Recombinational DNA Repair in Bacteria and the RecA Protein

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In bacteria, the major function of homologous genetic recombination is recombinational DNA repair. This is not a process reserved only for rare double-strand breaks caused by ionizing radiation, nor is it limited to situations in which the SOS response has been induced. Recombinational DNA repair in bacteria is closely tied to the cellular replication systems, and it functions to repair damage at stalled replication forks, Studies with a variety of *rec* mutants, carried out under normal aerobic growth conditions, consistently suggest that at least 10–30% of all replication forks originating at the bacterial origin of replication are halted by DNA damage and must undergo recombinational DNA repair. The actual frequency may be much higher. Recombinational DNA repair is both the most complex and the least understood of bacterial DNA repair processes. When replication forks encounter a DNA lesion or strand break, repair is mediated by an adaptable set of pathways encompassing most of the enzymes involved in DNA metabolism. There are five separate enzymatic processes involved in these repair events: (1) The replication fork assembled at OriC stalls and/or collapses when encountering DNA damage. (2) Recombination enzymes provide a complementary strand for a lesion isolated in a single-strand gap, or reconstruct a branched DNA at the site of a double-strand break. (3) The ϕX174-type primosome (or re-

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pair primosome) functions in the origin-independent reassembly of the replication fork. (4) The XerCD site-specific recombination system resolves the dimeric chromosomes that are the inevitable by-product of frequent recombination associated with recombinational DNA repair. (5) DNA excision repair and other repair systems eliminate lesions left behind in double-stranded DNA. The RecA protein plays a central role in the recombination phase of the process. Among its many activities, RecA protein is a motor protein, coupling the hydrolysis of ATP to the movement of DNA branches. © 2000 Academic Press.

I. Recombinational DNA Repair in Bacteria

Homologous genetic recombination represents the molecular foundation of much of classical genetics, as well as modern technologies used to map chromosomes and manipulate cellular genomes. Recombination is also a major pathway for DNA repair. For these reasons, the process has received decades of attention from geneticists and biochemists. Much of the biochemistry carried out on this process has focused on bacterial enzymes, and this review will be confined to the homologous genetic recombination system of bacteria.

In bacteria, the major function of homologous genetic recombination is recombinational DNA repair (*1–11*). The connection between recombination and DNA repair was evident in the first studies of bacterial mutants with defects in conjugational recombination (*12, 13*), because a major phenotype of the mutants was a much-elevated sensitivity to DNA-damaging agents. Subsequent discovery of recombination functions in bacteria has relied heavily on a paradigm focusing on conjugational recombination, leading to the definition of a hierarchical set of recombination pathways with more than a dozen separate enzymatic participants (*8, 14–18*).

The traditional focus on conjugational recombination has provided decades of discovery. However, it has historically left an important area of DNA metabolism in relative obscurity. A full understanding of the functions of recombination enzymes, as well as an improved understanding of cellular DNA metabolism, now requires that the conjugation paradigm be supplanted with one focused on DNA repair. DNA repair can be more demanding in its requirements than recombination during conjugation and transduction. Certain mutants of RecA protein, which reduce but do not abolish RecA function, can support conjugational recombination but not recombinational DNA repair (*19–21*). As described in more detail below, some *rec* functions, such as RecF, RecO, and RecR, have minimal roles in recombination during conjugation but important roles in recombinational DNA repair. In addition, the functions and evolution of a variety of enzymatic functions in DNA metabolism are most easily rationalized from the standpoint of recombination-

al DNA repair. The lessons learned in studies of conjugational recombination do not always translate faithfully when the focus shifts to recombinational DNA repair.

The bacterial chromosome can be damaged by external agents that might be added in a laboratory for mutagenesis or other purposes. However, recombinational DNA repair is not limited to situations whereby DNA damage is artificially elevated and the SOS response has been induced. It is also not a process reserved for rare double-strand breaks caused by ionizing radiation. Recombinational DNA repair in bacteria is closely tied to the cellular replication system, and it functions primarily to repair damage at stalled replication forks. The frequency with which this occurs is underappreciated. Studies with a variety of *rec* mutants, all carried out under normal aerobic growth conditions, consistently indicate that at least 10–30% of all replication forks originating at the bacterial origin of replication are halted by DNA damage and must undergo recombinational DNA repair (*7, 10, 22*). As described below, the frequency may be much higher. This repair is mediated by a complex set of recombination functions that can adapt to a variety of damage situations, operating through multiple alternative pathways. Following recombination, additional enzymes function to restart replication and reverse some of the adverse genomic effects of recombination. These include systems now associated with recombinational DNA repair, such as the ϕX174-type primosome (perhaps better described as a restart primosome) and the XerCD site-specific recombination system. The result is a repair system encompassing almost every enzymatic function in bacterial DNA metabolism.

Some of the ideas discussed below are also developed in a number of articles, books, and reviews (*6–8, 10, 23–31*). These should be consulted for more detailed discussions of some individual points and alternative views. The recombinational repair system includes a large number of proteins, and a thorough treatment of all of them is not possible here. RecA protein alone is described at some length. Additional information is available elsewhere about the replication fork originating at OriC (*32–38*), the RecFOR proteins (*8, 17, 29, 39, 40*), the RecBCD enzyme (*17, 30, 41, 42*), the RuvABC proteins (*8, 17, 29, 43–45*), the RecG protein (*46, 47*), the Rus protein (*48, 49*), the ϕX174-type primosome (*31*), and the XerCD recombinase (*27, 50*).

A. DNA Damage and Repair at Replication Forks

1. Prevalence of DNA Damage

Although there are very few good direct *in vivo* measurements, DNA damage is common in bacterial DNA, as it is in every genome. Under normal aerobic growth conditions, the best numbers available suggest that an *Escherichia coli* culture suffers 3000–5000 DNA lesions per cell per genera-

tion. Spent culture medium from an *E. coli* culture harvested at $A_{600} = 1.0$ yields sufficient 8-oxo-7,8-dihydro-2′-deoxyguanosine (oxo8dG) to account for several hundred of the corresponding lesions per cell per generation (*51*). Other work indicates that oxo8dG represents about 5% of the oxidative lesions in a typical spectrum of oxidative damage (*52*), providing the final estimate cited above. In the absence of artificial insults, the oxidative damage should be the predominant DNA damage present in the cell. These numbers are quite approximate, and no systematic effort has been made to estimate the overall incidence of damage as a function of growth conditions or relevant mutant background.

2. Link between Damage and Recombination

Much of this damage is repaired via classical pathways of nucleotide and base excision repair. Recombinational DNA repair is required to repair lesions that occur in single-strand gaps (and therefore have no complementary strand to act as a template for accurate excision repair), or where double-strand breaks and cross-links occur (when both DNA strands are damaged). Lesions in single-strand gaps occur most often when a replication fork encounters an unrepaired DNA lesion. Double-strand breaks (DSBs) occur most often when a replication fork encounters a break in one strand, such as the transient breaks introduced when a lesion is undergoing excision repair. We first connect recombination to DNA damage, and then more specifically to the encounter of replication forks with DNA damage.

In general, DNA recombination is initiated at DNA breaks, and breaks are associated with DNA damage in bacteria. However, nothing has yet been found in bacteria that is comparable to the directed double-strand break generation system that operates during meiosis in eukaryotes (*53, 54*). The link between recombination and DNA damage is evident in a wide range of bacterial studies. Most important, the prototypical phenotype of *rec* mutants is an enhanced sensitivity to DNA-damaging agents. A partial listing of other results would include the following events:

1. Mutations in genes such as *ung, xth, lig, polA,* and *dam* cause an increase in the level of DSBs. An increase in DNA breaks produces a hyperrec phenotype (*55, 56*). Strains combining one of these mutations with a *rec* mutation such as *recA, recB,* or *recC* are not viable (*57–61*). Introducing DNA breaks in cells lacking a repair path for them is clearly lethal.
2. Undamaged and unreplicated bacteriophage λ DNA is essentially inert to recombination *in vivo* unless it is damaged prior to infection (*62*).
3. There is a clear correlation between levels of DNA damage and the frequency of homologous genetic recombination (*63–66*).

4. There is no correlation between the levels of RecA protein present in the cell and the frequency of recombination (*62*). Bacterial recombination systems are activated by breaks and gaps, and recombination does not occur in their absence.
5. A wild-type *E. coli* cell can survive treatments that produce 65 or more DNA cross-links. A *recAuvrA6* mutant cell cannot survive one cross-link (*67, 68*).
6. Null *recA* mutants can be grown but have a much reduced viability. Close to half of the cells in an aerobically grown *recA E. coli* culture are dead and about 10% contain no DNA (*69*).
7. The bacterium *Deinococcus radiodurans* is capable of surviving a 1.5 Mrad dose of cobalt-60 radiation. The treatment fragments its DNA, but a sophisticated DNA repair process allows the bacterium to reconstruct its genome and begin again to grow and divide (*70–72*). This repair capacity is completely dependent on the resident *recA* function (*73*).

During conjugation or transduction, the cell is effectively presented with predamaged (cleaved) DNA molecules, with the DNA ends needed to trigger the RecBCD path for recombination. When you take away processes that introduce DNA with free ends into the cell, there is no evidence that homologous recombination ever occurs in an *E. coli* cell without an initiating DNA damage event.

In the absence of artificial insults, oxidative DNA damage provides the major contribution to the initiation of recombination. There are several results that lead to this conclusion:

1. Many of the inviable mutant strains that include a *rec* mutation are able to grow anaerobically (*59, 74*).
2. The oxidative damage observed in a *fur* mutant, which causes an overload of iron and an accompanying oxidative stress, is lethal in a *recA* mutant. However, the *fur recA* double mutant can also be grown anaerobically (*75*). The *fur* mutation leads directly to an increase in the observed levels of chromosomal recombination resulting in sister chromatid exchange (*76*).
3. Perhaps most dramatically, a search for new *rec* mutants that do not carry out recombination between two direct-order chromosomal repeats in *Salmonella* turned up not only the usual *recA* and other *rec* mutants, but also a substantial number of mutations in intermediary metabolism focusing on respiration (*11;* J. R. Roth, personal communication). The elimination of certain genes for citrate cycle and electron transport functions can effectively suppress homologous recom-

bination. This is presumably because the most significant cellular sources of oxygen radicals and other reactive oxygen species involved in oxidative DNA damage are thereby removed.

3. Link between Recombination and Replication Forks

The connection between the induction of recombination and replication forks is also very strong. Replication forks are halted at DNA damage *in vitro* (77–79). The first suggestion that a replication fork might collapse at the site of a DNA strand break came in 1966 (80). This idea was elaborated upon by Skalka, who first suggested that a stalled replication fork might be reassembled via recombination (81). The general idea that replication fork progress is halted by many types of DNA damage is now supported by an array of experimental observations (82). A relatively high dose of UV light triggers a transient pause in DNA synthesis *in vivo* (24).

Replication is required after recombination, as well as before. The induction of origin-independent replication in a recombination-dependent fashion has been associated with the later steps of the repair of collapsed replication forks (83, 84). Replication is restarted with a specialized initiation system requiring PriA and other proteins of the ϕX174-type primosome (26, 31, 85, 86). The RecF and RecR proteins also play a role in replication restart (87).

Some of the links between replication and recombination are merely circumstantial but are nevertheless intriguing. The *recF* gene is cotranscribed with two major replication genes, *dnaA* and *dnaN*. The latter encodes the sliding clamp that renders DNA polymerase III highly processive (88, 89). Similarly, the *recR* gene is part of an operon also including the *dnaX* gene, which encodes the γ and τ subunits of DNA polymerase III (90).

Some of the DNA damage requiring recombinational DNA repair is not readily observed under normal growth conditions unless DNA is being actively replicated. For example, unrepaired DSBs appear at elevated levels in *recBC* mutants, but only if the chromosomal DNA is being replicated (91). Replication can also enhance the requirement for recombination enzymes for particular repair situations. The repair of DNA gaps is *recA* dependent only during chromosome replication (92, 93).

DNA gaps or processed DSBs provide loading points for RecA protein, and can thereby lead to the induction of the SOS response (10, 24, 25). Studies of the requirements for SOS induction can therefore reinforce the link between the appearance of SOS-inducing signals and replication. Replication is required for the induction of SOS (25, 94). DNA damage alone is insufficient (95, 96).

The same RecA protein that binds to single-stranded DNA and induces the SOS response also plays a direct role in repair. It is the recombination activities of RecA that are required for recombinational DNA repair, as opposed

to the RecA-mediated induction of repair functions associated with the SOS system (*64, 97, 98*). As elaborated upon below, recombinational DNA repair is needed under normal growth conditions, and does not require the induction of SOS.

B. Pathways

1. Overview

There are at least two major pathways for recombinational DNA repair (Fig. 1). These are distinguished primarily according to the recombination functions involved. The pathways approximately parallel the RecBCD and

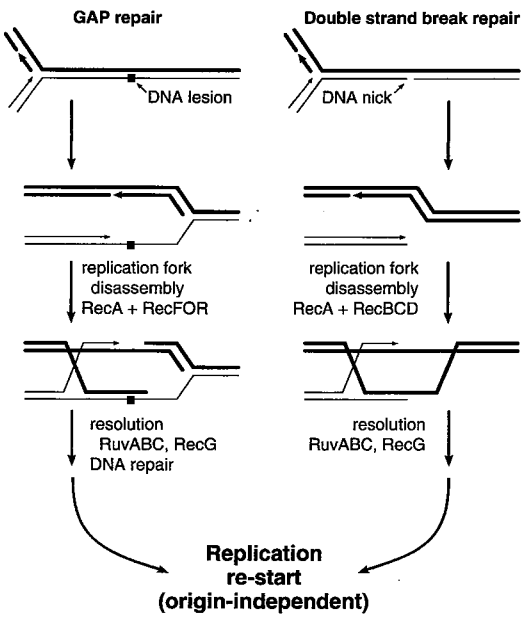


FIG. 1. Pathways for recombinational DNA repair. The two paths shown are distinguished by the recombination functions involved. If the replication fork encounters an unrepaired DNA lesion, a lesion-containing gap is created that is repaired by the RecFOR pathway. If the replication fork encounters a lesion undergoing repair such that there is a break in one DNA strand, a double-strand break is created that is repaired by the RecBCD pathway. In both cases, the replication fork must yield the DNA to the recombination enzymes, and that may usually involve disassembly of the replication fork. After the recombination steps are complete, both pathways require replication restart mediated by PriA protein and the ϕX174-type primosome, which recognizes and assembles on recombination intermediates. One consequence of recombinational DNA repair is the creation of chromosomal dimers, which must be resolved prior to or during cell division by the XerCD site-specific recombination system. (From Ref. *165*. Copyright 1997 National Academy of Sciences, U.S.A.)

RecF pathways defined by Clark and co-workers for conjugational recombination (*8, 99, 100*). However, the pathway hierarchy may be quite different. In conjugational recombination, the RecBCD pathway is clearly predominant, with the RecF pathway generally evident only in mutant backgrounds whereby RecBCD is inactive and the *recBC* defect is suppressed (*8, 15, 100*). Studies based on this hierarchy are directly responsible for the definition of alternative recombination pathways and for the discovery of many recombination functions. However, conjugational recombination is quite rare in natural bacterial populations (*101, 102*). Analysis of recombination between direct chromosomal repeats in *Salmonella*, which is almost certainly initiated by DNA damage, suggests more of a parity between the RecBCD and RecF pathways for recombination needed in moment-to-moment DNA metabolism (*11*). RecF protein is more important than RecBCD in the RecA-dependent repair of lesions generated by UV light in replicated DNA (*93*). An analysis of chromosomal recombination resulting in sister chromatid exchange associated with recombinational DNA repair in *E. coli* also indicates that the RecF pathway is as important as the RecBCD pathway in this context (*76*). The work on conjugational recombination clearly underestimates the importance of the RecF pathway in bacterial DNA metabolism (*11, 76, 93, 103*).

If the replication fork encounters an unrepaired DNA lesion, a gap in the DNA is generated and the gap repair process involves the RecF, RecO, and RecR protein functions. This path parallels the RecF pathway for recombination. If the replication fork encounters a break in one strand (as might be the case when a lesion was undergoing repair), a double-strand break is generated. In this situation, the RecBCD pathway predominates. There are doubtless many variations of the pathways of Fig. 1, adapting to the DNA substrates presented for repair. The RecBCD pathway of recombination must predominate during conjugation simply because of the DNA substrates—DNA ends—presented to the cell.

When bacterial cells are subjected to abnormally high levels of DNA damage, the SOS system of repair processes is induced. This includes a new and RecA-dependent mechanism for the replicative bypass of DNA lesions. Unlike the process we are referring to as recombinational DNA repair, the replicative lesion bypass process is highly error prone. This and other processes associated with the SOS system are beyond the scope of this review, and the reader should consult other sources (*24, 25, 79, 104–106*) for information.

2. DNA Damage and the Fate of Replication Forks

Some potential variations in the pathways may arise depending upon the actual fate of the replication forks upon encountering a break or lesion. In principle, a replication fork might stall and/or disassemble. Alternatively, the

same replication complex might reinitiate synthesis beyond the lesion and continue on (Fig. 2). *In vivo,* there is no direct evidence for or against replication fork collapse brought on by an encounter with DNA damage of any type, presumably because the experiment is very hard to do. Nonetheless, it seems quite reasonable to suppose that the replication fork collapses upon encountering a DNA break (*82*). In addition, some DSBs are created at the sites of stalled replication forks (*91, 107, 108*). Whether the replication fork always halts at the site of an unrepaired lesion, or instead bypasses it as in Fig. 2, is less clear. Replicative lesion bypass in this context, to the extent that it occurs, should not be confused with the mutagenic lesion bypass that is observed when the SOS system is induced. Under normal growth conditions, bypass would generally mean reinitiation of DNA synthesis downstream of the lesion, leaving the lesion in a single-stranded gap. Recombinational DNA repair would then provide a pathway for accurate repair.

The examination of DNA following UV irradiation suggests that the daughter strands have gaps at intervals approximating the location of lesions on the template strands (*109–112*). The result suggests that replication somehow bypassed the lesions, leaving them in the gaps, and gap repair is generally presented as a postreplication process between the gapped DNA and another homologous duplex away from a replication fork. However, heavy UV irradiation leads to a rather general cessation of DNA replication, indicating a problem with lesion bypass (*24*), and RecA protein is required for recovery of DNA replication (*113, 114*). Much of this cessation of DNA replication could reflect collapse of forks at DNA breaks, although *recB* function is not required for replication recovery (*113*). Work done *in vitro* indicates that

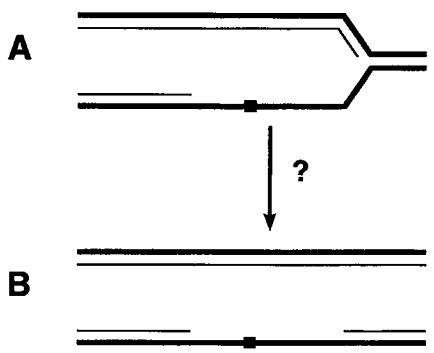


FIG. 2. Alternative fates for the replication fork. Upon encountering a DNA lesion, the replication fork might (A) stall and disassemble or (B) prime and reinitiate DNA synthesis downstream. In either case, the lesion is left in a single-strand gap. If the replication fork stalls, the DNA branch remains nearby and might affect the course of repair (see Fig. 3B).

