the nuclease domain is purely hypothetical. RecBCD enzyme initiates unwinding after binding to blunt or nearly blunt dsDNA.

When the concentration of ATP is greater than that of Mg^{+2} (left images), DNA unwinding produces an ssDNA loop and two ss tails (A). (B) RecBCD enzyme makes an ss-endonucleolytic cut a few nucleotides to the 3′-side of Chi, and RecA is loaded onto this ssDNA end. (C) The enzyme continues unwinding DNA without further nucleolytic activity.

When the concentration of Mg^{+2} exceeds that of ATP (right images), RecBCD degrades the strand with a 3′ terminus at which it initiates unwinding, so that only one ssDNA tail with a 5′-end is formed (A). (B) Upon reaching Chi, the 3′ to 5′nuclease activity is reduced, and RecA is loaded on ssDNA produced by continued unwinding. (C) The complementary strand is cut and degraded following Chi's activation of the 5′ to 3′ nuclease activity.

A Hybrid Machine—Recombination by the Sum of Interchangeable Parts

The interchangeability of parts of the RecBCD and RecF recombination machines was revealed recently by analysis of the nuclease-deficient *recB1080* mutant in strains with and without RecF pathway functions. The initial genetic analysis demonstrated that *recB1080* was recombination-deficient (Amundsen et al., 2000; Table 1, line 3), consistent with the observed inability of $RecB^{1080}CD$ enzyme to load RecA protein onto ssDNA (Amundsen et al., 2000; Anderson et al., 1999). In contrast, Jockovich and Myers (2001) later reported that *recB1080* was recombination-proficient (Table 1, line 4). An explanation for these contradictory results was offered recently when Ivančić-Baće et al. (2003) noted that the strains used in the two reports differed in their *recF* genotype, and this group went on to show that activities from the RecF recombination machine could suppress the *recB1080* (*recF*$^-$) recombination-deficient phenotype.

The parts of the RecF recombination machine promoting recombination in the *recB1080* mutant were elucidated by Ivančić-Baće et al. (2003) using strains with mutations in individual RecF pathway genes (Table 1). As expected from the observation that the $RecB^{1080}CD$ enzyme lacks both nuclease activity and the ability to load RecA protein, elimination of RecF pathway nuclease activity by mutation of *recJ*, or loss of the ability to produce a RecA-ssDNA filament by mutation of *recF*, *recO*, or *recR*, left the *recB1080* strain recombination-deficient (Table 1, lines 3 and 5). A mutation in *recQ*, encoding the RecF pathway helicase, had no effect on recombination, consistent with retention of helicase activity by $RecB^{1080}CD$ enzyme (Table 1, line 6). These data indicate that RecA-ssDNA filament formation by RecFOR can substitute for the RecA-loading activity missing in $RecB^{1080}CD$ enzyme and that RecJ provides the missing nuclease activity. As expected, mutations in *recF*, *recO*, or *recR* did not significantly reduce the recombination proficiency of a *recB1080* Δ*recD* strain (Table 1, line 7): removal of the inhibitory RecD subunit allows $RecB^{1080}C(D^-)$ enzyme to load RecA onto ssDNA independently of RecFOR. Although not tested to date in conjugational crosses, it seems likely that *recB1080* Δ*recD* would require RecJ to be recombination-proficient, because $RecB^{1080}C(D^-)$ can load RecA but lacks nuclease activity (Table 1, line 8; Table 2). This outcome would be consistent with the observation that

Table 1. Repair and Recombination Phenotypes of *E. coli* Mutants Lacking Parts of the Recombination Machinery

E.coli genotype		
RecBCD pathway[a]	RecF pathway[b]	DNA repair and recombination proficiency[c]
+	*recF* (+ or −)	+
recB	*recF* (+ or −)	−
recB1080 (Nuc−)	*recF*−	−
"	*recF*+	+
"	*recO*−, *R*−, *or J*−	−
"	*recQ*−	+
recB1080 Δ*recD*	*recF*, *O*, *R* (+ or −)	+
"	*recJ*−	−(?)[d]
recD	*recF*, *O*, *R*, *or Q* (+ or −)	+
"	*recJ*−	−[e]

[a] "+" indicates wild type; other alleles are null, except for *recB1080*, which abolishes nuclease activity.

