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Chapter 3

The Role of RecA Protein in Homologous Genetic Recombination

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Homologous genetic recombination is the process by which genetic information is rearranged between chromosomes that possess similar sequences. This type of recombination is responsible, in prokaryotes, for DNA rearrangements that occur during transformation or conjugation, postreplication repair of DNA, and a number of other processes [1]. Additional roles have been identified in eukaryotic cells [1,2]. About 30 homologous recombination events occur somewhere in the human genome during each meiosis, scattered more or less randomly about the chromosomes [3]. In bacteria, the probability of recombination occurring in a given length of DNA is increased about 500-fold relative to human meiotic chromosomes. A recombination event takes at least 35 minutes in bacteria [4] and perhaps 8 to 10 times longer in fungi [5].

A variety of models for homologous genetic recombination have been proposed, based primarily on genetic observations in fungi [2,5,6]. Although these models differ in important details, in most cases they share a set of fundamental steps. These are outlined in Figure 3.1, and include (1) cutting or creation of a gap in the DNA molecules to be recombined; (2) alignment or pairing of homologous sequences in two different DNA molecules; (3) the creation of a crossover or Holliday junction between the molecules in which DNA strands from each are paired, creating short regions of heteroduplex DNA; (4) extension of heteroduplex DNA (branch migration); and (5) resolution of the crossover junction to yield products [2,5].

This is an extremely complicated reaction that requires the action of several different proteins. Moreover, the reaction must occur in a cellular environment filled with structural, topological, and energetic barriers. Recombination proteins must compete for binding sites with a plethora of other cellular DNA-binding proteins. The helical nature of DNA places topological constraints on the reaction. Energy is required to confer order and a driving force in a reaction where products and substrates can be chemically indistinguishable or nearly so.

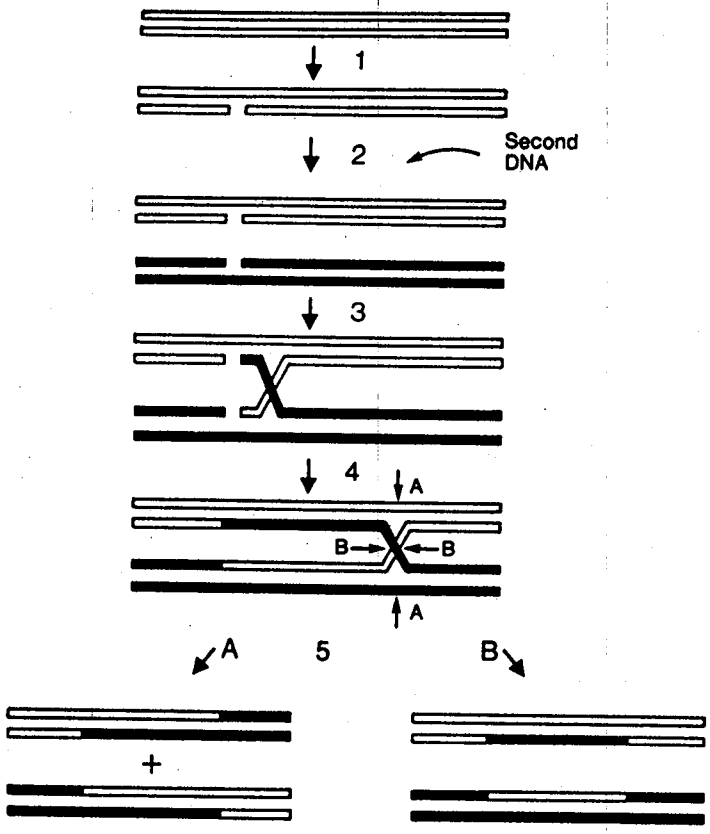


Figure 3.1. A model for homologous genetic recombination. The model is arranged to highlight the contribution of recA protein. Steps are (1) creation of a nick or gap in at least one strand of a duplex DNA; (2) alignment of two DNAs cut in this way; (3) creation of a crossover structure or Holliday junction; (4) branch migration; and (5) resolution of the Holliday junction. A ligation step sealing the original DNA nicks is included in step 4 but could occur at another point. Step 5 is a combination of cleavage by a specialized resolution nuclease and religation. The two sets of products are those generated by cleavage at the points labeled A and B, respectively. Additional steps, likely to occur, that require additional nucleases and DNA polymerase are not shown. [For variations, see references 3 and 6.]

been purified from many sources and studied in vitro [7]. These studies are quite advanced in the case of bacteria. This chapter will focus on the recA protein of *E. coli* as a model system for key events in homologous recombination.