DNA polymerases are halted by lesions on the template strand (*77–79*). *In vivo,* the blocking DNA lesions halt DNA replication on plasmid DNAs. Mutagenic bypass of these lesions does not occur unless the SOS system is induced (*115, 116*). Under normal growth conditions, when no SOS-inducing treatments have been applied, replication forks should usually halt at DNA lesions.

If lesions are bypassed to any extent, how might this take place? On the lagging strand, discontinuous DNA synthesis might permit priming and reinitiation of replication away from the lesion, providing a potential avenue for some bypass. However, it is hard to envision a bypass mechanism for continuous synthesis on the leading strand. The answer suggested by some workers is that both DNA strands are synthesized discontinuously *in vivo* (*117–120*), as reviewed by Kuzminov (*10*). In the published studies of discontinuous DNA synthesis *in vivo* (*117–120*), DNA in growing cells was briefly pulse labeled, usually with [^{3}H]thymidine. The DNA was then isolated and centrifuged in alkaline sucrose gradients. Well over half the label was found in short, Okazaki-fragment-sized DNA pieces, rather than the 50% in large DNA fragments predicted if synthesis on the leading strand were continuous. In *polA* mutants, short fragments have been shown directly to arise from the leading strand (*119*). If this interpretation is correct, the discontinuous DNA synthesis could provide a potentially nonmutagenic mechanism for lesion bypass on both DNA strands.

However, discontinuous DNA synthesis is not the only possible explanation for the generation of short DNA fragments during replication on the leading DNA strand. Short fragments could be largely accounted for by uracil incorporation into the DNA. Under normal growth conditions, the presence of low levels of dUTP in the *E. coli* cell leads to the incorporation of about one U residue for every 2000–3000 nucleotides of newly synthesized DNA (*121*). The rapid repair of this DNA via a base excision pathway may make a significant contribution to the number of short fragments originating from both leading and lagging strands (*121, 122*). The number of short fragments is greatly increased and they are much longer lived in *lig* or *polA* mutants (used in many of the studies exploring discontinous DNA synthesis *in vivo*), because the enzyme products of both of these genes are needed for the latter stages of the base excision repair pathway (*121, 123, 124*).

The *in vitro* work is unambiguous on this issue, and clearly supports continuous DNA synthesis on the leading strand with discontinuous synthesis only on the lagging strand (*32, 38, 125, 126*). Continuous DNA synthesis on the leading strand is also seen in work done on cellophane disks, whereby the cells are gently lysed and essentially all cellular macromolecules are retained in the experiments (*127*). The report by Louarn and Bird (*119*) indicates that on bacteriophage λ templates, DNA synthesis is normally discon-

tinuous on the lagging strand only. Shorter fragments are seen on the leading strand as well in a *polA* strain, but the discontinuities occur up to 40 times less frequently than on the lagging strand. As already noted, the fragments seen in the *polA* strain could as easily arise from DNA repair processes as from Okazaki fragment processing within a discontinuous DNA synthesis mechanism.

Other factors may also contribute to the apparent generation of short DNA fragments *in vivo*. In the cell, the incorporation of a brief pulse of label into the leading strand might generate a broad range of labeled DNA sizes in a bacterial population, as long as at least some were spared the scissions associated with DNA repair processes. The distribution of these longer DNAs over an alkaline sucrose gradient might lead to an underestimation of their contribution compared to the more discretely sized Okazaki fragments from the lagging strand and repair processes. It is also unclear to what extent the *in vivo* replication work is affected by replication blockage associated with other repair processes, such as base excision repair of altered bases other than uracil, or even recombinational DNA repair. Slow-migrating labeled material from the *in vivo* experiments could be generated by damage to the DNA inflicted by experimental procedures, and the incorporation of label into non-DNA macromolecules. Whether in the context of leading-strand DNA synthesis (*117–120*) or the DNA present after UV irradiation (*109–112*), all of these factors make the DNA fragment sizes seen *in vivo* somewhat difficult to interpret at best. There is no clear evidence for discontinuous synthesis of DNA on the leading strand during replication, but further work on this question may be useful.

At present, it is probably reasonable to assume that both of the situations laid out in Fig. 2 occur to some extent. Replicational lesion bypass (leaving behind a gap opposite the lesion) may be possible in some cases without the aid of other proteins, at least on the lagging strand. Future work may help determine how repair events partition between these alternatives, and how that partitioning is affected by growth conditions and the type of DNA lesion involved. In addition, much of the *in vivo* work to date has focused on lesions easily generated in the laboratory, such a pyrimidine dimers. More work is needed to determine the fate of replication enzymes that encounter oxidative lesions and DNA breaks *in vivo*.

3. The Recombination Steps

Once the replication fork has halted or bypassed a lesion, the recombination functions must be loaded onto the DNA at the site of the DSB or the DNA gap. In particular, RecA protein must be loaded onto the DNA to promote the key DNA pairing steps. For gap repair, RecA loading is modulated by the RecF, RecO, and RecR proteins (*39, 40, 128*). For DSB repair, the

RecBCD enzyme facilitates the loading of RecA protein onto the prepared ssDNA (*129*). The RecA protein promotes DNA pairing and formation of a Holliday structure. Additional enzymes, including the RuvA, RuvB, and RecG proteins, bind to the Holliday junction and promote additional branch migration (*43, 44, 130–133*). The Holliday junction can be resolved by one of at least two Holliday junction resolvases, the RuvC and Rus proteins (*48, 49, 134–137*). Alternatively, the Holliday structure might be resolved by reverse branch migration (*131, 138*).

For gap repair (Figs. 1 and 2), a complementary strand must be transferred to the DNA in the gap. This DNA comes from the template strand on the opposite side of the replication fork. Whether or not the replication fork has bypassed the damage, the usual pathway envisioned is one requiring that a break be introduced into the transferred strand by an endonuclease. The hypothetical endonuclease has not been identified. In principle, a topoisomerase could facilitate the transfer without an endonuclease. In addition, the reverse branch migration pathway (*131, 138*) provides an alternative path that does not require the action of an endonuclease (Fig. 3). The replication complex would presumably have to disassemble, and recombination enzymes would then effectively reverse the replication process via branch migration. The newly synthesized strands would be displaced and a Holliday junction would be created as the branch moved past the lesion. The free 3′ end would serve as a primer for DNA polymerase I, using the undamaged DNA as a template to fill the gap. Another reverse branch migration, perhaps promoted by the RecG helicase, would resolve the Holliday junction and provide a site for replication restart, without utilizing RuvC or Rus. Notably, this pathway could only be available if the replication fork stalled at the lesion and reinitiation of replication did not occur until the recombination steps were completed. Similar models have been used to account for translesion DNA synthesis in *E. coli* (*108, 115*), as well as recombination-dependent replication in microorganisms (*139, 140*).

4. The Completion of DNA Replication Following Recombination

Once the recombination phase is complete, a new replication fork must be assembled. This must occur away from the bacterial origin of replication, at essentially any site in the chromosome where repair may have taken place. Replication restart involves a specialized replication initiation complex, much of it consisting of the proteins formerly known as the ϕX174-type primosome. Much of the work leading to this conclusion, as well as the properties of the primosome, are reviewed elsewhere in this volume (*31*), and will only be summarized here. The emphasis in this discussion is on a scheme in which replication restart follows recombination. However, it should be not-

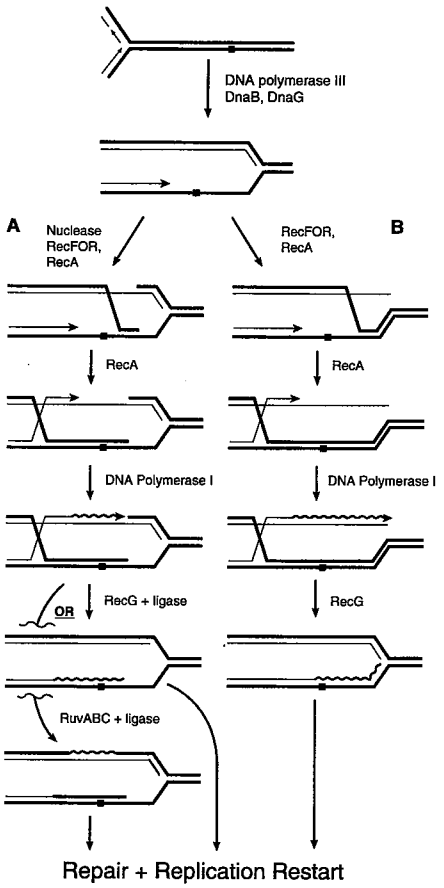


FIG. 3. Two possible paths for gap repair. These differ in the mechanism by which a complementary strand is provided for the lesion-containing gap. (A) An endonuclease (not yet identified) cleaves the template strand on the opposite side of the replication fork. This strand is then transferred to the gap by the action of RecA and other recombination enzymes. (B) A copy-choice/reverse branch migration path. After replication fork disassembly, branch migration is used to transfer the strand complementary to the gap without endonuclease action. Once the branch migration has proceeded beyond the lesion, DNA polymerase I would then fill in the gap, using the undamaged DNA strand as a template. The replication fork is then set up for reinitiation by reverse branch migration of a type catalyzed by the RecG helicase. This pathway is available only if the replication fork remains nearby after encountering the lesion as in Fig. 2A.

ed that if replication is restarted prior to recombination, such as to leave behind a DNA gap (Fig. 2), then the systems described below might take part in that process as well.

Primosome assembly on ϕX174 DNA requires seven different proteins: PriA, PriB, PriC, DnaB, DnaC, DnaG, and DnaT (*32, 33*). The DnaB protein is a helicase required at the replication fork. The DnaG protein is the lagging-strand primase. These two proteins, along with the DNA polymerase III holoenzyme, are the only ones required for chromosomal replication originating from OriC (*141*). The DnaC protein is required for assembly of the replication fork at OriC. The remaining four proteins, all produced by *E. coli* and implicated in replication, were left without clear functions in chromosomal DNA replication. This observation led to questions about the function of the seven-protein primosome.

An examination of the phenotypes of mutants lacking one of these functions, in particular PriA, has implicated the larger primosome in the restart of DNA replication following the recombination phase in recombinational DNA repair. The *priA* null mutant strains are not deficient in OriC-dependent replication. Instead, the *priA* strains are constitutively induced for SOS, and exhibit substantial defects in recombination and recombinational DNA repair processes (*142, 143*). This led to the suggestion that replication forks originating at OriC often did not complete replication, and that a new complex requiring PriA was needed to complete the process (*26*). This was elaborated to include a need to assemble replication complexes on recombination intermediates (*83, 142*). The idea was neatly borne out by experiments *in vitro* demonstrating that PriA protein facilitated the assembly of new replication forks at branched DNA structures that provide models for recombination intermediates (*85, 86*). The branched DNA intermediates created by recombination are preferred sites for the PriA-directed primosome assembly (*85;* K. Marians, personal communication). The ϕX174-type primosome is thus gradually being revealed as a specialized complex directing the origin-independent assembly of replication forks for the purpose of replication restart following recombination.

The DNA polymerase II of *E. coli,* whose function in DNA metabolism has long been obscure (*25, 33*), may also have a specialized function in the restart of replication following DNA damage and recombinational DNA repair. After UV irradiation of cells, replication restart is delayed by 45 min in a *polB* deletion strain (S. Rangarjan, R. Woodgate, and M. F. Goodman, personal communication). Replication restart in irradiated cells is blocked altogether in *polB umuCD* double mutants (S. Rangarjan, R. Woodgate, and M. F. Goodman, personal communication). Expression of the *polB* gene is induced by the SOS response. It is unclear whether DNA polymerase II functions in the context of error-prone replication during SOS, plays a role in error-free replication restart associated with recombinational DNA repair, or both.

Origin-independent but recombination-dependent DNA replication processes, called induced and constitutive stable DNA replication (abbreviated iSDR and cSDR, respectively), have been characterized extensively by Kogoma and co-workers (*28, 83*). These replication modes are most readily observed after the induction of SOS. They generally require the central recombination activities such as RecA protein, as well as the components of the ϕX174 primosome, clearly associating them with recombinational DNA repair (*28, 83, 84, 143, 144*). It seems very likely that these replication processes are manifestations of the general cellular system for origin-independent replication restart that must occur as part of recombinational DNA repair.

5. Effects of Recombinational DNA Repair on Chromosome Partitioning at Cell Division

Recombinational DNA repair involves the transient formation of Holliday junctions. Resolution of these structures can lead to the formation of chromosomal dimers that cannot be partitioned into daughter cells at cell division (Fig. 4). The conversion of chromosome dimers to monomers is mediated by a specialized site-specific recombination system in *E. coli*, the XerCD system (*27*). Mutations that inactivate the XerCD site-specific recombinase, or its chromosomal binding site called *dif*, lead to a lengthening of the average cell, severe filamentation of a substantial fraction of the cells, and abnormal nucleoids (*145, 146*). These and other observations have led several groups to propose that the function of the XerCD system is to convert chromosomal dimers, resulting from recombination, to monomers (*10, 27*). The requirement for Xer-mediated recombination is reduced greatly in *recA*$^-$ cells, reinforcing a connection between the XerCD system and recombinational DNA repair (*145, 146*). Using a variety of growth conditions and mutant strains, it is possible to demonstrate a direct relationship between the levels of recombinational DNA repair and the requirements for Xer-mediated site-specific recombination (*76*).

Within the *E. coli* chromosome, the *dif* site functions only when positioned within a relatively short region near the terminus of replication (*147–150*). In this location, it can be replaced by some other site-specific recombination systems (*147*). When replication is complete, site-specific recombination at *dif* sites might proceed repeatedly, with final chromosome segregation occurring at a point where the chromosomes are monomeric. Such a model works if the *dif* sites are positioned within the last part of the chromosome to be separated at segregation (*151*). The detailed *in vivo* function of the XerCD system may also rely upon the properties of at least some sequences surrounding *dif* (*149*).

The evolution of this system to repair a secondary but potentially detri-

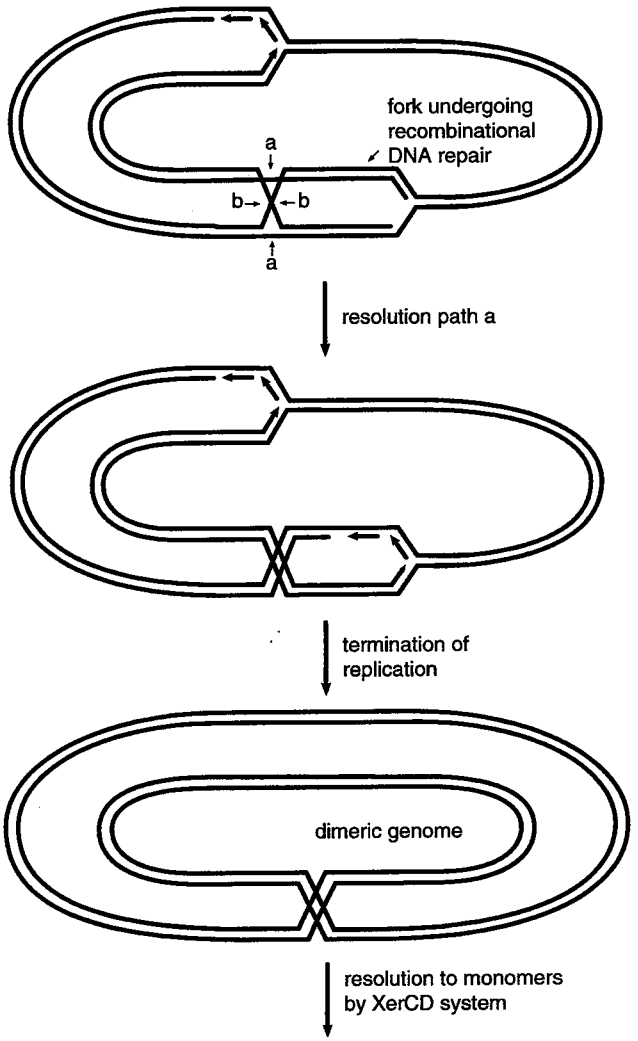


FIG. 4. Creation of chromosomal dimers as a by-product of recombinational DNA repair, and their resolution by XerCD. Holliday structures will be created in the course of recombinational DNA repair (Figs. 1 and 3). Those that are resolved by RuvC or Rus may be cut so as to create a cross-over. This in turn creates a chromosomal dimer upon completion of replication. Chromosomal partitioning requires the monomerization of the dimer by the XerCD site-specific recombination system. (From Ref. 7, by permission of Blackwell Science Ltd.)

mental effect of recombinational DNA repair helps to underscore the importance to bacterial DNA metabolism of the pathways shown in Fig. 1.

C. Frequency of Recombinational DNA Repair

Quantifying the frequency of recombinational DNA repair is increasingly feasible. Estimates can be derived from a variety of direct and indirect observations. Most of the best clues can be derived from studies of cells lacking one or more of the recombinational repair functions mentioned above. The presence of stalled replication forks, unrepaired DSBs, or dimeric chromosomes leads to a number of chromosome and cellular conditions easily scored in a light microscope, including abnormal nucleoids, lengthened cells, and filamented cells. We have already suggested that at least 10–30% of the replication forks originating at OriC are halted by DNA lesions or breaks before they complete their synthetic assignment. This estimate is derived from cells with defects in one or more steps of the recombinational repair process described above, and is probably quite conservative.

If a replication fork encounters a lesion at a stage in repair in which a DNA strand break exists, the encounter will result in the formation of a double-strand break that cannot readily be repaired in a *recA* or *recBC* mutant. A quantification of linearized *E. coli* chromosomal DNA (*91*) indicated that double-strand breaks appeared in about 15% of the chromosomes of a *recB* or *recC* mutant, and over 20% of the chromosomes from a *recA recD* double mutant. In this study, cells were grown aerobically in a minimal medium. The frequency of double-strand breaks can be interpreted as one measure of the encounters between replication forks and genomic strand breaks. Some fraction of these breaks may also arise at the sites of replication forks stalled at DNA lesions (*108*) [see Kuminov (*107*) for a review]. Inasmuch as DSBs may not be associated with every stalled replication fork, these studies suggest that 10–20% should be regarded as a lower limit for the premature disruption of replication forks originating at OriC.

Additional studies of RecA-deficient cells yield at least qualitatively consistent information, but tend to support more frequent stalling and collapse of replication forks. A defect in DNA replication and cell division in *recA* cells was first reported by Inouye (*152*). An examination of the cells in a *recA* mutant strain suggests major problems in the completion of DNA replication. Over 50% of the cells in a culture of a *recA* null mutant are nonviable under some normal growth conditions (*69*). The frequency of productive replication initiation at oriC (producing complete chromosomes) is significantly reduced (*153*). More than 10% of *recA* cells contain no DNA (*154, 155*), and many more have abnormal numbers of chromosomes (*153, 156*). The observation of anucleate cells has been explained as reflecting a defect in chromosomal partitioning (*154*), but chromosome loss due to the degradation of chromosomes with breaks is equally plausible and provides a quantitative explanation for the appearance of anucleate cells as well as those with odd

numbers of chromosomes (*156*). These studies were done with bacterial cells grown aerobically in a variety of standard media. As already noted, oxidative DNA damage is the major source of replication-halting lesions in cells grown under standard laboratory conditions.