[b] The alleles (−) are apparently null, except for wild-type (+) *recF*, *recO*, *recR*, and *recQ* where indicated.

[c] Survival following UV or γ irradiation relative to wild type and/or recombination relative to wild type in conjugational recombination or in phage λ *red*− *gam*− crosses. See Low and Lloyd (1996) for references.

[d] Predicted but not yet tested.

[e] Recombination-proficient for λ replication-permitted crosses, perhaps due to ssDNA substrates produced during replication.

Table 2. Interchangeable Parts of the *E. coli* Recombination Machinery

Recombination-Proficient *E. coli* Strain	Operative Pathway[a]	Recombination Protein Providing the Required Activity		
		Helicase	Nuclease	RecA-ssDNA Filament Formation
Wild-type	RecBCD	RecBCD	RecBCD	RecBCD
ΔrecB sbcB sbcC	RecF	RecQ	RecJ	RecF, O, R
ΔrecD	Hybrid (‡)	RecBC	RecJ	RecBC
recB1080	Hybrid	$RecB^{1080}CD$	RecJ	RecF, O, R
recB1080 ΔrecD	Hybrid	$RecB^{1080}C$	RecJ (?)[b]	$RecB^{1080}C$

[a] Hybrid indicates activities coming from both RecBCD and RecF pathway components. ‡ ("double dagger") designates the recombination-proficient, nuclease-deficient mutant class (Amundsen et al., 1986).
[b] Predicted but not yet tested.

recombination-proficiency in *recD* mutants requires RecJ (Lovett et al., 1988; Table 1, line 10).

Parts of the RecBCD and RecF pathways can act together to form hybrid recombination machines when the nuclease or RecA-loading activities of RecBCD enzyme are absent (Figure 1, Table 2). Analysis of nuclease-deficient strains carrying *recD* and/or the *recB1080* mutation suggests that at least three hybrid machines can operate in one or another circumstance. All of these hybrid machines appear to use RecJ nuclease to produce ssDNA and, in the absence of RecBCD's RecA-loading activity, to use RecF, RecO, and RecR to complete presynaptic filament formation.

Why Parts of the RecF Recombination Machine Are Not Used for dsDNA Break Repair When the RecBCD Machine Is Operative

The interchangeability of parts of the RecBCD and RecF recombination machines is striking in *recB1080* cells, but in wild-type cells the pathways are largely independent; their parts are not obviously interchangeable. This is likely a reflection of the relative potencies of RecBCD enzyme and RecF pathway components. RecBCD enzyme's rate of unwinding is 100 to 1000 times faster than that of RecQ and is probably more processive (Harmon and Kowalczykowski, 2001). RecF pathway recombination is detected only after inactivation of exonuclease I (SbcB) and SbcCD nuclease, suggesting that substrates or intermediates in recombination must be protected from nucleolytic degradation to be processed into recombinants by the RecF pathway. The presence of the same nucleases in wild-type cells has no effect on RecBCD pathway recombination, however, suggesting that DNA molecules are either protected from nuclease degradation (perhaps after being actively loaded with RecA) or processed too quickly to be degraded.

DNA Damage Repair: Roles of the RecBCD and RecF Recombination Machines

One prime function of homologous recombination is the repair of DNA damage induced by physical agents or arising during replication and transcription. The role of homologous recombination in DNA repair is indicated by the reduced viability of recombination-deficient mutants and the sensitivity of these mutants to UV light, X-rays, and other DNA damaging agents. At least two types of DNA lesions, dsDNA breaks and ssDNA gaps, must be repaired to maintain chromosomal integrity and cellular viability. The RecBCD and RecF recombination machines appear to act on different types of lesions; thus, both machines have a role in DNA damage repair. dsDNA break repair is carried out primarily, or perhaps solely, by the RecBCD pathway. In contrast, ssDNA gaps appear to be repaired primarily by the RecF pathway. As outlined here, the RecBCD and RecF recombination machines act separately to repair damaged DNA, but in certain *recBCD* mutant strains the two machines can interchange activities. Perhaps in wild-type cells also, there are situations of altered DNA metabolism not yet recognized in which activities from the two recombination machines interchange to maintain chromosomal integrity.

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