The recA protein promotes the reactions depicted in steps 2 through 4 of Figure 3.1. These steps are the heart of a recombination event. Steps 1 and 5 and the depicted ligation reactions can be rationalized as the

activities of specialized versions of familiar enzyme classes such as helicases, nucleases, and ligases. Several nucleases have been purified with the unusual and interesting substrate specificities required for these steps in recombination [7]. Steps 2 through 4 could similarly be rationalized as an unusual combination of helicase and strand-annealing activities. In this case, however, the rationalization falls far short of the biochemical mark. The recA protein appears to be a prototype of a completely distinct class of enzyme.

The activity of recA protein is not easily categorized. An in vitro reaction that mimics recombination steps 2 through 4 and that has been widely employed in recA studies is illustrated in Figure 3.2. The reaction involves an exchange of strands between linear duplex DNAs and homologous circular single strands. RecA protein will also promote strand exchanges between two duplex DNAs (a four-strand exchange as opposed to the three-strand exchange described here) if one of the duplexes is partially single stranded [8–10]. The substrates are generally derived from bacteriophages. The mechanism by which recA protein carries out this reaction is currently a focus of research in many laboratories. The three reaction phases illustrated in Figure 3.2 are easily distinguished experimentally. The system offers a number of biochemical problems that can be most easily described in a summary of these reaction phases.

The first phase is the formation of a stoichiometric, filamentous complex of recA protein on the ssDNA [11,12]. This complex contains approximately one recA monomer per three or four nucleotides of DNA, arranged in a right-handed helical array [13–17]. An example is illustrated in Figure 3.3. Formation of this complex is facilitated by a second protein, the single-stranded DNA-binding protein of *E. coli* (SSB). The role of SSB in this reaction is discussed at length elsewhere [7,18,19] and will not be considered here. During strand exchange, the SSB is transferred to the displaced linear "+" strand, as shown in Figure 3.2 (B.C. Schutte and M.M. Cox, unpublished).

In the second phase, the ssDNA in the complex is aligned with homologous sequences in the duplex. Several steps have been identified in this process [20,21]. Initial interactions with nonhomologous sequences are manifested by the formation of aggregates of complexes and duplex DNA molecules that may facilitate the search for homology [21]. Additional studies initiated by the Radding group have defined two types of homologous interaction. The first is a paranemic joint in which the ssDNA and duplex DNA are aligned homologously but no strand interwinding to produce heteroduplex DNA has occurred [20,22,23]. Recent evidence has demonstrated that paranemic joints can be thousands of base pairs long [24–26]. The structure of this paranemic joint is a central issue in understanding the overall reaction. Paranemic joints are formed fast enough to be considered intermediates in the pathway leading to the

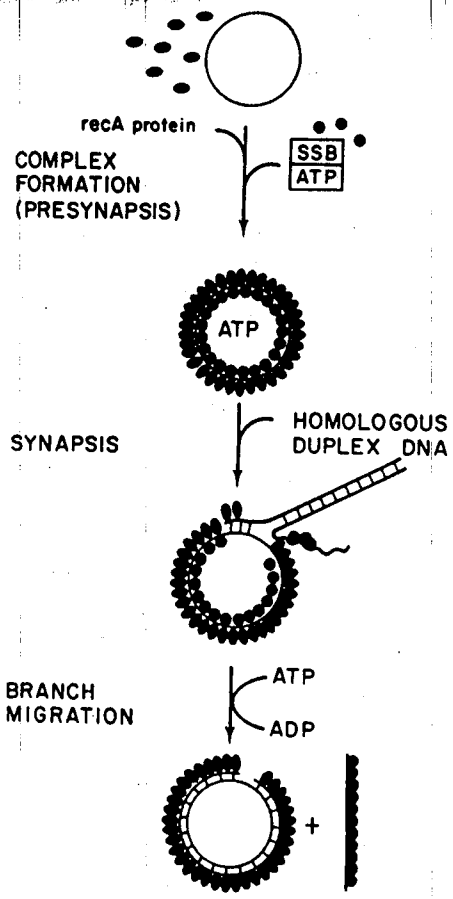


Figure 3.2. RecA protein-promoted DNA strand exchange. The three phases of the reaction are described in the text. RecA protein and SSB are denoted by ovals and circles, respectively.