If a *ter* site for replication termination is placed in the *lac* operon, well away from its normal position in the terminus region, replication forks are forced to halt at a higher frequency than normal (*155*). This results in constitutive induction of the SOS response and cell growth becomes almost completely dependent on RecA function (*155*).

If replication restart is disrupted, a similar picture again emerges. However, studies of *priA* mutants again suggest a much higher frequency of fork collapse. Cells that entirely lack PriA function exhibit a viability about 1% that of wild-type strains, under normal growth conditions. The SOS response is expressed constitutively (*157*), suggesting that the cellular RecA protein is always bound to DNA (as would be the case at stalled replication forks). The cells form long filaments and are very difficult to grow (*142, 144, 157*). They rapidly pick up suppressor mutations in the *dnaC* gene (*142, 144*), bypassing the requirement for PriA in the assembly of the ϕX174-type primosome at branched DNA recombination intermediates (*86*). The properties of *priA* mutants suggest that a vast majority of replication forks originating at OriC are halted by DNA damage, even when no external DNA-damaging treatments are applied (*86*). In the mutant cells, recombination is carried out to effect DNA repair, but replication cannot be restarted.

If a high proportion of replication forks undergo recombinational DNA repair at some point in their travels, and the absence of RecBC function results in unrepaired DSBs appearing in only 10–20% of the cells, then significant fractions of the stalled replication forks are repaired without the aid of the RecBCD pathway. Compared to *recBC* cells, a much more extreme growth phenotype is observed when both the RecBCD and RecFOR paths for the recombination portion of recombinational DNA repair are genetically blocked (*158, 159*). Cell viability and other phenotypes now more closely match that seen with the *priA* mutants. These results again argue for very high-frequency replication fork collapse. They also reinforce the notion that there is a variety of structures that might appear at a stalled replication fork and multiple ways to repair them.

Null *priA* mutations appear to be even more compromised than *recA* null mutations, suggesting that there are at least some pathways for recombinational DNA repair that do not require RecA protein. RecA-independent recombination between nearby repeated sequences in the genome is readily detected, most if not all of it associated with replication (*160–162*). Some of this may involve the RecE (+ RecT) pathway of recombination (*8, 163*), or a process such as replicational slippage or copy choice (*160–162*). There is also an SOS-inducible pathway for recombination that suppresses the lethality of

recA polA double mutants (*84*). It is interesting to speculate that the SOS-inducible functions are the RuvA and RuvB proteins. In principle, the RuvAB proteins could mediate recombinational DNA repair via a pathway like that in Fig. 3. The process might not be as unidirectional and thus not as efficient as it would probably be with RecA protein, but the substitution of RuvAB in a pathway like this might rescue stalled replication forks in many cells even in the absence of SOS induction.

The relative severity of the *priA* phenotype probably reflects the involvement of the ϕX174-type primosome in replication restart regardless of the recombination path employed. In contrast, the absence of a particular *rec* function (with the possible exception of RecA protein) would affect only a subset of the available recombinational DNA repair pathways and thus would often lead to less severe deficiencies. For recombination functions that might play auxiliary roles, such as the RecFOR functions, the affected pathways may be leaky to the extent that RecA can carry out DNA strand exchange, and replication restart can occur in their absence. This could also lead to an underestimation of the importance of a given pathway to DNA metabolism as a whole.

Some of the most quantitative results come from an examination of the frequency of XerCD resolution of dimeric chromosomes in *E. coli* cell cultures by Kuempel and co-workers (*164*), using a density label assay. In cells grown in a rich medium under normal aerobic conditions, about 14% of the cells per generation required XerCD resolution of chromosomal dimers created by homologous recombination (*164*). This actually translates into a frequency of about a 17% for recombination to generate dimers (sister chromatid exchange; SCE), because double cross-overs will occur in 0.17×0.17, or 3%, of the chromosomes and cancel each other out (*164*).

In a follow-up study, the same workers (*76*) examined the effects of a range of recombination and repair mutations on the SCE frequency as measured by the frequency of required XerCD recombination. The baseline SCE frequency in cells growing in a rich medium was 15.6%, similar to that in the earlier work. A minimal medium with an acetate carbon source resulted in a decrease to about 10.5%. The increase in double-strand breaks associated with *dut, uvrD,* and *polA* mutants brings about an increase in SCE frequency to 23–31% (with different growth media having a modest effect). Oxidative DNA damage has a major role in triggering the SCE events, because the frequency increases to nearly 25% in a *fur* mutant (*76*). As expected, the elimination of *rec* functions decreases the observed frequency of SCE. The exchanges are virtually eliminated in both *recA* and *recB recF* mutant strains. Alone, both *recF* and *recB* null mutations eliminated about half of the SCE events, again indicating that the two recombination pathways are about equally important in this context (*76*).

It is important to emphasize that the measured frequency of sister chro-

matid exchange necessarily underestimates the frequency of recombinational DNA repair. The generation of a cross-over should depend largely on the resolution of Holliday junctions by RuvC, Rus, and perhaps other enzymes. If only half of the cleaved Holliday structures result in cross-overs, then the frequency of recombinational DNA repair is at least twice the frequency of sister chromatid exchange. If the resolution of Holliday junctions were skewed to favor non-cross-overs, the frequency of recombinational DNA repair would be further underestimated. If other pathways are available for Holliday junction resolution that do not involve cleavage by an enzyme such as RuvC or Rus, then the events that use these pathways would also not appear in the sister chromatid exchange data.

Overall, the results suggest that a large fraction of replication forks originating at OriC, perhaps one or more per cell, are halted at some point and undergo recombinational DNA repair. In the absence of unusual cellular insults, about 15–20% of the cells have a replication fork that encounters a break requiring repair by the RecBCD pathway (*91*). About an equal number have forks that encounter lesions requiring repair by the RecFOR pathway (*11, 76*). Many of these events generate Holliday junctions that are resolved by RuvC or Rus, and thus show up in the SCE frequency data (*76, 154*). To account for the very low viability of *priA, recA,* and *recBC recF* mutant strains, it seems likely that additional forks undergo recombinational DNA repair by a pathway that does not involve resolution by RuvC or Rus, perhaps involving a reverse branch migration mechanism like that in Fig. 3. Some of these events occur even in the absence of RecA protein. The 10–30% per generation estimate offered at the beginning of this discussion for the rate of replication fork collapse must be viewed as a very conservative minimum.

Recombinational DNA repair thus occurs often, and is a moment-to-moment problem with which the cell must deal. Conjugational recombination occurs rarely, is unessential for survival of the cell, and may merely be an incidental by-product of the cellular recombinational DNA repair processes (*7, 155, 165*).

D. Bacteriophage T4 Provides Another Look at a System in Which Replication and Recombination Are Tightly Coupled

Whereas the integration of DNA metabolism seen in bacterial recombinational DNA repair has become broadly apparent only over the past decade or so, a similar integration of replication, recombination, and repair has long been evident in the DNA metabolism of bacteirophage T4. There are robust pathways for recombinational DNA repair of bacteriophage T4 DNA. At some stages of a T4 infection, DNA replication is largely initiated by recom-

bination. These pathways have been thoroughly reviewed elsewhere (*166–168*). The importance of T4 as a model system for DNA metabolism should only be enhanced as analogous bacterial pathways for recombinational DNA repair are unraveled.

II. The RecA Protein

The RecA protein of *E. coli* is a multifunctional protein, with role sin the induction of the SOS response to DNA damage, SOS mutagenesis, homologous genetic recombination, and recombinational DNA repair (*7, 8, 17, 29, 169–171*). The reader is referred elsewhere for information on the coprotease activity of RecA protein, involved in the induction of SOS (*104, 105*), and the mutagenic lesion bypass function associated with SOS induction (*79, 106*). This discussion focuses on the recombination functions involved directly in recombinational DNA repair.

The RecA protein is found in virtually all bacteria, and an activity of this type has been a part of bacterial physiology for over 1.5 billion years (*29, 171, 172*). True structural and functional analogs of RecA have been found in bacteriophage (*173*), in archaea (*174, 175*), and in eukaryotic cells (*176–182*). Homologous genetic recombination and recombinational DNA repair appear to be ubiquitous processes.

A. Activities Overview

In bacteria, RecA promotes the central steps in recombination: alignment of homologous DNAs, strand exchange to establish a cross-over, and branch migration. RecA protein promotes a DNA strand exchange reaction *in vitro* that mimics these postulated central steps in homologous genetic recombination *in vivo*. The reaction is stimulated by the single-stranded DNA-binding protein of *E. coli* (SSB). RecA is also a DNA-dependent ATPase, and some aspects of the DNA strand exchange reaction require ATP hydrolysis.

The most common model reactions used for *in vitro* studies of RecA protein-mediated DNA strand exchange are outlined in Fig. 5. DNA substrates are generally derived from bacteriophage DNA and the reaction can involve either three or four strands. Two decades of research in many laboratories have provided much information about this reaction (*8, 17, 29, 169, 170*). In the first step (not shown in Fig. 6), a RecA helical filament forms on the ssDNA (or the gapped duplex DNA in B), assembling in the 5′ to 3′ direction relative to the ssDNA or the single-stranded gap (*183*) (Fig. 6). This filament contains one RecA monomer per three DNA nucleotides or base pairs. A 24-monomer segment of a RecA filament (without bound DNA), developed from the structure published by Steitz and co-workers (*184, 185*), is shown in Fig.

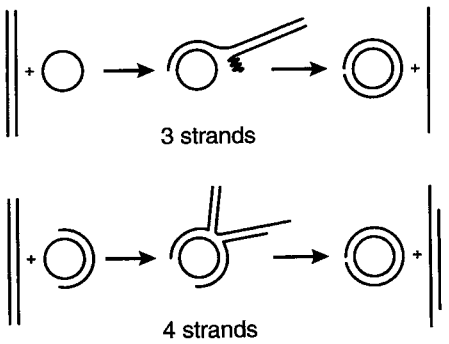


FIG. 5. The common DNA strand exchange reactions promoted by RecA protein *in vitro*. The RecA filament is formed on the circular single strand or the gapped circular duplex. In the four-stranded reaction, both RecA filament assembly and DNA pairing are initiated in the single-strand gap.

6. The nucleoprotein filament then binds to a second homologous duplex DNA and aligns it with the first. A strand switch occurs to form a region of hybrid duplex. When ATP is hydrolyzed, this process is propagated unidirectionally until strand exchange is completed, 5′ to 3′ relative to the ssDNA within the original nucleoprotein filament. RecA protein hydrolyzes ATP during this reaction with a monomer k_{cat} approaching 20 min^{-1}. In unidirectional DNA strand exchange, the ends of the homologous linear duplex where DNA strand exchange begins and terminates are sometimes referred to as the proximal and distal ends, respectively.

B. Structure and Properties

An *E. coli* RecA protein monomer is a polypeptide with 352 amino acid residues with a total M_r of 37,842. Bacterial strains engineered to produce the RecA protein at very high levels are widely available. Purification is straightforward (*186–190*), although a persistent contamination with exonuclease I can be a problem (*191*). The protein is quite soluble in aqueous buffers, and stable for many months if stored at −70°C. The isoelectric point of the purified protein has been measured in at least six studies, giving p*I* values form 5.0 (*192*) to 6.2 (*193*). Combining all the measurements (*192–197*) gives both an average and a generally representative p*I* of 5.6. In solution, the protein has a tendency to aggregate into oligomers, filaments, and bundles of filaments (*195, 198–202*). The aggregation state of a given solution of the protein is affected by pH, salt concentration, and temperature. Concentrated solutions of the protein can appear almost opalescent as a result of light scattering by filamentous structures.

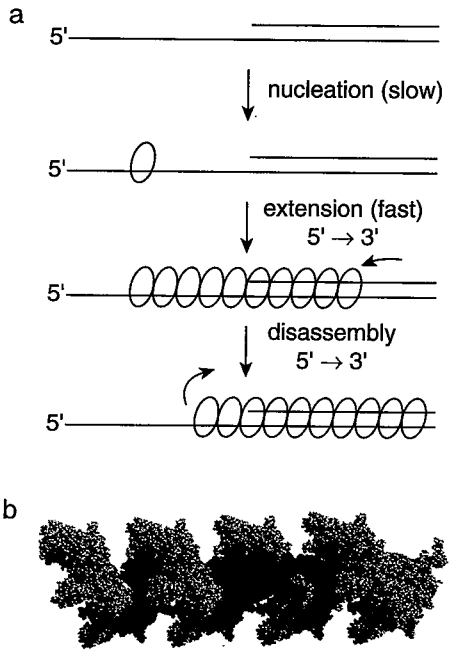


FIG. 6. RecA filament assembly and disassembly. (a) The rate-limiting step in assembly is nucleation, and the best nucleation site is a single-stranded DNA. Extension of the filament occurs 5′ to 3′ relative to the single strand. On gapped DNA, extension will continue and envelop any contiguous double-stranded DNA. Disassembly also occurs 5′ to 3′ on ssDNA, such that filaments grow on one end and are shortened on the other. Filament extension is generally much faster than end-dependent disassembly. Assembly of a filament in its active state requires ATP but not its hydrolysis. End-dependent DNA disassembly is coupled to ATP hydrolysis. (b) A portion of a RecA filament (24 monomers total) based on the structure of Story and Steitz (*184, 185*).

The structure of RecA protein without DNA bound has been determined at 2.3 Å resolution (*184, 185*). In each monomer, there is a major central domain flanked by two smaller subdomains at the N and C termini. Monomers in the crystal are packed to form a continuous spiral filament, with six monomers per right-handed helical turn (Fig. 6). These results are consistent with other studies showing that RecA protein forms a helical nucleoprotein filament on DNA, also with six monomers per helical turn. The filament seen in the crystal exhibits a deep helical groove. The structure was solved in two forms, one with bound ADP (*185*) and the other with no nucleotide (*184*). Both are highly informative. However, the filament seen in the crystal is not as extended as that observed on DNA in the presence of ATP, and hence the structure may reflect an inactive form of the protein (*177, 203–206*).

The availability of *recA* sequences from many bacterial species, as well as from a variety of archaeans and eukaryotes, has led to extensive sequence alignment efforts (*29, 171, 172, 207*). These sources may be consulted for a more detailed structural analysis. The RecA proteins from bacteria are all closely related, with none having less than a 49% amino acid sequence identity to the *E. coli* RecA protein. In one comparison of 64 bacterial *recA* genes (*29*), there were 59 invariant residues and another 100 that could be classified as chemically conserved. The protein has a binding site for ATP and less well-defined binding sites for up to three strands of DNA withn the helical groove. Many of the conserved residues are involved in the binding of DNA or ATP, or are at the monomer–monomer interface in the filaments. The RecA protein also has a novel conserved structural motif, called the MAW motif (residues 42–65), that is conserved among all *recA* homologs (*29*). The MAW motif may be involved in an ATP-induced conformation change.

Most of the central core domain of RecA protein, from residues 61 to 246, exhibits an intriguing structural similarity to the F1-ATPase, even though the two proteins share very little sequence similarity (*208*). The same structural folding pattern is seen in hexameric helicases (*208–210*). Although the functional significance of this structure is not understood in any detail, all of these proteins can be considered rotary ATPase motors. The case for the assignment of a motor function to the RecA protein is made below.

C. Mutant RecA Proteins

The *E. coli* RecA protein has been subjected to extensive mutational analyses, with hundreds of mutants now available (*29, 172*). Particularly comprehensive efforts at mutational investigation of RecA structure/function have been carried out by Knight and co-workers (*20, 211–213*). Only a few RecA mutants that have been or are likely to be especially useful in mechanistic studies, RecAK72R, RecAH163W, RecAY103W, RecAY264W, RecA441, and RecA803, will be considered here.

RecAK72R is one of the best characterized RecA mutant proteins (*214–216*). The mutation is at a conserved site in the Walker A box (or P-loop) for ATP binding (*217, 218*). The mutant protein binds but does not hydrolyze ATP and dATP. It exhibits many functions of the wild-type RecA protein, forming filaments on DNA and promoting limited DNA strand exchange, but works well only in the presence of dATP (*214*). RecAK72R will form random mixed filaments with the wild-type protein on both ssDNA and dsDNA (*215*). The properties of this mutant have helped in the effort to determine the function of RecA-mediated ATP hydrolysis (*214, 216*).

The RecAH163W mutant protein was constructed by Bryant and coworkers (*219*). The mutant protein carries out RecA activities with essentially wild-type efficiency. The alteration is useful in that it introduces a fluo-

rescent Trp reporter group into the central nucleotide and DNA-binding domains of the protein, and the intrinsic fluorescence properties of the protein become sensitive to nucleotide-induced conformation changes (*219–221*). The conformation changes can then be monitored in real time, and have been used to follow the change in conformation associated with the activation of RecA by ATP (*219–221*).

The RecAY103W and RecAY264W mutants were constructed for similar reasons (*222*). Both mutations leave the cell with wild-type UV resistance. The mutant proteins provide a fluorescence signal that is sensitive to the binding of ADP and ATP (more so for Y103W than for Y264W) while exhibiting only a modest reduction in strand exchange activity *in vitro*.

The RecA441 (formerly tif; E38K, I298V) and RecA803 (V37M) mutants are proteins with somewhat enhanced DNA-binding properties. In particular, they are better able to displace single-strand break (SSB) protein from ssDNA than is the wild-type RecA protein (*223, 224*). Both of these mutants suppress the UV sensitivity phenotype of *recF, recO,* and *recR* mutants *in vivo* (*225*).

D. ATP Hydrolysis

RecA-mediated ATP hydrolysis is almost entirely DNA dependent. On ssDNA, RecA protein hydrolyzes ATP with a monomer k_{cat} of about 30 min^{-1}. On dsDNA, the rate decreases to 16–20 min^{-1}. The maximum reported value for k_{cat}/K_M at 37°C is about 2×10^4 sec^{-1} M^{-1}, some four orders of magnitude below the diffusion-controlled limit. The kinetics are complicated by cooperativity, and the K_M is more accurately reported as $S_{0.5}$.

Two methods are commonly used to monitor ATP hydrolysis by RecA. One employs labeled ATP and thin-layer chromatography to separate ATP, ADP, and Pi (*226, 227*). The other is a spetrophotometric assay in which ATP hydrolysis is enzymatically coupled to an NAD–NADH interconversion 9*227–231*).

RecA-mediated ATP hydrolysis in the absence of DNA is slow, with a maximum k_{cat} of 0.1 min^{-1} observed at about pH 6.0 (*232*). The rate is stimulated markedly by high (1.5–2 *M*) concentrations of a wide range of salts (*201*). The salt appears to induce a conformation change that to some degree mimics that induced by the binding of DNA to RecA protein (*233, 234*).

The DNA-dependent ATPase activity exhibits no dependence on pH between 5.5 and 9.0 (*232*). The effects of temperature between 25 and 45°C on ssDNA-dependent ATP hydrolysis produce a linear Arrhenius plot, from which an Arrhenius activation energy of 11.8 ± 0.3 kcal mol^{-1} can be derived (*227*) for the reaction. The ATP hydrolysis occurs uniformly throughout the RecA filament, with no enhancement at filament ends (*235–237*). The relationship between ATP hydrolysis and DNA binding is sufficiently

constant when longer DNAs are present that ATP hydrolysis can be used as an indirect measure of DNA binding (*39, 238*).

ATP is hydrolyzed to ADP and Pi. ADP acts as a competitive inhibitor of ATP hydrolysis. Another common inhibitor is the ATP analog ATPγS [adenosine 5′-*O*-(3-thio)triphosphate], which is bound but not appreciably hydrolyzed by RecA protein and acts as a potent competitive inhibitor. The inhibition patterns in both cases are greatly complicated by additional effects of these two nucleotides (*239, 240*). Mixtures of ATP and ADP result in collapse and dissociation of RecA filaments from both ssDNA and dsDNA when the ADP/ATP ratio approached 50% (*239–241*). ATP and ADP tend to stabilize cooperatively different conformations of a RecA filament and these forms are not directly interconvertible via ATP hydrolysis (*240, 242, 243*).

A variety of rNTPs and dNTPs are hydrolyzed by RecA protein, including dATP, UTP, PTP (purine ribonucleoside triphosphate), ITP, CTP, dCTP, GTP, and dUTP (*232, 244, 245*). Only those with a measured $S_{0.5}$ below 100 μM serve as cofactors in the DNA strand exchange reaction (*232, 244–246*).

The hydrolysis of dATP tends to enhance some RecA reactions. With dATP, hydrolytic rates are increased about 20% relative to ATP hydrolysis. RecA filament stability and the rates of DNA strand exchange are also enhanced (*215, 247*). As already noted, the RecA mutant RecA K72R will promote a limited DNA strand exchange reaction only if dATP is used as the nucleotide cofactor (*214, 216*). The use of dATP also prevents an end-dependent disassembly of filaments from ssDNA as described below (*39*).

E. Filament Assembly and Disassembly

An understanding of RecA filament assembly and disassembly is a prerequisite for a broader description of how RecA filaments form where they are needed for recombinational activities on cellular DNA. RecA protein filaments both assemble and disassemble in a unidirectional and end-dependent manner, with protomers added at one end and subtracted from the other (Fig. 6) (*39, 248*). This is true on both ssDNA and dsDNA. The oligomeric species that is added and subtracted has not been defined for *E. coli* RecA protein. However, work with the RecA protein from *Thermus thermophilus* indicates that monomeric RecA protein is an intermediate in filament assembly on ssDNA (*249*).

The binding of RecA protein to ssDNA is a cooperative process (*169, 250*). In addition, the binding of ATP or ATPγS to individual filament subunits can stimulate NTP hydrolysis in neighboring subunits (*239, 251*). These interactions tend to maintain the entire filament in an extended active state. However, for filaments on ssDNA, the ATP hydrolytic cycles of adjacent monomers are not coordinated in the sense that a given step in the hydrolytic cycle of one monomer triggers a particular step in its neighbor (*215, 238*).

This changes for filaments on the dsDNA, where filaments behave as though there was a tight coordination of the ATP hydrolytic cycles of adjacent monomers (*215;* T. A. Arenson and M. M. Cox, unpublished results). This change in filament state when a second complementary DNA strand is present in the filament is a theme of the continuing discussion, because the change mirrors a change in filament state that is also seen upon the initiation of DNA strand exchange.

In the assembly process, nucleation of filament formation is rate limiting (*236, 252, 253*). The slow binding of RecA to dsDNA at neutral pH and above is due to slow nucleation (*236, 252, 253*). Nucleation on dsDNA is facilitated by DNA treatments that tend to unwind the DNA. The most effective nucleation site is a ssDNA gap (*236, 252, 253*). Nucleation on ssDNA is much faster than on dsDNA, a fact that should target RecA filament formation to ssDNA gaps in the cell. Nucleation is not enhanced by DNA damage as suggested in some reports (*254–257*), but by the perturbations in DNA structure (in particular DNA unwinding) that are caused by DNA damage (*253*).

On ssDNA, extension of the filament is uniquely 5′ to 3′ (*39, 183*). In a ssDNA gap, the extension of the filament continues into the adjoining dsDNA, continuing until the available contiguous DNA or RecA protein is depleted. On dsDNA, the two strands of the DNA are bound asymmetrically. The strand occupying the binding site normally occupied by ssDNA (site I) is protected from nuclease digestion two to three times better than its complement (site II) (*231, 258*). The strand bound in site I determines the orientation of the entire filament, with assembly processes proceeding 5′ to 3′ relative to that strand.

End-dependent RecA filament disassembly occurs on the end opposite to that at which assembly occurs (*39, 248*). On ssDNA, this process had been obscured for many years, in part because of the widespread use of circular ssDNA in RecA studies. The process is important in that it potentially affects the status of RecA filaments in all *in vivo* situations requiring recombinational DNA repair.

The net addition of RecA monomers to one end and their deletion from the other end in the same test tube has some thermodynamic implications. The monomer–monomer interfaces are presumably the same at either end (and everywhere else) in the filament. As pointed out by Wegner (*259*), the K_D for monomer addition to either filament end cannot be different unless an independent source of chemical energy is provided to affect the binding to one end or the other. ATP is hydrolyzed by RecA monomers uniformly throughout RecA filaments (*235*), and there is no evidence for enhanced rates at either filament end. RecA filament assembly, but not disassembly, proceeds readily in the presence of ATP analogs, such as ATPγS, that are bound but not hydrolyzed by RecA. Presumably, ATP hydrolysis is somehow

coupled to disassembly. The hydrolysis of ATP by interior monomers does not generally result in dissociation, and under some conditions ATP hydrolysis can proceed with no evident dissociation of RecA monomers (*39, 215, 260*). A simple model arises. ATP hydrolysis occurs everywhere, resulting in dissociation only for monomers at the disassembling end. ATP hydrolysis is coupled to end-dependent disassembly, and occurs with a probability that is some function of reaction conditions.

The methods used initially to characterize end-dependent disassembly from both dsDNA (*231, 248*) and ssDNA (*39*) provided only very rough estimates of the disassembly rates. The process on ssDNA has now been placed on a more quantitative footing (*238*). In RecA filaments, ATP is hydrolyzed uniformly throughout the filament (*235*), but disassembly generally results only if the hydrolysis occurs at the monomer on the disassembling end (*238*). On ssDNA, the maximum observed rate of disassembly (seen above pH 7.5) is 60–65 monomers per end per minute (*238*). Given a k_{cat} for ATP hydrolysis of 30 min^{-1} by all the RecA monomers in the filament (or one ATP every 2 sec), this is the rate of disassembly one would expect if adjacent monomers in a filament hydrolyze ATP independently (i.e., the ATP hydrolytic cycles of adjacent monomers are not coupled in any way). If a monomer has just dissociated, the next monomer in line should be on average halfway through its hydrolytic cycle, and will thus reach the point of dissociation (on average) in 1 sec, giving a hypothetical 60 min^{-1} rate that is very close to what is observed. The situation changes with filaments on dsDNA. Now the k_{cat} for ATP hydrolysis is lower (−16–20 min^{-1}), but the rate of end-dependent disassembly increases to about 100 monomers per minute per filament end (about three times the rate expected if adjacent monomers hydrolyzed ATP independently) (T. A. Arenson and M. M. Cox, unpublished results). Unlike the situation on ssDNA, this rate implies that on dsDNA, the ATP hydrolytic cycles of adjacent monomers are coordinated. The simplest model invokes unidirectional waves of ATP hydrolysis moving down the filament.

Filament dynamics can also be studied with the aid of mixed filaments of wild-type RecA protein and a mutant (*215*). Mixed filaments are formed with wild-type RecA protein and the mutant protein RecAK72R, which does not hydrolyze dATP (*215*). When the proteins are added together, proportions of mutant and wild-type proteins in the resulting mixed filaments are the same as the proportions in the overall experiment. The presence of the mutant affects the observed levels of dATP hydrolysis. In filaments on ssDNA, the reduction in dATP hydrolysis is exactly proportional to the reduction in bound wild-type protein, indicating that the mutant has little effect on the dATP hydrolytic cycles of its wild-type neighbors (Fig. 7). This reinforces the notion that the ATP hydrolytic cycles of adjacent monomers in a filament on

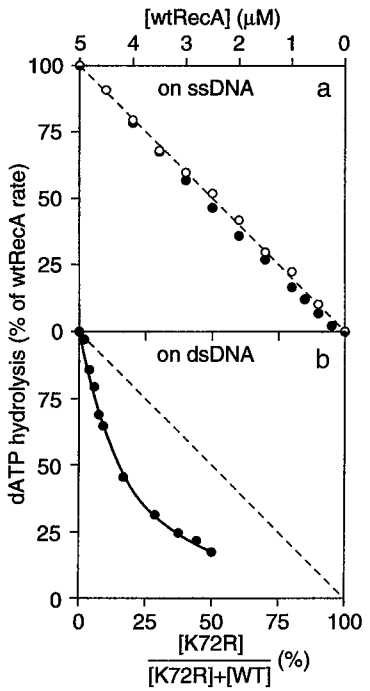


FIG. 7. Monomer–monomer effects in RecA filaments. Mixed filaments can be formed with wild-type and K72R mutant RecA protein (*215*). The mutant protein does not hydrolyze ATP, and it decreases total ATP hydrolysis by displacing wild-type protein from the DNA. If both proteins are maintained in excess relative to the total DNA-binding sites available, as in these experiments, the fraction of RecK72R in the filaments is the same as its fraction in the total RecA pool (*215*). (a) RecA mixed filaments on ssDNA. The effect of increasing the RecAK72R mutant in the filaments is a simple linear function of its relative concentration. there is no indication that the mutant affects the ATP hydrolytic cycles of its neighboring wild-type monomers in the filament. (b) RecA mixed filaments on dsDNA. The ATPase activity of wild-type RecA protein is decreased to an extent greater than the fraction of protein displaced by the mutant. The mutant appears to have a direct and adverse effect on ATP hydrolysis by its neighboring wild-type monomers. Data are derived from Shan and Cox (*215*).

ssDNA are not coordinated in any way. In filaments on dsDNA, the reduction in dATP hydrolysis is much greater than the reduction in bound wild-type protein. The ATP hydrolytic cycles of adjacent monomers are therefore coupled in some way in filaments on dsDNA (Fig. 7).

If the mutant protein is added after wild-type filaments are formed on ssDNA, little exchange of mutant protein into the filaments is observed. On dsDNA, there is a rather facile exchange of mutant protein into the filaments. In general, the filaments on dsDNA are more dynamic with respect to

monomer exchange than those on ssDNA (*215*). This is another manifestation of the different states of filaments bound to ssDNA vs. dsDNA.

Most models for recombination focus on the invasion of 3′ ends (*15, 261–265*), in part because of their potential utility in priming DNA synthesis. A 3′-end bias in DNA pairing reactions has been demonstrated with bacterial RecA protein *in vitro* (*264, 266, 267*), attributed to the 5′ to 3′ polarity of RecA filament assembly (*39, 183*), which would be expected to leave 3′ ends more uniformly coated with RecA protein compared to 5′ ends. The end-dependent disassembly process would tend to enhance these effects. Other factors, notably the presence of the RecO and RecR proteins (see below), can stabilize RecA protein at the 5′ ends of DNA strands and reduce or eliminate the end bias of pairing (J. M. Bork and M. M. Cox, unpublished results).

F. DNA Binding Parameters

In a RecA filament, there is one RecA monomer per three bound nucleotides or base pairs of DNA. A few titration experiments have provided a stoichiometry twice as high (*247, 268–270*). These results could be explained by an intrinsic site size of six nucleotides per RecA monomer, with the lower site size resulting from the binding of a second set of RecA monomers to the first. Alternatively, the intrinsic site size could be three nucleotides per monomer, and the higher site size could reflect the binding of a second DNA strand to another DNA binding site within the filament. The issue has been resolved in favor of the latter interpretation by Brenner and colleagues (*271*), an outcome consistent with the result obtained consistently with electron microscopy (*177, 272–274*).

Without nucleotide cofactor or with ADP, RecA protein binds to ssDNA in a compact form called a "collapsed" filament, with a helical pitch of 64 Å and an axial rise of 2.1 Å per nucleotide (*275, 276*). An extended right-handed helical filament is formed when ATP, dATP, ATPγS, or ADP·AlF_4^- is added, with six RecA monomers per turn, a pitch of 95 Å, and a diameter of 100 Å (*243, 277–279*). This single strand of DNA binds deep within the filament groove and near the filament axis (*272*), to the site desginated strand-binding site I (*280, 281*). The DNA is bound along the ribose phosphate backbone, with the DNA bases displayed in the filament groove (*282–284*). RecA binding to ssDNA is generally nonspecific with respect to sequence, although the protein exhibits an enhanced affinity to certain homopolymers such as poly(dT) (*285–287*). RecA also exhibits a higher affinity for a range of GT-enriched sequences, among them the recombination hot spot Chi (*288, 289*). Optimal binding of wild-type RecA protein to poly(dT) generally occurs only with polymers more than 50 nucleotides in length (*235*), although somewhat shorter polymers can be employed in concert with the addition of ATPγS.

RecA filaments can actually bind up to three DNA strands within the fil-

ament groove, with three distinct DNA strand-binding sites (*280, 281, 290–292*). The sites are usually called simply I, II, and III (*280, 281, 290–292*). Site I has the highest affinity for a DNA strand and, as already described, is generally the site at which ssDNA is bound to RecA protein. Site II is the site generally occupied by a strand complementary to that in site I, although heterologous sequences are tolerated. Site III would then be the site occupied by a DNA strand displaced during DNA strand exchange. The numbering of the sites varies somewhat from research group to research group. When strands of identical ssDNA are added successively to a RecA filament, site III actually binds to the DNA with an affinity higher than site II (when identifying sites by the system used here), and is therefore sometimes referred to as site II.

In a competing formalism, the filament groove is described by some workers to have two DNA binding sites, each of which can bind to either a single- or double-stranded DNA (*293–296*). Because no more than three total strands can be bound at one time, the two sites as defined this way presumably overlap.

In the context of recombinational DNA repair, RecA protein brings two homologous DNA molecules together and promotes a DNA strand exchange reaction between them. Binding of the first DNA can be thought of as primary binding, involving either ssDNA to strand-binding site I or dsDNA to sites I and II. Binding of the second DNA can be considered secondary binding. When ssDNA is bound at site I, secondary binding can involve dsDNA binding at strand-binding sites II and III.

G. DNA Strand Exchange

1. DNA Pairing Intermediates

The fundamental process of DNA pairing occurs within the filament groove, as originally proposed by Howard-Flanders and co-workers (*297*) and repeatedly confirmed in a decade and a half of experiments. Analysis of the kinetic of DNA pairing reveals at least two steps, a rapid second-order alignment of the two homologous DNA molecules, followed by a slow first-order process (*298–300*). The homologous alignment does not involve a sliding of the RecA–ssDNA complex along a duplex DNA, but instead is best explained by random collision (*301*).

A four-stranded DNA quadruplex structure has often been promoted as a potential recombination intermediate in this context (*136, 169, 297, 302–305*). However, the idea of a four-stranded DNA pairing intermediate playing a role in RecA-mediated DNA pairing reactions (*297*) must be modified. Many data now indicate that four DNA strands (two duplexes) cannot be accommodated in the filament interior (*216, 280, 281, 290–293, 306–309*). Al-

though RecA promotes an efficient four-strand exchange reaction, pairing in these reactions is always initiated within the single-strand gap; i.e., four-strand exchanges must be initiated as three-strand reactions (*308, 310, 311*), and there is no unambiguous evidence for such a quadruplex DNA intermediate in RecA reactions (*306–308, 312*).

The problem of DNA pairing inside a RecA filament is thus reduced to an interaction between a RecA-bound ssDNA and a homologous duplex. In principles, the duplex could approach the ssDNA within the filament via either its major or minor groove, providing two potential pathways (Fig. 8). In the major-groove-first path, the potential exists for a novel triplex DNA intermediate to be formed prior to strand switch, providing one interesting mechanism for homologous recognition. This proposed intermediate has

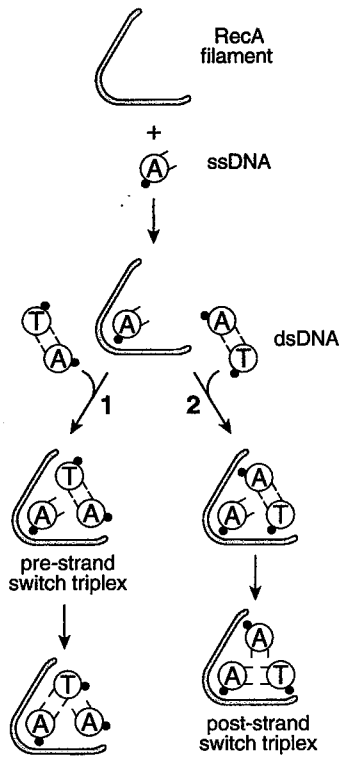


FIG. 8. Two alternative pathways for DNA alignment by the RecA protein. The small closed circles represent the DNA backbone. Pathway 1 is the major groove first path, which may involve the formation of a major groove triplex DNA as a pairing intermediate. Pathway 2 is the minor groove first pathway. In this case, the homologous alignment would involve the rotation of bases and homology sampling via standard Watson–Crick interaction.

been called R-form DNA (*313, 314*). In the alternative minor-groove-first path, homologous alignment would involve standard Watson–Crick base pairing. As the duplex bound, it would be extended and underwound such that its bases would be free to rotate and "sample" the bound ssDNA for complementarity. Even in this path, an R-form triplex might appear transiently after the strand switch was complete, although one might question its importance to the overall reaction.

The R-form triplex as proposed would be a novel DNA structure. Computer modeling suggests that an R-form triplex DNA should be stable in aqueous solution (*313, 315*) (Fig. 9). There are reports of a putative triplex species that persists and is very stable after RecA protein is removed in a strand exchange reaction in which pairing is limited to the distal end of an R-form triplex in aqueous solution (*317*). All of this work has been questioned following reports that support a minor-groove-first pathway for DNA pairing (*318–323*). The RecA filament appears to stabilize the products of DNA strand exchange, using binding energy to promote the strand switch (*318–323*). The RecA filament appears to stabilize the products of DNA strand exchange, using binding energy to promote the strand switch (*324*). The existence of a triplex DNA intermediate on the reaction pathway remains very controversial.