second type of homologous interaction in which some interwinding of strands from the two DNA molecules occurs [20,22,23]. This species is a plectonemic joint [22]. The formation of plectonemic joints is limited only by topological considerations [27]. RecA protein is a DNA-dependent ATPase, and ATP is required for the pairing process. Paranemic joints, however, can be formed in the presence of ATP analogs that are not hydrolyzed by recA protein [23,28,29].

ATP hydrolysis is required only in the final phase illustrated in Figure 3.2. This is a branch migration reaction in which the linear "+" strand of the duplex is progressively replaced by the circular "+" strand until products are formed. This is probably the least well understood phase of the DNA strand exchange reaction. As pointed out elsewhere [7], a major contribution of recA protein to this phase of the reaction is the absolute polarity it exhibits. Branch migration proceeds only in the 5′ → 3′ direc-

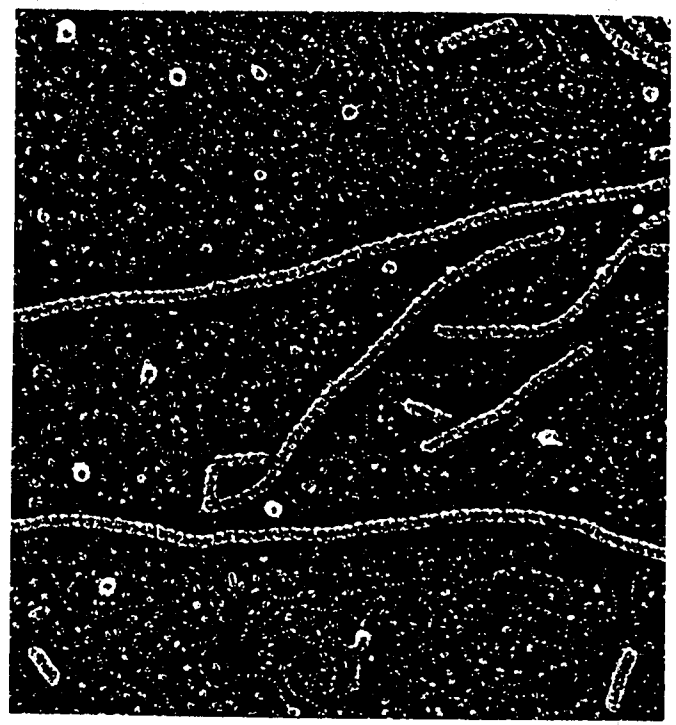


Figure 3.3. RecA protein helical filaments formed on duplex DNA in the presence of ATPγS. The filaments are right-handed helices. Unbound DNA is evident in the background. (Courtesy of J. Heuser, Washington University.)

tion relative to the incoming ssDNA within the complex [30–33]. The process is much slower than spontaneous branch migration (5 to 20 bp sec^{-1} vs. 6,000 bp sec^{-1}) [7]. By giving the process a direction, however, the efficiency of an extensive branch migration reaction is greatly increased relative to the faster, but random, uncatalyzed process. This raises a classical biochemical question concerning the coupling of chemical energy to a unidirectional process. The problem is analogous, in this respect, to muscle contraction and ATP-driven ion pumps. The problem is made more complicated by the fact that the active species is a long nucleoprotein filament.

A complete understanding of this reaction has proved elusive. What follows is a short overview of the fundamental properties and activities of recA protein. This is followed by an attempt to rationalize a wealth of data in terms of some possible mechanisms for strand exchange. Of necessity, this treatment will be somewhat selective, and the reader is encouraged to seek out several recent reviews [7,18,19] for different perspectives.

General Properties of recA Protein

Binding to Single-Stranded DNA

RecA protein will bind readily to single-stranded DNA at pHs ranging from 5 to 9. The binding stoichiometry appears to be 1 recA monomer per 4 nucleotides (nt) when solution methods are used for measurement [7]. A somewhat smaller binding site is seen with electron microscopic measurements of complexes on duplex DNA [15] (see below). The reason for the apparent discrepancy is not clear. ATP is not required for binding to ssDNA. The protein is bound along the phosphate backbone of the DNA [34,35], with the bases exposed in a large helical groove evident in image reconstructions derived from electron micrographs of filaments formed in the presence of ATPγS [36]. An inherent polarity in the filament is evident both from image reconstructions and from the experimental observation that the filament is assembled in a 5′ → 3′ direction along the DNA [37,38].

ATP Hydrolysis

At physiological salt concentrations recA protein is a DNA-dependent ATPase, exhibiting a turnover number (per monomer) varying between 20 and 35 min^{-1} depending on conditions and the DNA cofactor [7]. A key characteristic of this reaction is that it occurs throughout recA nucleoprotein filaments [7,19,39]. There is no relationship between the location of a recA monomer with respect to the filament ends and its rate of ATP hydrolysis.

This reaction (and many other recA activities) is inhibited by low concentrations (20 to 100 mM) of NaCl [7]. The reaction is much less sensitive to glutamate, recently shown to be the physiologically relevant anion in *E. coli* [40]. The ATPase activity is activated in the absence of DNA by very high concentrations (2 M) of a variety of salts [41]. The turnover number under high salt conditions is at least as high as that observed in the presence of DNA at low salt concentrations.

ATP hydrolysis is inhibited by ADP and ATPγS [7]. Both act as competitive inhibitors, but the inhibition patterns are complicated by cooperativity between adjacent recA monomers in the presence of both high salt and DNA. A slow conformation change mediated by ADP leads to recA protein dissociation when the ADP/ATP ratio exceeds 1.0 [42; J. Lee and M. Cox, unpublished].

Binding to Duplex DNA

Recent findings [43–48] have clarified many aspects of this interaction. RecA protein binds to duplex DNA via a multisegment pathway (Fig. 3.4). This association exhibits an absolute requirement for ATP or an ATP analog. The first segment is probably a weak binding of recA monomers or small oligomeric units that does not alter the structure of the DNA. This step is rapid, reversible, and dependent on recA protein concentration [44,45]. In the second reaction segment, a tighter association of a recA monomer or small oligomeric unit occurs that results in the underwinding of a corresponding region of DNA. This nucleation event is rate limiting overall [43–45]. Nucleation is followed by a very rapid propagation segment in which the DNA is completely covered with recA protein. On nicked circular duplex DNA the final complex is stable, and ATP hydrolysis occurs throughout with a k_{cat} (monomer) of 20 to 25 min^{-1} [44,45]. The recA protein is arranged in a right-handed helical structure, following the phosphate backbone of the DNA. There is a net uptake of two protons in the first two reaction segments of Figure 3.4, resulting in a very slow rate of de novo association at pHs above 7.0 [44,45].

The DNA within this complex is underwound by 39.6% in the presence of ATP [48]. This corresponds to an underwinding of 13.1°/bp and a helical periodicity of 17.6 bp per turn. This underwinding increases slightly, to 43% or 18.6 bp per turn, when ATPγS is substituted for ATP [48,49]. Underwinding of DNA in the recA-dsDNA complexes in the presence of ATPγS is manifested in the electron microscope by a 1.6-fold extension of the complexes relative to B-form DNA [13–17,49]. There is an 8 to 10-nm repeat in the filament structure that corresponds to the ~18 bp per turn helical repeat of the DNA. There are approximately six recA monomers per repeat in the filament [15,49].