2. RecA Is a Motor Protein

Summarizing some of the results already described, we can set the stage for DNA strand exchange. The reaction begins by binding RecA protein to ssDNA, forming an extended helical filament in the presence of ATP. The nucleoprotein filament hydrolyzes ATP with the monomer k_{cat} of 30 min^{-1}. Fil-

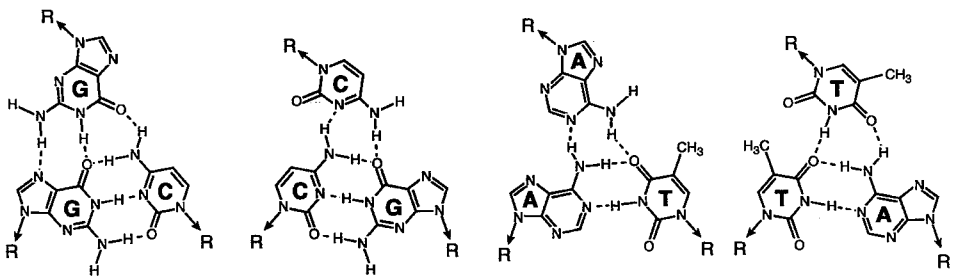


FIG. 9. Hydrogen bonding patterns proposed for the R-form triplex DNA structure. Each triplet is formed, in the major groove, using non-Watson–Crick hydrogen bonds that are both complementary and unique. The structure has the advantage that the position of the phosphoribose backbone is approximately the same for the third strand of each triplet, allowing for a fairly uniform structure (*313, 341*). A structure of this type was first proposed by Lacks in 1966 (*342*). However, there is little evidence to date that the structure exists.

ament assembly is cooperative, but individual monomers hydrolyze ATP independently. When a homologous duplex DNA is added to initiate strand exchange, a rapid decrease in ATP hydrolysis (by one-third) is observed, to a rate characteristic of filaments on dsDNA (*237*). During DNA strand exchange, the RecA filaments are in a state analogous to that observed when filaments are formed on dsDNA (*325*). The filaments become more dynamic and monomer exchange is observed, as is seen with filaments on dsDNA (*325*). This induced exchange between bound and free RecA monomers occurs only when a complementary strand is already paired with the ssDNA in the filament; i.e., strand exchange must be complete. It is not seen when the duplex DNA is heterologous (*325*) and thus has no role in the "search for homology" that must occur prior to homologous alignment of the two DNAs.

ATP hydrolysis is not required to promote the fundamental process of DNA strand exchange within the filament. Many studies have demonstrated that RecA protein can promote limited DNA strand exchange (typically yielding about 1 kbp of hybrid DNA) under conditions in which ATP is not hydrolyzed. These conditions include the use of ATP analogs that are bound but not hydrolyzed by wild-type RecA protein (ATPγS and an ADP·AlF_4^- complex) (*309, 326–329*), as well as the use of the mutant RecAK72R, which binds but does not hydrolyze dATP (*214, 216*).

However, when ATP is not hydrolyzed, DNA strand exchange halts long before the reaction reaches completion with the bacteriophage DNAs commonly employed (*216, 326, 330*). ATP hydrolysis allows the reaction to go to completion, renders the DNA strand exchange reaction unidirectional (*216, 330*), and allows it to bypass substantial DNA structural barriers (*216, 328, 331*). These structural barriers can include a heterologous insertion of 100 bp or more in the duplex DNA substrate, which is unwound as it is bypassed by RecA protein-mediated strand exchange. In addition, ATP hydrolysis is required for any DNA strand exchange involving two duplex DNAs (the four-strand reaction; Fig. 5) (*216, 309*). These results imply that ATP hydrolysis is directly coupled to the later stages of DNA strand exchange, and provide clues to the molecular basis of the coupling. During these ATP-dependent stages, the strand exchange proceeds uniquely 5′ to 3′ relative to the ssDNA to which RecA protein initially binds.

NTPases can generally be classified according to one of three biological functions: motor proteins, molecular timing devices, or recycling functions (*332*). The ATPase activity of RecA is often portrayed as a recycling function (*17, 169, 302, 332*), causing the dissociation of RecA monomers from the filament after DNA strand exchange has occurred. As already noted, ATP hydrolysis is coupled to the end-dependent disassembly of RecA filaments. A number of early models for RecA-mediated DNA strand exchange proposed that RecA filament disassembly, and/or filament reassembly on the displaced

single strand, played a mechanistic role in the movement of the DNA branch (*297, 333, 334*). Both processes proceed in the same direction as DNA strand exchange. However, under optimal conditions, there is no net dissociation of RecA protein during DNA strand exchange, and the filament is found on the hybrid DNA product rather than on the displaced single strand (*335*). Rates of end-dependent filament assembly and/or disassembly are affected greatly by changes in reaction conditions (pH, dilution, addition of very small amounts of ATPγS) that have little or no effect on the rates of DNA strand exchange (*248*). No attempt has been made to explain how the proposed assembly/disassembly mechanisms would permit the bypass of DNA lesions and heterologous insertions during DNA strand exchange. Finally, most of these models envision that two duplex DNAs are both inside the filament during a four-strand exchange reaction, conferring on the RecA filament interior a binding capacity it does not appear to have. For these and other reasons the models that employ filament assembly or disassembly as the enabling process in ATP-dependent DNA strand exchange must be abandoned.

Any successful model for the coupling of ATP hydrolysis to DNA strand exchange must explain how heterologous insertions in the DNA are bypassed and how four-strand exchange reactions come about. Both of these processes are completely dependent on ATP hydrolysis. The bypass of heterologous insertions in a three-strand reaction has two further requirements. First, homologous DNA must be present on both sides of the heterology (*334, 336*). Even 22 bp of heterology at the distal end of the duplex DNA cannot be unwound (*334*). Second, when the heterologous insert is in the duplex DNA substrate, a torsional stress is placed on the insert DNA to effect the unwinding needed for bypass. A nick in one of the two DNA strands completely blocks the bypass (*336*).

The two remaining models can be introduced in the context of a single question: why does DNA strand exchange come to a halt before it is completed when ATP is not hydrolyzed?

Model 1: The RecA redistribution model. In this model, it is proposed that RecA filaments are discontinuous, that DNA strand exchange halts at the discontinuities when ATP is not hydrolyzed, and that ATP hydrolysis serves to recycle RecA protein so as to fill in the discontinuities (*214, 326, 329*). This idea is subtly different from the ideas considered above, because the filament is seen to stay largely intact and bound to the hybrid DNA duplex at the end of a reaction, as consistent with observation. Also, the recycling of RecA protein need not occur at the migrating branch point of a DNA strand exchange reaction. The model can explain how strand exchange might bypass a heterologous insertion in the duplex DNA substrate (*18*) (Fig. 10). The RecA protein underwinds bound DNA. If the dsDNA substrate is bound within the fil-

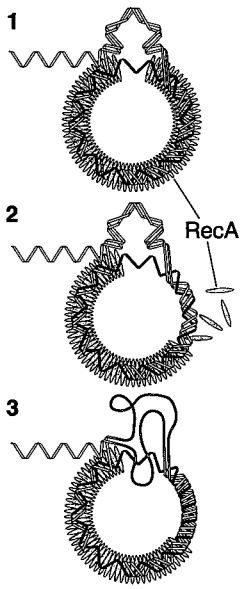


FIG. 10. Mechanism for bypassing heterologous DNA insertions in the duplex DNA substrate during strand exchange, using RecA redistribution. The duplex DNA is bound to a RecA nucleoprotein filament and aligned with the single-stranded DNA. The alignment causes the heterologous DNA in the duplex to be looped out. The DNA within the filament is underwound to 18 bp per turn. If some of the RecA monomers in the filament dissociate, this underwound DNA is release. In the circular RecA filament, there are no free ends to allow DNA rotation and relaxation of the released DNA. Hence, when the released DNA collapses to 10 bp per turn, its underwinding can be translated into strand separation in the heterologous insert. This, in turn, would permit DNA strand exchange to be completed beyond the insert. This mechanism should be precluded for RecA filaments formed on linear ssDNA, because any underwound DNA released by RecA dissociation would be relaxed via simple rotation at the filament ends. Note that the gap in the RecA filament shown at the point of the heterologous insertion in the top panel need not be present.

ament on both sides of the insertion, and the filament had no ends with a potential for free rotation (as would be the case on the standard circular DNA substrates), then a modest dissociation of RecA protein from the filament might release underwound DNA and allow the underwinding to be translated into unwinding of the heterologous insertion (Fig. 10). The model of Fig. 10 also readily explains why homologous DNA must be present on both sides of the heterologous insertion in order for bypass to occur, and also provides a mechanism for the induction of torsional stress needed to unwind the heterologous DNA. There has been no attempt to explain how four-strand exchange reactions might proceed with the RecA redistribution model, or why

ATP hydrolysis might be required for them. In addition, RecA dissociation from DNA rarely accounts for more than a small fraction of the ATP hydrolysis observed, so that the model provides an incomplete explanation for why ATP is hydrolyzed by RecA.

Model 2: The facilitated DNA rotation model. This model has been evolving for over a decade (*29, 170, 216, 337, 338*). It depicts the RecA ATPase as a motor activity with RecA recycling being a secondary function. The model proposes that without ATP hydrolysis, DNA strand exchange stops because of a discontinuous DNA pairing intermediate rather than a discontinuous filament (Fig. 11). The discontinuities take the form of DNA loops external to the filament, and are created as one duplex DNA is aligned with a bound ssDNA at multiple sites. The contiguous region of strand exchange that occurs in the absence of ATP hydrolysis is limited to the region between the end of the duplex DNA and the first such external loop. When ATP is hydrolyzed, the loops are resolved by rotating this external DNA around the outside of the filament, keeping the DNA more or less parallel to the filament axis (Fig. 11, III). Unidirectional rotation will bring about a unidirectional migration of

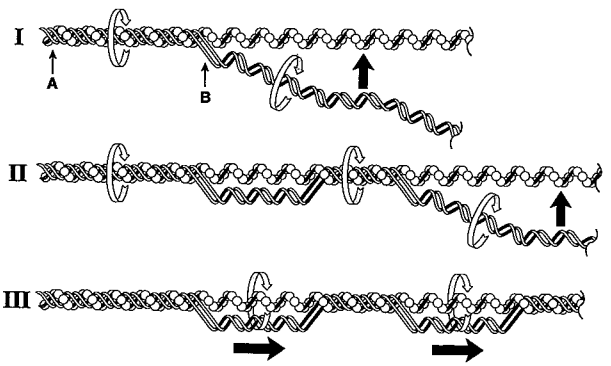


FIG. 11. Facilitated DNA rotation model for the coupling of ATP hydrolysis to RecA-mediated DNA strand exchange. The duplex DNA is paired with the ssDNA within the filament. New duplex DNA is spooled into the filament to extend the paired region. As this occurs, new pairing events downstream provide new points for the spooling of duplex DNA into the filament and trap segments of the duplex DNA on the exterior of the RecA filament. These external loops can be resolved only by rotating the DNA about the exterior of the RecA filament, and the rotation can be coupled to ATP hydrolysis. The external loops of DNA are arrayed longitudinally along the filament exterior, bound to every sixth monomer. Rotation about the filament axis is brought about by passing the external loop of DNA from one $n/6$ set of RecA monomers to the next. Six such steps provide one rotation of 360° and require that each monomer hydrolyze one ATP. If the rotation is unidirectional, the branches will migrate unidirectionally, with DNA spooling into the filament on one end of each loop and out of the filament on the other end. The strand exchange reaction is rendered unidirectional, and sufficient energy is provided to bypass structural barriers that might be encountered in the context of recombinational DNA repair.

the loop, with DNA spooling into the filament on one end and out on the other. The rotation is driven by the ATPase motor. With six RecA monomers per helical turn of the filament, the looped DNA would be bound on the exterior of every sixth monomer at any given time. Those monomers hydrolyze ATP and pass the DNA to the adjacent set of monomers, always keeping the external DNA oriented longitudinally along the filament axis. If it worked as advertised, the ATP hydrolytic cycles of adjacent monomers would be coordinated so that waves of ATP hydrolysis migrated unidirectionally down the filament, with individual waves offset by six monomers (Fig. 12).

In a model related to Model 2, Jwang and Radding have proposed the two DNAs are interwound within the RecA filament, and that RecA promotes a coaxial DNA rotation coupled to ATP hydrolysis (336). This idea is consistent with many of the observations made to date. However, it requires that

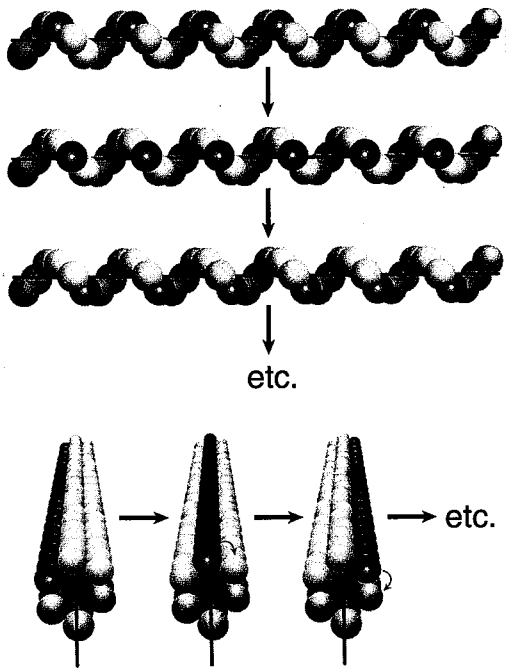


FIG. 12. The facilitated DNA rotation model requires a unique coordination of ATP hydrolysis within the RecA filament. If the external looped DNA is bound to every sixth monomer in the filament (Fig. 11), then the rotation of the looped DNA would require that every sixth RecA monomer hydrolyze an ATP more or less coincidentally. The ATP hydrolytic cycles of the monomers would have to be organized so that ATP hydrolysis proceeded in waves along the filament, with individual waves staggered at six-monomer intervals.

two duplexes be bound within the filament to effect a four-strand exchange and is thus inconsistent with evidence that the filament will not accommodate two duplexes.

A rotational motor working as shown in Fig. 11 would provide the energy for the bypass (and unwinding) of heterologous inserts in the duplex DNA substrate, and would render the strand exchange unidirectional. The torsional stress used to unwind the heterologous DNA would be generated by the ATP-driven rotation. The process provides a mechanism by which RecA protein can promote a four-strand exchange reaction without accommodating four strands in the filament interior [only one duplex need be in the filament, and the other can be rotated around it (Fig. 13)], and explains why these reactions require ATP hydrolysis. Some coordination would be required throughout the filament to bring about the facilitated DNA rotation, and the model provides a more complete explanation for the hydrolysis of ATP by RecA.

In a standard three-strand exchange reaction, the displaced strand is bound by SSBs. It seems unlikely that an SSB-bound ssDNA strand, or perhaps any ssDNA, could be rotated about the outside of the filament. The substrate for rotation in this model is dsDNA. In a three-strand exchange reaction, this would explain why a heterology at the distal end of the duplex cannot be unwound, because it would have to come about via rotation of the displaced ssDNA about the filament.

Both of these models make a number of testable predictions. The RecA redistribution model makes three predictions:

1. The RecA protein should be exchanged between bound and unbound forms during DNA strand exchange. As outlined above, this clearly

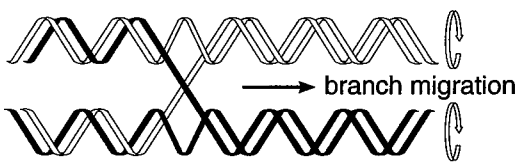


FIG. 13. The facilitated DNA rotation model provides a mechanism for promoting four-strand DNA exchange reactions, even though RecA filaments cannot accommodate all four strands within a RecA filament. If RecA filament is bound to the bottom duplex as shown, and the top duplex is rotated around it, the branch will move from left to right with no need to accommodate more than two DNA strands in the filament interior at any one time. Unlike the three-strand reaction, RecA protein does not spontaneously align two duplex DNAs in the absence of ATP hydrolysis. Instead, DNA pairing is always initiated within a single-strand gap. The exchange is moved out of the gap and converted to a reciprocal exchange between duplexes only if ATP is hydrolyzed (*216, 309*).

happens. The filament becomes more dynamic and RecA monomer exchange is observed when strand exchange is initiated (*325*). The exchange represents a relatively small subset of the ATP hydrolytic events in the filament, but it seems sufficient to explain the ATP dependence of a normal DNA strand exchange reaction. This observation is consistent with the redistribution model.

2. If filament discontinuities bring DNA strand exchange to a halt when ATP is not hydrolyzed, excess RecA protein could fill in the gaps and permit strand exchange to go to completion without hydrolyzing ATP. When ATP is hydrolyzed, redistribution of RecA monomers should be sufficient, in and of itself, to permit the completion of DNA strand exchange and the bypass of structural barriers in the DNA. The RecAK72R mutant protein does not hydrolyze ATP but does promote limited DNA strand exchange that halts after formation of a kilobase or so of hybrid DNA is formed under normal reaction conditions (*214, 216*). Excess RecAK72R mutant protein does not allow the strand exchange reaction to go to completion, and in fact has no discernible effect on the reaction (*216*). Although discontinuities might remain that reflect DNA secondary structure or some other barrier difficult to fill in, these experiments provide no support for the model. In addition, redistribution of RecA protein alone is not enough to allow completion of strand exchange. Using mixed filaments of wild-type and K72R mutant RecA, complete DNA strand exchange is suppressed when one in three monomers is mutant, even when the other monomers are hydrolyzing ATP (*325*). When a short heterologous insertion is placed in the dsDNA substrate, complete strand exchange is suppressed when only one in six monomers is mutant. Under these conditions, there is still a facile exchange of RecA monomers (including mutant protein) into and out of the mixed filaments once DNA strand exchange is initiated (*325*), but it does not permit completion of strand exchange.
3. Any model for the use of ATP hydrolysis must explain why ATP hydrolysis is required to advance strand exchange past a heterologous insertion in the duplex DNA substrate (unwinding it in the process) (*216, 328*). The RecA redistribution model provides one potential way to do this (*18*) (Fig. 10). When RecA protein binds to DNA, it underwinds it extensively, to 18 bp per turn. If the dsDNA substrate were taken into the filament on either side of the heterologous insertion (Fig. 10), then a limited disassembly of the RecA filament might release underwound DNA, allowing the underwinding to be translated into unwinding of the DNA in the insertion. The limited disassembly would presumably require ATP hydrolysis, and strand exchange beyond the insertion could proceed once the insertion DNA was unwound. To translate the

underwinding into unwinding of DNA elsewhere, there would have to be no free rotation of DNA or filament ends that might relax the underwound DNA as soon as it was released by filament disassembly. This condition is met with the circular ssDNA substrates normally used to monitor RecA reactions *in vitro*. However, if RecA uses this mechanism to bypass heterologous insertions, then such bypass should not occur if linear ssDNA is used instead of circular. This idea is simple to test, and the bypass of heterologous insertions goes just as well with linear ssDNA substrates as it does with circular ssDNA substrates (*339*). This result argues against the RecA redistribution model.

The facilitated DNA rotation model also makes three predictions:

1. In any motor, there should be a finite payoff for each unit of chemical energy invested. In the case of this proposed RecA motor, there should be a quantitative relationship between the rate of ATP hydrolysis and the rate of DNA branch movement. The DNA inside a RecA filament is underwound to 18 bp per turn. One rotation of the looped DNA (Fig. 11) will thus move the branch point by 18 base pairs. To effect this one rotation, each RecA monomer would have to hydrolyze one ATP. From the vantage point of a single monomer in the filament, one ATP would be hydrolyzed each time the DNA rotated past that point. Thus, the k_{cat} for ATP hydrolysis (min^{-1}) should be related to the rate of branch movement (bp min^{-1}) by a factor of 18 bp. If the k_{cat} for ATP hydrolysis during strand exchange is 20 min^{-1} (as is the case at 37°C), then the DNA branch should move at 360 bp min^{-1}. ATP hydrolysis and DNA branch movement during DNA strand exchange were measured under a range of temperatures; the results are shown in Fig. 14. The two lines in this Arrhenius plot are parallel and separated by exactly a factor of 18 bp. The results are quantitatively consistent with the model.
2. ATP hydrolysis should move unidirectionally through the filament in the form of coordinated waves, offset by six monomers as shown in Fig. 15. With a k_{cat} of 16–20 min^{-1}, a wave must reach a given monomer every 3–3.8 seconds. If the waves are offset by six monomers, each wave must move from one monomer to the next at intervals of 0.5–0.6 sec. Each of the individual waves would then be traveling at a numerical rate of six times the k_{cat} for ATP hydrolysis, or 96–120 monomers per minute. There are no techniques with which to monitor ATP hydrolytic waves within a RecA filament. However, the final wave should be coupled to end-dependent disassembly, and that process can be measured. As described under filament disassem-

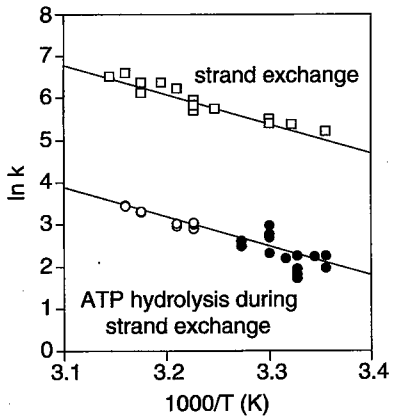


FIG. 14. Coupling of ATP hydrolysis to DNA branch movement in DNA strand exchange. Rates of DNA branch movement (□; bp min^{-1}) and ATP hydrolysis (○, ●; min^{-1}) during RecA-mediated DNA strand exchange were measured at a range of temperatures. The data describe two parallel straight lines. The lines shown are separated by exactly a factor of 18 bp, fulfilling a key quantitative prediction of the facilitated DNA rotation model. [Data from Bedale and Cox (227).]

bly, the measured rates of end-dependent disassembly from dsDNA (in monomers per end per minute) are five to seven times the numerical rate for the ATPase k_{cat}, fitting the prediction quite well (T. A. Arenson and M. M. Cox, unpublished results).

3. RecA has no classical helicase activity associated with it, and it generally does not bring about complete strand separation in duplex DNAs

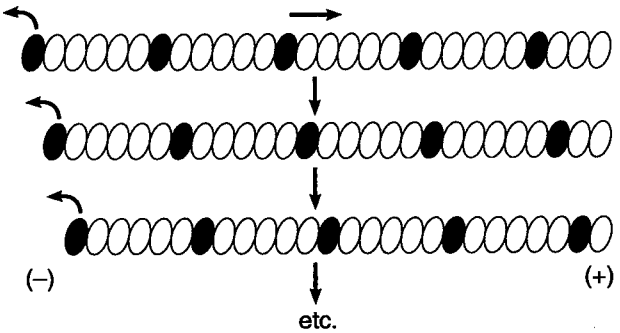


FIG. 15. The waves of ATP hydrolysis described in Fig. 12 would travel unidirectionally down the RecA filament. The interior waves cannot be monitored readily. However, the final wave is coupled to monomer dissociation under some conditions, and the disassembly process can be monitored.

to which it is bound. However, if RecA protein promotes the proposed rotation, then we should be able to make the protein act like a kind of helicase under some conditions. For a branch such as that in Fig. 16, if RecA is bound to the bottom DNA and rotates the top DNA around it as shown, the DNA to the right of the branch point should be actively unwound. The strand separation would be brought about indirectly by the upstream rotation of attached DNAs. In the RecA version of helicase action, the reaction should be abolished if a break is placed in the branched DNA as shown. To test this idea, RecA can be used to promote DNA strand exchange with the substrates shown in Fig. 17. RecA protein promotes this "indirect helicase" activity, unwinding up to several hundred base pairs in the process (*339*). As predicted, the reaction is abolished when a strand break separates the rotated DNA from that being unwound. The activity is consistent with the facilitated DNA rotation model. The indirect helicase activity is quite different from the blocked unwinding of a heterologous DNA segment in a three-strand exchange reaction (*334*), because the former reaction can come about by rotating a segment of dsDNA rather than ssDNA about the filament.

In Summary, recycling of RecA protein occurs during DNA strand exchange and must be regarded as one of the functions of ATP hydrolysis. However, it accounts for only a small fraction of the ATP hydrolysis under most conditions. The overall results suggest that the recycling is incidental to strand exchange rather than mechanistically related. They also provide support for facilitated DNA rotation, a role for ATP hydrolysis in fueling a RecA motor.

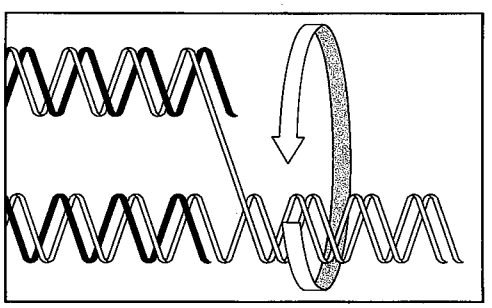


FIG. 16. An indirect helicase activity. If the two strands of the duplex DNA at right are separated and both provided complementary strands as shown at left, the rotation of the two DNAs at left about each other should unwind the DNA at right. (From Ref. *339*.)

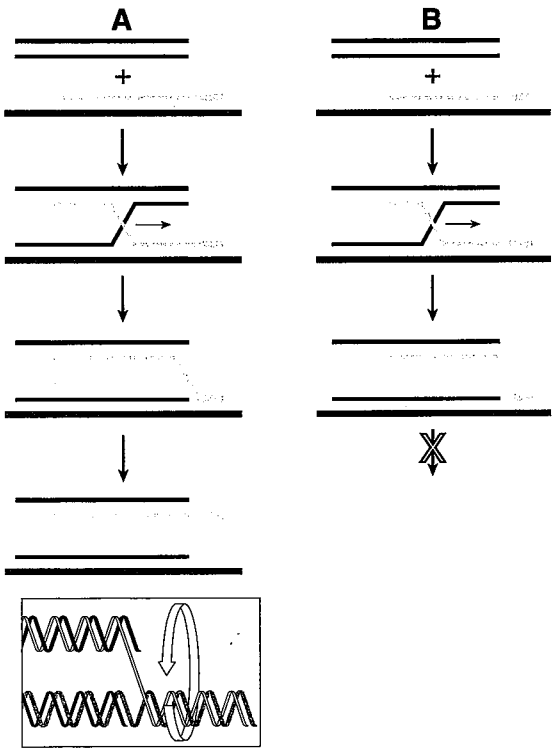


FIG. 17. RecA protein has an indirect helicase activity. (A) Using DNA substrates such as those shown, RecA protein is bound to the gapped DNA substrate and a four-strand exchange reaction is initiated in the gap. Note that the bottom strand of the gapped molecule is actually circular, and is shown here as linear to simplify the illustration. As the strand exchange proceeds, a Holliday structure is created. The discontinous strand of the gapped DNA extends beyond the region of reciprocal exchange (thickened DNA segment). If RecA rotates one DNA about the other, this distal segment will be unwound. RecA unwinds up to 400 bp of DNA in this context. (B) If the distal segment is separated from the exchanging DNA by a break, it is no longer unwound (*339*).

3. No Four-Stranded DNA Pairing Intermediates

For nearly a decade and a half, the generally consistent demonstrations that no more than three DNA strands can be accommodated within the RecA filament groove have been countered by one compelling result. West and Howard-Flanders (*340*) demonstrated that in a RecA-promoted four-strand exchange reaction, a double-strand break in the linear duplex substrate could be bypassed. Because the second DNA fragment would have to initiate exchange in a region where the gapped DNA substrate was duplex (Fig. 18), it was argued

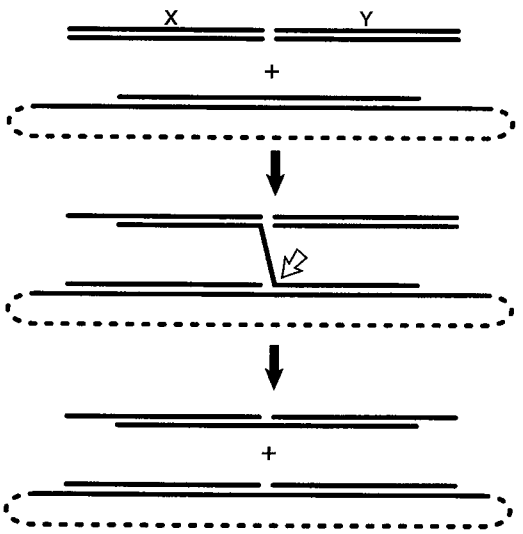


FIG. 18. The double-strand break bypass reaction. During a four-strand exchange reaction, a double-strand break in the linear duplex is bypassed, as first demonstrated by West and Howard-Flanders (*340*). The dashed line is meant to show that the gapped DNA substrate is actually circular, and the single-stranded DNA is oriented 5′ to 3′, left to right, as drawn. Because the second DNA fragment (Y) must initiate strand exchange in a position where the DNA within the filament is duplex (open arrow), this result was initially interpreted to mean that RecA protein can align two duplex DNAs within the filament interior (*340*). The four-strand reaction is completely dependent on ATP hydrolysis, and proceeds unidirectionally, so that fragment X is always exchanged before fragment Y (*307, 340*). (From Ref. *307*, by permission of Cell Press.)

that two duplexes must be aligned within the filament. The double-strand break bypass result is readily confirmed (*307*). However, the indirect helicase activity of RecA protein (*339*) (Fig. 16) suggested an alternative explanation for this phenomenon. A double-strand break separates the linear dsDNA substrate into two fragments. If strand exchange proceeds unidirectionally, then fragments X and Y must exchange in succession [it has been confirmed that they do (*307, 340*)]. If only the exchange with fragment 1 is considered, the experimental situation is very similar to the set up to examine the indirect helicase activity (Figs. 16 and 17). After fragment X has undergone exchange, a rotatory motor should unwind the gapped duplex for some distance beyond the DNA complementary to fragment X (Fig. 19). This would open up a region of ssDNA in which fragment Y could pair and initiate DNA strand exchange, with the initiation occurring as a three-strand rather than a four-strand reaction. If the gapped duplex substrate had a break in one strand coincident with the double-strand break in the linear DNA, then the indirect helicase-like unwinding

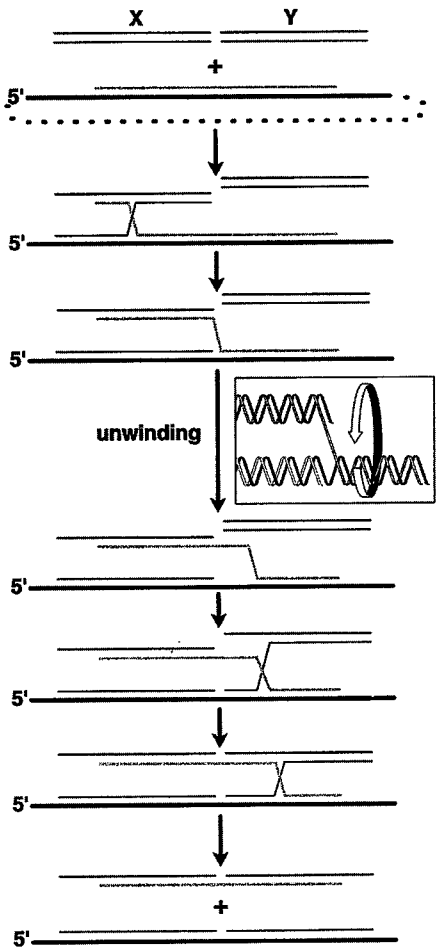


FIG. 19. Mechanism of RecA-mediated bypass of double-strand breaks in a four-strand exchange reaction. RecA protein is bound to the gapped DNA. The four-strand exchange is initiated with fragment X. Once the exchange with fragment X is complete, the indirect helicase activity of RecA protein continues to rotate one product duplex DNA on the outside of the filament around the product DNA on the inside, resulting in the unwinding of the bound substrate duplex DNA downstream. This creates a region of single-stranded DNA within the filament where the exchange of fragment Y can be initiated to complete the bypass. A break in the gapped duplex that is coincident with the break in the linear duplex abolishes the bypass reaction (*307*). (From Ref. *307*, by permission of Cell Press.)

of the DNA could not occur (the rotating DNA would be separated from the DNA to be unwound). If bypass requires only a duplex–duplex alignment inside the filament, then this break should not affect the result, but if the bypass requires the motor-dependent unwinding of the gapped substrate to allow pair-

ing of fragment Y, then a break should block the bypass. The break does abolish the bypass entirely (*307*), providing an alternative explanation for the double-strand break bypass that does not require duplex–duplex DNA alignment within the RecA filament groove.

III. Summary and Prospects

Recombinational DNA repair is arguably the most complex and least understood DNA repair pathway. In the present decade the picture has begun to coalesce, as this repair path provides a formerly missing link between different aspects of DNA metabolism. However, the discontinuities in understanding are large and numerous. The pathway is likely to require an extraordinary level of molecular coordination, a topic that has not yet been addressed adequately. When a replication fork stalls, the mechanisms by which replication enzymes are replaced with recombination functions, and then the recombination functions are removed for replication restart, ought to provide an interesting challenge for future research. The effort should also continue to illuminate the function of proteins not yet well-understood, and provide a role for a few yet undiscovered.

The picture *in vivo* is also incomplete. A systematic effort to assess the levels and types of DNA damage in bacteria as a function of growth conditions would be invaluable. Renewed efforts are needed to assess the roles of different recombination systems, not during conjugation but during normal replication. New approaches are needed to determine the fate of replication forks *in vitro* with better resolution, and provide a better understanding of the circumstances under which each recombination system is employed. The study of recombinational DNA repair should inevitably promote a view of replication and recombination systems as linked components of an integrated DNA metabolism.

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References

1. E. C. Dougherty, *Syst. Zool.* **4**, 145–169, 190 (1955).
2. J. Maynard Smith, "The Evolution of sex." Cambridge Univ. Press, London, 1978.

3. H. Bernstein, H. C. Byerly, F. A. Hopf, and R. E. Michod, *Science* **229,** 1277–1281 (1985).
4. H. Bernstein, F. A. Hopf, and R. E. Michod, *In* "The Evolution of Sex: An Examination of Current Ideas" (R. E. Michod and B. R. Levin, eds.), pp. 139–160. Sinauer Assoc. Inc., Sunderland, Massachusetts, 1988.
5. H. Potter and D. Dressler, *In* "The Recombination of Genetic Material" (K. B. Low, ed.), pp. 217–282. Academic Press, San Diego, 1988.
6. M. M. Cox, *BioEssays* **15,** 617–623 (1993).
7. M. M. Cox, *Genes Cells* **3,** 65–78 (1998).
8. A. J. Clark and S. J. Sandler, *Crit. Rev. Microbiol.* **20,** 125–142 (1994).
9. R. E. Michod, "Eros and Evolution: A Natural Philosophy of Sex." Addison Wesley, Menlo Park, California, 1995.
10. A. Kuzminov, "Recombinational Repair of DNA Damage." R. G. Landes Company, Georgetown, Texas, 1996.
11. T. Galitski and J. R. Roth, *Genetics* **146,** 751–767 (1997).
12. A. J. Clark and A. D. Margulies, *Proc. Natl. Acad. Sci. U.S.A.* **53,** 451–459 (1965).
13. P. Howard-Flanders and L. Theriot, *Genetics* **53,** 1137–1150 (1966).
14. A. J. Clark, *Biochimie* **73,** 523–532 (1991).
15. G. R. Smith, *Cell* **64,** 19–27 (1991).
16. G. R. Smith, S. K. Amundsen, P. Dabert, and A. F. Taylor, *Philos. Trans. Royal Soc. London, Ser. B: Biol. Sci.* **347,** 13–20 (1995).
17. S. C. Kowalczykowski, D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer, *Microbiol. Rev.* **58,** 401–465 (1994).
18. P. R. Bianco, R. B. Tracy, and S. C. Kowalczykowski, *Frontiers Biosci.* (online journal) **3,** 560–603 (1998).
19. K. Ishimori, S. Sommer, A. Bailone, M. Takahashi, M. M. Cox, and R. Devoret, *J. Mol. Biol.* **264,** 696–712 (1996).
20. J. T. Konola, K. M. Logan, and K. L. Knight, *J. Mol. Biol.* **237,** 20–34 (1994).
21. J. T. Konola, H. G. Nastri, K. M. Logan, and K. L. Knight, *J. Biol. Chem.* **270,** 8411–8419 (1995).
22. K. H. Zavitz and K. J. Marians, *J. Biol. Chem.* **267,** 6933–6940 (1992).
23. T. Asai, D. B. Bates, and T. Kogoma, *Cell* **78,** 1051–1061 (1994).
24. Z. Livneh, F. O. Cohen, R. Skaliter, and T. Elizur, *Crit. Rev. Biochem. Mol. Biol.* **28,** 465–513 (1993).
25. E. C. Friedberg, G. C. Walker, and W. Siede, "DNA Repair and Mutagenesis." ASM Press, Washington, D.C., 1995.
26. K. H. Zavitz and K. J. Marians, *Mol. Microbiol.* **5,** 2869–2873 (1991).
27. D. J. Sherratt, L. K. Arciszewska, G. Blakely, S. Colloms, K. Grant, N. Leslie, and R. McCulloch, *Philos. Trans. Royal Soc. London, Ser. B: Biol. Sci.* **347,** 37–42 (1995).
28. T. Kogoma, *Microbiol. Mol. Biol. Rev.* **61,** 212–238 (1997).
29. A. I. Roca and M. M. Cox, *Prog. Nucleic Acids Res. Mol. Biol.* **56,** 129–223 (1997).
30. G. R. Smith, *In* "DNA Damage and Repair, Vol. 1: DNA Repair in Prokaryotes and Lower Eukaryotes" (J. A. Nickoloff and M. F. Hoekstra, eds.), pp. 135–162. Humana Press, Totowa, New Jersey, 1998.
31. K. J. Marians, *Prog. Nucleic Acids Res. Mol. Biol.* **63,** 39–67 (1999).
32. K. J. Marians, *Annu. Rev. Biochem.* **61,** 673–719 (1992).
33. A. Kornberg and T. A. Baker, "DNA Replication." W. H. Freeman, New York, 1992.
34. A. Yuzhakov, J. Turner, and M. O'Donnell, *Cell* **86,** 877–886 (1996).
35. F. B. Dean and M. O'Donnell, *Curr. Biol.* **6,** 931–934 (1996).
36. V. Naktinis, J. Turner, and M. O'Donnell, *Cell* **84,** 137–145 (1996).
37. S. Kim, H. G. Dallmann, C. S. McHenry, and K. J. Marians, *Cell* **84,** 643–650 (1996).

38. S. Kim, H. G. Dallmann, C. S. McHenry, and K. J. Marians, *J. Biol. Chem.* **271,** 21406–21412 (1996).
39. Q. Shan, J. M. Bork, B. L. Webb, R. B. Inman, and M. M. Cox, *J. Mol. Biol.* **265,** 519–540 (1997).
40. B. L. Webb, M. M. Cox, and R. B. Inman, *Cell* **91,** 347–356 (1997).
41. A. K. Eggleston and S. C. West, *Curr. Biol.* **7,** R745–R749 (1997).
42. S. C. Kowalczykowski, *Experientia* **50,** 204–215 (1994).
43. S. C. West, *Cell* **76,** 9–15 (1994).
44. S. C. West, *Annu. Rev. Genet.* **31,** 213–244 (1997).
45. D. Zerbib, C. Mezard, H. George, and S. C. West, *J. Mol. Biol.* **281,** 621–630 (1998).
46. M. C. Whitby and R. G. Lloyd, *J. Biol. Chem.* **273,** 19729–19739 (1998).
47. A. A. Mahdi, P. McGlynn, S. D. Levett, and R. G. Lloyd, *Nucleic Acids Res.* **25,** 3875–3880 (1997).
48. S. N. Chan, S. D. Vincent, and R. G. Lloyd, *Nucleic Acids Res.* **26,** 1560–1566 (1998).
49. G. J. Sharples, S. N. Chan, A. A. Mahdi, M. C. Whitby, and R. G. Lloyd, *EMBO J.* **13,** 6133–42 (1994).
50. B. Hallet and D. J. Sherratt, *FEMS Microbiol. Rev.* **21,** 157–178 (1997).
51. E. M. Park, M. K. Shigenaga, R. Degan, T. S. Korn, J. W. Kitzler, C. M. Wehr, P. Kolachana, and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* **89,** 3375–9 (1992).
52. C. Richter, J. W. Park, and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* **85,** 6465–6467 (1988).
53. L. Cao, E. Alani, and N. Kleckner, *Cell* **61,** 1089–101 (1990).
54. S. Keeney, C. N. Giroux, and N. Kleckner, *Cell* **88,** 375–84 (1997).
55. E. B. Konrad, *J. Bacteriol.* **130,** 167–72 (1977).
56. J. Zieg, V. F. Maples, and S. R. Kushner, *J. Bacteriol.* **134,** 958–966 (1978).
57. T. C. Wang and K. C. Smith, *J. Bacteriol.* **165,** 1023–1025 (1986).
58. E. M. Witkin and M. V. Roegner, *J. Bacteriol.* **174,** 4166–4168 (1992).
59. S. Linn and J. A. Imlay, *J. Cell Sci. (Suppl)* **6,** 289–301 (1987).
60. A. G. Miguel and R. M. Tyrrell, *Biophys. J.* **49,** 485–491 (1986).
61. L. S. Morse and C. Pauling, *Proc. Natl. Acad. Sci. U.S.A.* **72,** 4645–4649 (1975).
62. W. Y. Feng, E. H. Lee, and J. B. Hays, *Genetics* **129,** 1007–1020 (1991).
63. W. D. Rupp, C. E d. Wilde, D. L. Reno, and P. Howard-Flanders, *J. Mol. Biol.* **61,** 25–44 (1971).
64. K. C. Smith and T. C. Wang, *BioEssays* **10,** 12–6 (1989).
65. J. B. Hays and J. G. Hays, *Biopolymers* **31,** 1565–79 (1991).
66. W. Y. Feng and J. B. Hays, *Genetics* **140,** 1175–86 (1995).
67. R. R. Sinden and R. S. Cole, *J. Bacteriol.* **136,** 538–47 (1978).
68. R. S. Cole, *J. Bacteriol.* **107,** 846–852 (1971).
69. F. N. Capaldo, G. Ramsey, and S. D. Barbour, *J. Bacteriol.* **118,** 242–249 (1974).
70. M. J. Daly, L. Ouyang, P. Fuchs, and K. W. Minton, *J. Bacteriol.* **176,** 3508–3517 (1994).
71. M. J. Daly and K .W. Minton, *J. Bacteriol.* **177,** 5495–5505 (1995).
72. K. W. Minton, *Mutat. Res.* **363,** 1–7 (1996).
73. K. W. Minton, *Mol. Microbiol.* **13,** 9–15 (1994).
74. M. Morimyo, *J. Bacteriol.* **152,** 208–214 (1982).
75. D. Touati, M. Jacques, B. Tardat, L. Bouchard, and S. Despied, *J. Bacteriol.* **177,** 2305–2314 (1995).
76. W. W. Steiner and P. L. Kuempel, *J. Bacteriol.* **180,** 6269–6275 (1998).
77. M. Rajagopalan *et al., Proc. Natl. Acad. Sci. U.S.A.* **89,** 10777–10781 (1992).
78. P. Belguise-Valladier, H. Maki, M. Sekiguchi, and R. P. Fuchs, *J. Mol. Biol.* **236,** 151–164 (1994).

79. M. Tang, I. Bruck, R. Eritja, J. Turner, E. G. Frank, R. Woodgate, M. O'Donnell, and M. F. Goodman, *Proc. Natl. Acad. Sci. U.S.A.* **95,** 9755–9760 (1998).
80. P. C. Hanawalt, *Photochem. Photobiol.* **5,** 1–12 (1966).
81. A. Skalka, *In* "Mechanisms in Recombination" (R. F. Grell, ed.), pp. 421–432. Plenum, New York, 1974.
82. A. Kuzminov, *Mol. Microbiol.* **16,** 373–384 (1995).
83. T. Kogoma, *Cell* **85,** 625–627 (1996).
84. Y. Cao and T. Kogoma, *Genetics* **139,** 1483–1494 (1995).
85. P. McGlynn, D. A. Al, J. Liu, K. J. Marians, and R. G. Lloyd, *J. Mol. Biol.* **270,** 212–221 (1997).
86. J. Liu, L. Xu, S. J. Sandler, and K. J. Marians, *Proc. Natl. Acad. Sci. U.S.A.* **96,** 3552–3555 (1999).
87. J. Courcelle, C. Carswell-Crumpton, and P. Hanawalt, *Proc. Natl. Acad. Sci. U.S.A.* **94,** 3714–3719 (1997).
88. M. A. Blanar, S. J. Sandler, M. E. Armengod, L. W. Ream, and A. J. Clark, *Proc. Natl. Acad. Sci. U.S.A.* **81,** 4622–4626 (1984).
89. R. L. Perez-Roge, M. Garcia-Sogo, J. Navarro-Avino, C. Lopez-Acedo, F. Macian, and M. E. Armengod, *Biochimie* **73,** 329–334 (1991).
90. A. M. Flower and C. S. McHenry, *J. Mol. Biol.* **220,** 649–658 (1991).
91. B. Michel, S. D. Ehrlich, and M. Uzest, *EMBO J.* **16,** 430–8 (1997).
92. K. C. Smith, T. V. Wang, and R. C. Sharma, *J. Photochem. Photobiol. B, Biol* **1,** 1–11 (1987).
93. K. C. Smith and R. C. Sharma, *Mutat. Res.* **183,** 1–9 (1987).
94. M. Sassanfar and J. W. Roberts, *J. Mol. Biol.* **212,** 79–96 (1990).
95. S. Sommer, A. Leitao, A. Bernardi, A. Bailone, and R. Devoret, *Mutat. Res.* **254,** 107–117 (1991).
96. R. D'Ari and O. Huisman, *Biochimie* **64,** 623–627 (1982).
97. M. V. Madiraju, A. Templin, and A. J. Clark, *Proc. Natl. Acad. Sci. U.S.A.* **85,** 6592–6596 (1988).
98. T. Asai, S. Sommer, A. Bailone, and T. Kogoma, *EMBO J.* **12,** 3287–3295 (1993).
99. Z. Horii and A. J. Clark, *J. Mol. Biol.* **80,** 237–344 (1973).
100. A. J. Clark, *Genetics* **78,** 259–271 (1974).
101. H. Ochman and R. K. Selander, *Proc. Natl. Acad. Sci. U.S.A.* **81,** 198–201 (1984).
102. H. Ochman and A. C. Wilson, *In "Escherichia coli* and *Salmonella typhimurium:* Cellular and Molecular Biology" (F. C. Neidhardt *et al.,* eds.), pp. 1649–1654. American Society for Microbiology, Washington, D.C., 1987.
103. T. Asai and T. Kogoma, *J. Bacteriol.* **176,** 7113–7114 (1994).
104. J. W. Little, *Biochimie* **73,** 411–422 (1991).
105. H. Shinagawa, *Exs* **77,** 221–235 (1996).
106. G. C. Walker, *Trends Biochem. Sci.* **20,** 416–420 (1995).
107. A. Kuzminov, *BioEssays* **17,** 733–741 (1995).
108. M. Seigneur, V. Bidnenko, S. D. Ehrlich, and B. Michel, *Cell* **95,** 419–430 (1998).
109. W. D. Rupp and P. Howard-Flanders, *J. Mol. Biol.* **31,** 291–304 (1968).
110. R. S. Cole, *J. Bacteriol.* **106,** 143–149 (1971).
111. S. G. Sedgwick, *Proc. Natl. Acad. Sci. U.S.A.* **72,** 2753–2757 (1975).
112. D. A. Youngs and K. C. Smith, *J. Bacteriol.* **125,** 102–110 (1976).
113. M. A. Khidhir, S. Casaregola, and I. B. Holland, *Mol. Gen. Genet.* **199,** 133–140 (1985).
114. E. M. Witkin, M. V. Roegner, J. B. Sweasy, and J. O. McCall, *Proc. Natl. Acad. Sci. U.S.A.* **84,** 6805–6809 (1987).
115. N. Koffel-Schwartz, F. Coin, X. Veaute, and R. P. P. Fuchs, *Proc. Natl. Acad. Sci. U.S.A.* **93,** 7805–7810 (1996).

116. R. L. Napolitano, I. B. Lambert, and R. P. P. Fuchs, *Proc. Natl. Acad. Sci. U.S.A.* **94,** 5733–5738 (1997).
117. R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto, R. Kainuma, A. Sugino, and N. Iwatsuki, *Cold Spring Harb. Symp. Quant. Biol.* **33,** 129–143 (1968).
118. C. Pauling and L. Hamm, *Proc. Natl. Acad. Sci. U.S.A.* **64,** 1195–1202 (1969).
119. J. M. Louarn and R. E. Bird, *Proc. Natl. Acad. Sci. U.S.A.* **71,** 329–333 (1974).
120. T. C. Wang and K. C. Smith, *Biochem. Biophys. Res. Commun.* **165,** 685–688 (1989).
121. B. K. Tye, J. Chien, I. R. Lehman, B. K. Duncan, and H. R. Warner, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 233–237 (1978).
122. B. K. Tye, P. O. Nyman, I. R. Lehman, S. Hochhauser, and B. Weiss, *Proc. Natl. Acad. Sci. U.S.A.* **74,** 154–157 (1977).
123. B. K. Tye and I. R. Lehman, *J. Mol. Biol.* **117,** 293–306 (1977).
124. B. M. Olivera, P. Manlapaz-Ramos, H. R. Warner, and B. K. Duncan, *J. Mol. Biol.* **128,** 265–275 (1979).
125. C. A. Wu, E. L. Zechner, J. A. Reems, C. S. McHenry, and K. J. Marians, *J. Biol. Chem.* **267,** 4074–483 (1992).
126. H. Hiasa and K. J. Marians, *J. Biol. Chem.* **269,** 6058–6063 (1994).
127. B. M. Olivera, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 238–242 (1978).
128. K. Umezu and R. D. Kolodner, *J. Biol. Chem.* **269,** 30005–30013 (1994).
129. D. G. Anderson and S. C. Kowalczykowski, *Cell* **90,** 77–86 (1997).
130. A. J. Vangool, R. Shah, C. Mezard, and S. C. West, *EMBO J.* **17,** 1838–1845 (1998).
131. M. C. Whitby, L. Ryder, and R. G. Lloyd, *Cell* **75,** 341–350 (1993).
132. M. C. Whitby, S. D. Vincent, and R. G. Lloyd, *EMBO J.* **13,** 5220–5228 (1994).
133. B. Müller and S. C. West, *Experientia* **50,** 216–222 (1994).
134. D. Zerbib, S. D. Colloms, D. J. Sherratt, and S. C. West, *J. Mol. Biol.* **270,** 663–673 (1997).
135. A. Kuzminov, *BioEssays* **15,** 355–8 (1993).
136. A. K. Eggleston, A. H. Mitchell, and S. C. West, *Cell* **89,** 607–617 (1997).
137. M. J. E. Giraudpanis and D. M. J. Lilley, *J. Mol. Biol.* **278,** 117–133 (1998).
138. N. P. Higgins, K. Kato, and B. Strauss, *J. Mol. Biol.* **101,** 417–425 (1976).
139. T. Formosa and B. M. Alberts, *Cell* **47,** 793–806 (1986).
140. H. Zou and R. Rothstein, *Cell* **90,** 87–96 (1997).
141. J. M. Kaguni and A. Kornberg, *Cell* **38,** 183–190 (1984).
142. S. J. Sandler, H. S. Samra, and A. J. Clark, *Genetics* **143,** 5–13 (1996).
143. T. Kogoma, G. W. Cadwell, K. G. Barnard, and T. Asai, *J. Bacteriol.* **178,** 1258–1264 (1996).
144. H. Masai, T. Asai, Y. Kubota, K. Arai, and T. Kogoma, *EMBO J.* **13,** 5338–5345 (1994).
145. G. Blakely, S. Colloms, G. May, M. Burke, and D. Sherratt, *New Biol.* **3,** 789–798 (1991).
146. P. L. Kuempel, J. M. Henson, L. Dircks, M. Tecklenburg, and D. F. Lim, *New Biol.* **3,** 799–811 (1991).
147. N. R. Leslie and D. J. Sherratt, *EMBO J.* **14,** 1561–1570 (1995).
148. M. Tecklenburg, A. Naumer, O. Nagappan, and P. Kuempel, *Proc. Natl. Acad. Sci. U.S.A.* **92,** 1352–1356 (1995).
149. F. Cornet, J. Oouarn, J. Patte, and J. M. Louarn, *Genes Dev.* **10,** 1152–1161 (1996).
150. P. Kuempel, A. Hogaard, M. Nielsen, O. Nagappan, and M. Tecklenburg, *Genes Dev.* **10,** 1162–1171 (1996).
151. T. A. Baker, *Nature (London)* **353,** 794–795 (1991).
152. M. Inouye, *J. Bacteriol.* **106,** 539–542 (1971).
153. K. Skarstad and E. Boye, *J. Bacteriol.* **170,** 2549–2554 (1988).
154. J. W. Zyskind, A. L. Svitil, W. B. Stine, M. C. Biery, and D. W. Smith, *Mol. Microbiol.* **6,** 2525–2537 (1992).
155. T. Hoiruchi and Y. Fujimura, *J. Bacteriol.* **177,** 783–791 (1995).

156. K. Skarstad and E. Boye, *J. Bacteriol.* **175,** 5505–5509 (1993).
157. P. Nurse, K. H. Zavitz, and K. J. Marians, *J. Bacteriol.* **173,** 6686–6693 (1991).
158. L. Ryder, G. J. Sharples, and R. G. Lloyd, *Genetics* **143,** 1101–1114 (1996).
159. K. Ishioka, H. Iwasaki, and H. Shinagawa, *Genes Genet. Syst.* **72,** 91–99 (1997).
160. X. Bi and L. F. Liu, *J. Mol. Biol.* **256,** 849–858 (1996).
161. X. Bi and L. F. Liu, *Proc. Natl. Acad. Sci. U.S.A.* **93,** 819–823 (1996).
162. S. T. Lovett and V. V. Feschenko, *Proc. Natl. Acad. Sci. U.S.A.* **93,** 7120–7124 (1996).
163. S. D. Hall, M. F. Kane, and R. D. Kolodner, *J. Bacteriol.* **175,** 277–287 (1993).
164. W. W. Steiner and P. L. Kuempel, *Mol. Microbiol.* **27,** 257–268 (1998).
165. M. M. Cox, *Proc. Natl. Acad. Sci. U.S.A.* **94,** 11764–11766 (1997).
166. K. N. Kreuzer and S. W. Morrical, *In* "Molecular Biology of Bacteriophage T4" (J. D. Karam, ed.), pp. 28–42. ASM Press, Washington, D.C., 1994.
167. G. Mosig, *In* "Molecular Biology of Bacteriophage T4" (J. D. Karam, ed.), pp. 54–82. ASM Press, Washington, D.C., 1994.
168. K. N. Kreuzer and J. W. Drake, *In* "Molecular Biology of Bacteriophage T4" (J. D. Karam, ed.), pp. 89–97. ASM Press, Washington, D.C., 1994.
169. S. C. Kowalczykowski and A. K. Eggleston, *Annu. Rev. Biochem.* **63,** 991–1043 (1994).
170. M. M. Cox, *Trends Biochem. Sci.* **19,** 217–222 (1994).
171. V. Brendel, L. Brocchieri, S. J. Sandler, A. J. Clark, and S. Karlin, *J. Mol. Evol.* **44,** 528–541 (1997).
172. A. I. Roca and M. M. Cox, *CRC Crit. Rev. Biochem. Mol. Biol.* **25,** 415–456 (1990).
173. H. Jiang, D. Giedroc, and T. Kodadek, *J. Biol. Chem.* **268,** 7904–7911 (1993).
174. S. J. Sandler, L. H. Satin, H. S. Samra, and A. J. Clark, *Nucleic Acids Res.* **24,** 2125–2132 (1996).
175. E. M. Seitz, J. P. Brockman, S. J. Sandler, A. J. Clark, and S. C. Kowalczykowski, *Genes Dev.* **12,** 1248–1253 (1998).
176. A. Shinohara, H. Ogawa, and T. Ogawa, *Cell* **69,** 457–470 (1992).
177. T. Ogawa, X. Yu, A. Shinohara, and E. H. Egelman, *Science* **259,** 1896–1899 (1993).
178. P. Baumann and S. C. West, *Trends Biochem. Sci.* **23,** 247–251 (1998).
179. D. K. Bishop, *Cell* **79,** 1081–1092 (1994).
180. R. C. Gupta, L. R. Bazemore, E. I. Golub, and C. M. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **94,** 463–468 (1997).
181. P. Sung, *Science* **265,** 1241–1243 (1994).
182. J. H. New, T. Sugiyama, E. Zaitseva, and S. C. Kowalczykowski, *Nature (London)* **391,** 337–338 (1998).
183. J. C. Register, III, and J. Griffith, *J. Biol. Chem.* **260,** 12308–12312 (1985).
184. R. M. Story, I. T. Weber, and T. A. Steitz, *Nature (London)* **355,** 318–325 (1992).
185. R. M. Story and T. A. Steitz, *Nature (London)* **355,** 374–376 (1992).
186. M. M. Cox, K. McEntee, and I. R. Lehman, *J. Biol. Chem.* **256,** 4676–4678 (1981).
187. T. Shibata, R. P. Cunningham, and C. M. Radding, *J. Biol. Chem.* **256,** 7557–7564 (1981).
188. S. M. Cotterill, A. C. Satterthwait, and A. R. Fersht, *Biochemistry* **21,** 4332–4337 (1982).
189. J. Griffith and C. G. Shores, *Biochemistry* **24,** 158–162 (1985).
190. S. Kuramitsu, K. Hamaguchi, T. Ogawa, and H. Ogawa, *J. Biochem.* **90,** 1033–1045 (1981).
191. W. A. Bedale, R. B. Inman, and M. M. Cox, *J. Biol. Chem.* **268,** 15004–15016 (1993).
192. T. Simonson, M. Kubista, R. Sjoback, H. Ryberg, and M. Takahashi, *J. Mol. Recogn.* **7,** 199–206 (1994).
193. L. J. Gudas and D. W. Mount, *Proc. Natl. Acad. Sci. U.S.A.* **74,** 5280–5284 (1977).
194. T. Ogawa, H. Wabiko, T. Tsurimoto, T. Horii, H. Masukata, and H. Ogawa, *Cold Spring Harb. Symp. Quant. Biol.* **2,** 909–915 (1979).
195. J. H. Krueger and G. C. Walker, *Proc. Natl. Acad. Sci. U.S.A.* **81,** 1499–1503 (1984).

196. N. Garvey, A. C. St. John, and E. M. Witkin, *J. Bacteriol.* **163,** 870–876 (1985).
197. C. Cazaux *et al.*, *J. Biol. Chem.* **269,** 8246–8254 (1994).
198. S. L. Brenner, A. Zlotnick, and J. D. Griffith, *J. Mol. Biol.* **204,** 959–972 (1988).
199. S. L. Brenner, A. Zlotnick, and W. F. Stafford, *J. Mol. Biol.* **216,** 949–964 (1990).
200. D. H. Wilson and A. S. Benight, *J. Biol. Chem.* **265,** 7351–7359 (1990).
201. B. F. Pugh and M. M. Cox, *J. Biol. Chem.* **263,** 76–83 (1988).
202. M. Takahashi, *J. Biol. Chem.* **264,** 288–295 (1989).
203. X. Yu and E. H. Egelman, *J. Mol. Biol.* **225,** 193–216 (1992).
204. E. H. Egelman and A. Stasiak, *Micron* **24,** 309–324 (1993).
205. E. H. Egelman, *Curr. Opin. Struct. Biol.* **3,** 189–197 (1993).
206. C. Ellouze, M. Takahashi, P. Wittung, K. Mortensen, M. Schnarr, and B. Norden, *Eur. J. Biochem.* **233,** 579–583 (1995).
207. S. Karlin and L. Brocchieri, *J. Bacteriol.* **178,** 1881–1894 (1996).
208. X. Yu and E. H. Egelman, *Nature Struct. Biol.* **4,** 101–104 (1997).
209. T. Amano, M. Yoshida, Y. Matsuo, and K. Nishikawa, *FEBS Lett.* **351,** 1–5 (1994).
210. M. A. Bianchet, Y. H. Ko, L. M. Amzel, and P. L. Pedersen, *J. Bioenerg. Biomembr.* **29,** 503–524 (1997).
211. H. G. Nastri and K. L. Knight, *J. Biol. Chem.* **269,** 26311–26322 (1994).
212. M. C. Skiba and K. L. Knight, *J. Biol. Chem.* **269,** 3823–3828 (1994).
213. K. M. Logan, M. C. Skiba, S. Eldin, and K. L. Knight, *J. Mol. Biol.* **266,** 306–316 (1997).
214. W. M. Rehrauer and S. C. Kowalczykowski, *J. Biol. Chem.* **268,** 1292–1297 (1993).
215. Q. Shan and M. M. Cox, *J. Mol. Biol.* **257,** 756–774 (1996).
216. Q. Shan, M. M. Cox, and R. B. Inman, *J. Biol. Chem.* **271,** 5712–5724 (1996).
217. J. E. Walker, M. Saraste, M. J. Runswick, and N. J. Gay, *EMBO J.* **1,** 945–951 (1982).
218. M. Saraste, P. R. Sibbald, and A. Wittinghofer, *Trends Biochem. Sci.* **15,** 430–434 (1990).
219. E. Stole and F. R. Bryant, *J. Biol. Chem.* **269,** 7919–7925 (1994).
220. E. Stole and F. R. Bryant, *J. Biol. Chem.* **270,** 20322–20328 (1995).
221. E. Stole and F. R. Bryant, *Biochemistry* **36,** 3483–3890 (1997).
222. K. Morimatsu, T. Horii, and M. Takahashi, *Eur. J. Biochem.* **228,** 779–785 (1995).
223. P. E. Lavery and S. C. Kowalczykowski, *J. Biol. Chem.* **265,** 4004–4010 (1990).
224. M. V. Madiraju, P. E. Lavery, S. C. Kowalczykowski, and A. J. Clark, *Biochemistry* **31,** 10529–10535 (1992).
225. T. C. Wang, H. Y. Chang, and J. L. Hung, *Mutat. Res.* **294,** 157–66 (1993).
226. G. M. Weinstock, K. McEntee, and I. R. Lehman, *J. Biol. Chem.* **256,** 8845–8849 (1981).
227. W. A. Bedale and M. Cox, *J. Biol. Chem.* **271,** 5725–5732 (1996).
228. S. W. Morrical, J. Lee, and M. M. Cox, *Biochemistry* **25,** 1482–1494 (1986).
229. L. J. Roman and S. C. Kowalczykowski, *Biochemistry* **25,** 7375–7385 (1986).
230. S. C. Kowalczykowski and R. A. Krupp, *J. Mol. Biol.* **193,** 97–113 (1987).
231. J. E. Lindsley and M. M. Cox, *J. Mol. Biol.* **205,** 695–711 (1989).
232. G. M. Weinstock, K. McEntee, and I. R. Lehman, *J. Biol. Chem.* **256,** 8829–8834 (1981).
233. K. A. Kumar, S. Mahalakshmi, and K. Muniyappa, *J. Biol. Chem.* **268,** 26162–26170 (1993).
234. P. Wittung, B. Norden, and M. Takahashi, *Eur. J. Biochem.* **228,** 149–154 (1995).
235. S. L. Brenner, R. S. Mitchell, S. W. Morrical, S. K. Neuendorf, B. C. Schutte, and M. M. Cox, *J. Biol. Chem.* **262,** 4011–4016 (1987).
236. S. C. Kowalczykowski, J. Clow, and R. A. Krupp, *Proc. Natl. Acad. Sci. U.S.A.* **84,** 3127–3131 (1987).
237. B. C. Schutte and M. M. Cox, *Biochemistry* **26,** 5616–5625 (1987).
238. T. A. Arenson, O. V. Tsodikov, and M. M. Cox, *J. Mol. Biol.* **288,** 391–401 (1999).
239. J. W. Lee and M. M. Cox, *Biochemistry* **29,** 7666–7676 (1990).

240. J. W. Lee and M. M. Cox, *Biochemistry* **29,** 7677–7683 (1990).
241. M. M. Cox, D. A. Soltis, I. R. Lehman, C. DeBrosse, and S. J. Benkovic, *J. Biol. Chem.* **258,** 2586–2592 (1983).
242. B. F. Pugh, B. C. Schutte, and M. M. Cox, *J. Mol. Biol.* **205,** 487–492 (1989).
243. X. Yu and E. H. Egelman, *J. Mol. Biol.* **227,** 334–346 (1992).
244. S. C. Kowalczykowski, *Biochemistry* **25,** 5872–5881 (1986).
245. K. L. Menge and F. R. Bryant, *Biochemistry* **31,** 5151–5157 (1992).
246. K. L. Menge and F. R. Bryant, *Biochemistry* **31,** 5158–5165 (1992).
247. J. P. Menetski and S. C. Kowalczykowski, *Biochemistry* **28,** 5871=5881 (1989).
248. J. E. Lindsley and M. M. Cox, *J. Biol. Chem.* **265,** 9043–9054 (1990).
249. R. Masui, T. Mikawa, R. Kato, and S. Kuramitsu, *Biochemistry* **37,** 14788–14797 (1998).
250. J. P. Menetski and S. C. Kowalczykowski, *J. Mol. Biol.* **181,** 281–295 (1985).
251. K. L. Menge and F. R. Bryant, *Biochemistry* **27,** 2635–2640 (1988).
252. B. F. Pugh and M. M. Cox, *J. Biol. Chem.* **262,** 1326–1336 (1987).
253. B. F. Pugh and M. M. Cox, *J. Mol. Biol.* **203,** 479–493 (1988).
254. C. Lu, R. H. Scheuermann and H. Echols, *Proc. Natl. Acad. Sci. U.S.A.* **83,** 619–623 (1986).
255. M. Kojima *et al.*, *Nucleic Acids Res.* **18,** 2707–2714 (1990).
256. Y. H. Wang, C. D. Bortner, and J. Griffith, *J. Biol. Chem.* **268,** 17571–17577 (1993).
257. S. K. Kim, M. Takahashi, B. Jernstrom, and B. Norden, *Carcinogenesis* **14,** 311–313 (1993).
258. S. A. Chow, S. M. Honigberg, and C. M. Radding, *J. Biol. Chem.* **263,** 3335–3347 (1988).
259. A. Wegner, *J. Mol. Biol.* **161,** 607–615 (1982).
260. S. K. Neuendorf and M. M. Cox, *J. Biol. Chem.* **261,** 8276–8282 (1986).
261. M. S. Meselson and C. M. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **72,** 358–361 (1975).
262. M. A. Resnick, *J. Theor. Biol.* **59,** 97–106 (1976).
263. J. W. Szostak, W. T. L. Orr, R. J. Rothstein, and F. W. Stahl, *Cell* **33,** 25–35 (1983).
264. D. A. Dixon and S. C. Kowalczykiwski, *Cell* **66,** 361–371 (1991).
265. D. G. Anderson and S. C. Kowalczykowski, *Genes Dev.* **11,** 571–581 (1997).
266. B. B. Konforti and R. W. Davis, *J. Biol. Chem.* **266,** 10112–10121 (1991).
267. M. Dutreix, B. J. Rao, and C. M. Radding, *J. Mol. Biol.* **219,** 645–654 (1991).
268. J. P. Menetski and S. C. Kowalczykowski, *J. Biol. Chem.* **262,** 2085–2092 (1987).
269. R. S. Mitchell, A. Zlotnick, and S. L. Brenner, *Biophys. J.* **53,** 220a (1988).
270. S. D. Lauder and S. C. Kowalczykowski, *J. Biol. Chem.* **266,** 5450–5458 (1991).
271. A. Zlotnick, R. S. Mitchell, R. K. Steed, and S. L. Brenner, *J. Biol. Chem.* **268,** 22525–30 (1993).
272. E. H. Egelman and X. Yu, *Science* **245,** 404–407 (1989).
273. E. H. Egelman and A. Stasiak, *J. Mol. Biol.* **200,** 329–349 (1988).
274. A. Stasiak, E. H. Egelman, and P. Howard-Flanders, *J. Mol. Biol.* **202,** 659–662 (1988).
275. A. Stasiak and E. H. Egelman, *In* "Genetic Recombination" (R. Kucherlapati and G. R. Smith, eds.), pp. 265–307. American Society for Microbiology, Washington, D.C., 1988.
276. J. Heuser and J. Griffith, *J. Mol. Biol.* **210,** 473–484 (1989).
277. E. H. Egelman and A. Stasiak, *J. Mol. Biol.* **191,** 677–697 (1986).
278. E. Di Capua, A. Engel, A. Stasiak, and T. Koller, *J. Mol. Biol.* **157,** 87–103 (1982).
279. B. Nordén, C. Elvingson, M. Kubista, B. Sjoberg, H. Ryberg, M. Ryberg, K. Mortensen, and M. Takahashi, *J. Mol. Biol.* **226,** 1175–1191 (1992).
280. M. Takahashi, M. Kubista, and B. Nordén, *Biochimie* **73,** 219–226 (1991).
281. M. Takahashi and B. Nordén, *Adv. Biophys.* **30,** 1–35 (1994).
282. D. F. Dombroski, D. G. Scraba, R. D. Bradley, and A. R. Morgan, *Nucleic Acids Res.* **11,** 7487–7504 (1983).

283. M. C. Leahy and C. M. Radding, *J. Biol. Chem.* **261,** 6954–6960 (1986).
284. E. Di Capua and B. Müller, *EMBO J.* **6,** 2493–2498 (1987).
285. K. McEntee, G. M. Weinstock, and I. R. Lehman, *J. Biol. Chem.* **256,** 8835–8844 (1981).
286. M. Amaratunga and A. S. Benight, *Biochem. Biophys. res. Commun.* **157,** 127–133 (1988).
287. C. Cazenave, M. Chabbert, J. J. Toulme, and C. Helene, *Biochim. Biophys. Acta* **781,** 7–13 (1984).
288. R. B. Tracy and S. C. Kowalczykowski, *Genes Dev.* **10,** 1890–1903 (1996).
289. R. B. Tracy, F. Chedin, and S. C. Kowalczykowski, *Cell* **90,** 205–206 (1997).
290. M. Kubista, T. Simonson, R. Sjöback, H. Widlund, and A. Johansson, *In* "Biological Structure and Function: Proceedings of the Ninth Conversation, The State University of New York" (R. H. Sarma and M. H. Sarma, eds.), pp. 49–59. Adenine Press, New York, 1996.
291. H. Kurumizaka, B. J. Rao, T. Ogawa, C. M. Radding, and T. Shibata, *Nucleic Acids Res.* **22,** 3387–3391 (1994).
292. H. Kurumizaka and T. Shibata, *J. Biochem.* **119,** 216–223 (1996).
293. B. Müller, T. Koller, and A. Stasiak, *J. Mol. Biol.* **212,** 97–112 (1990).
294. A. V. Mazin and S. C. Kowalczykowski, *Proc. Natl. Acad. Sci. U.S.A.* **93,** 10673–10678 (1996).
295. W. M. Rehrauer and S. C. Kowalczykowski, *J. Biol. Chem.* **271,** 11996–12002 (1996).
296. A. V. Mazin and S. C. Kowalczykowski, *EMBO J.* **17,** 1161–1168 (1998).
297. P. Howard-Flanders, S. C. West, and A. Stasiak, *Nature (London)* **309,** 215–219 (1984).
298. J. E. Yancey-Wrona and R. D. Camerini-Otero, *Curr. Biol.* **5,** 1149–1158 (1995).
299. L. R. Bazemore, M. Takahashi, and C. M. Radding, *J. Biol. Chem.* **272,** 14672–14682 (1997).
300. L. R. Bazemore, E. Foltastogniew, M. Takahashi, and C. M. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **94,** 11863–11868 (1997).
301. K. Adzuma, *J. Biol. Chem.* **273,** 31565–31573 (1998).
302. S. C. West, *Annu. Rev. Biochem.* **61,** 603–640 (1992).
303. S. McGavin, *J. Mol. Biol.* **55,** 293–298 (1971).
304. J. H. Wilson, *Proc. Natl. Acad. Sci. U.S.A.* **76,** 3641–3645 (1979).
305. R. A. Fishel and A. Rich, *In* "Mechanisms and Consequences of DNA Damage Processing" (E. C. Friedberg and P. C. Hanawalt, eds.), pp. 23–32. Alan R. Liss, New York, 1988.
306. M. M. Cox, *J. Biol. Chem.* **270,** 26021–26024 (1995).
307. Q. Shan and M. M. Cox, *Mol. Cell* **1,** 309–317 (1998).
308. J. E. Lindsley and M. M. Cox, *J. Biol. Chem.* **265,** 10164–10171 (1990).
309. J. I. Kim, M. M. Cox, and R. B. Inman, *J. Biol. Chem.* **267,** 16444–16449 (1992).
310. E. C. Conley and S. C. West, *J. Biol. Chem.* **265,** 10156–10163 (1990).
311. S. A. Chow, S. K. Chiu, and B. C. Wong, *J. Mol. Biol.* **223,** 79–93 (1992).
312. P. Wittung, B. Nordén, S. K. Kim, and M. Takahashi, *J. Biol. Chem.* **269,** 5799–5803 (1994).
313. V. B. Zhurkin, G. Raghunathan, N. B. Ulyanov, O. R. Camerini, and R. L. Jernigan, *J. Mol. Biol.* **239,** 181–200 (1994).
314. M. G. Kim, V. B. Zhurkin, R. L. Jernigan, and R. D. Camerini-Otero, *J. Mol. Biol.* **247,** 874–889 (1995).
315. M. R. Kiran and M. Bansal, *J. Biomol. Struct. Dyn.* **15,** 333–345 (1997).
316. B. J. Rao, S. K. Chiu, and C. M. Radding, *J. Mol. Biol.* **229,** 328–343 (1993).
317. A. K. Shchyolkina, E. N. Timofeev, O. F. Borisova, I. A. Il'icheva, E. E. Minyat, E. B. Khomyakova, and V. L. Florentiev, *FEBS Lett.* **339,** 113–118 (1994).
318. K. A. Kumar and K. Muniyappa, *J. Biol. Chem.* **267,** 24824–24832 (1992).
319. R. Baliga, J. W. Singleton, and P. B. Dervan, *Proc. Natl. Acad. Sci. U.S.A.* **92,** 10393–10397 (1995).

320. M. A. Podyminogin, R. B. Meyer, and H. B. Gamper, *Biochemistry* **34,** 13098–13108 (1995).
321. M. A. Podyminogin, R. B. Meyer, and H. B. Gamper, *Biochemistry* **35,** 7267–7274 (1996).
322. X. Zhou and K. Adzuma, *Biochemistry* **36,** 4650–4661 (1997).
323. M. D. Frank-Kamenetskii and S. M. Mirkin, *Annu. Rev. Biochem.* **64,** 65–95 (1995).
324. K. Adzuma, *Genes Dev.* **6,** 1679–1694 (1992).
325. Q. Shan and M. M. Cox, *J. Biol. Chem.* **272,** 11063–11073 (1997).
326. J. P. Menetski, D. G. Bear, and S. C. Kowalczykowski, *Proc. Natl. Acad. Sci. U.S.A.* **87,** 21–25 (1990).
327. W. Rosselli and A. Stasiak, *J. Mol. Biol.* **216,** 335–352 (1990).
328. J. I. Kim, M. M. Cox, and R. B. Inman, *J. Biol. Chem.* **267,** 16438–16443 (1992).
329. S. C. Kowalczykowski and R. A. Krupp, *Proc. Natl. Acad. Sci. U.S.A.* **92,** 3478–3482 (1995).
330. S. K. Jain, M. M. Cox, and R. B. Inman, *J. Biol. Chem.* **269,** 20653–20661 (1994).
331. W. Rosselli and A. Stasiak, *EMBO J.* **10,** 4391–4396 (1991).
332. B. Alberts and R. Miake-Lye, *Cell* **68,** 415–420 (1992).
333. B. B. Konforti and R. W. Davis, *J. Mol. Biol.* **227,** 38–53 (1992).
334. P. Morel, A. Stasiak, S. D. Ehrlich, and E. Cassuto, *J. Biol. Chem.* **269,** 19830–19835 (1994).
335. C. J. Ullsperger and M. M. Cox, *Biochemistry* **34,** 10859–10866 (1995).
336. B. Jwang and C. M. Radding, *Proc. Natl. Acad. Sci. U.S.A.* **89,** 7596–7600 (1992).
337. M. M. Cox, B. F. Pugh, B. C. Schutte, J. E. Lindsley, J. Lee, and S. W. Morrical, *In* "DNA Replication and REcombination" (R. McMacken and T. J. Kelly, eds.), pp. 597–607. Alan R. Liss, New York, 1987.
338. M. M. Cox, *In* "Molecular Biology of Chromosome Function" (K. W. Adolph, ed.), pp. 43–70. Springer-Verlag, Berlin and New York, 1989.
339. K. J. MacFarland, Q. Shan, R. B. Inman, and M. M. Cox, *J. Biol. Chem.* **272,** 17675–17685 (1997).
340. S. C. West and P. Howard-Flanders, *Cell* **37,** 683–691 (1984).
341. S. Umlauf, Ph.D., University of Wisconsin–Madison (1990).
342. S. Lacks, *Genetics* **53,** 207–235 (1966).