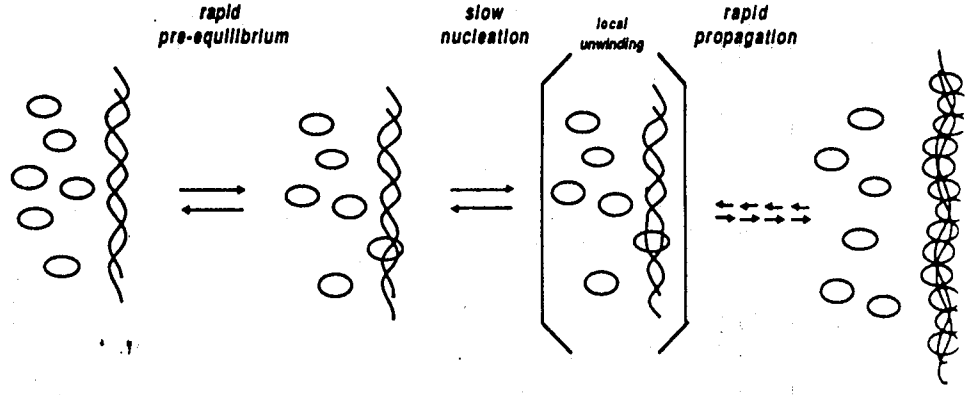


Figure 3.4. Pathway for recA protein binding to duplex DNA. The second step (nucleation) is generally rate limiting. ATP is hydrolyzed only after the complex is formed.

As with ssDNA, the recA-dsDNA nucleoprotein filament has an inherent polarity. The polarity reflects an asymmetry in binding to the two DNA strands. One of the two bound DNA strands exhibits a greater resistance to nuclease treatments than the other [46,50,51]. The more resistant strand can be referred to as the initiating strand. RecA protein filaments are assembled in the 5′ → 3′ direction relative to this strand [46,47,52]. It is reasonable to infer that the initiating strand occupies the same binding site occupied by ssDNA in recA-ssDNA complexes.

At pHs above 7.0, recA protein binds more rapidly to DNA with single-stranded gaps or tails, damaged DNA, DNA rich in A-T base pairs, and left-handed Z-form DNA than to B-form DNA [43,45–47,52,53]. This can be attributed to an enhancement in the rate of nucleation brought about by these structural features. Binding to Z-form DNA is much faster than to B-form DNA [53]. The final complex on Z DNA, however, is arranged in a right-handed helix structurally indistinguishable from that formed on B DNA [J. Kim, M. Cox, and J. Heuser, unpublished results]. The complex on Z DNA is also less stable than the complex formed on B DNA. When B and Z DNA are both present and compete for a limiting pool of recA protein, binding is ultimately restricted to the B DNA.

Once formed on duplex DNA, the recA nucleoprotein filament is stable at pHs between 5 and 9 as long as ATP is regenerated and the DNA substrate is circular (i.e., form II or nicked circular DNA). Complexes formed on linear duplex DNA are unstable at pHs above 6.8. This instability reflects a progressive dissociation of recA protein monomers (or other small units) from one end of the linear filament [46]. The maximum rate of this dissociation (observed at pHs above 8.0) is about 200 monomers min^{-1} per complex [46]. Relative to the initiating strand, dissociation occurs in the 5′ → 3′ direction. This is the same direction as assembly, so association and dissociation are largely restricted to opposite ends of the filament [46].

DNA Strand Exchange

Formation of a recA-DNA nucleoprotein filament that hydrolyzes ATP serves no useful end in itself. This complex is designed to find a second homologous DNA molecule, align it with the DNA already bound, and promote an exchange of DNA strands between these two DNA molecules. The mechanism of this reaction is not yet understood, but a wealth of experimental data is available to guide speculation. Approaching an understanding of this reaction requires a consideration of the cellular environment in which it takes place, the cellular problems it addresses, the structure of DNA, and the structure and kinetic properties of the recA nucleoprotein filament.

The Biological Problem

An analysis of the biological problem can begin with the question, Why would the cell employ a long protein filament to promote DNA pairing and branch migration? This strategy is expensive in two respects. A considerable energetic investment is made simply to assemble the 352 amino acids in each recA monomer in the filament. If each of the monomers then hydrolyzes ATP, the expense increases. At first glance, this investment seems unnecessary. Rates of uncatalyzed branch migration are sufficient to explain many of the genetic observations that require branch migration [54]. If directed pairing and branch migration are required, then it is easy to envision an enzyme with appropriate helicase and reannealing activities that could do the job. The in vitro evidence that the active species is a long filamentous complex, however, is extensive [7], and it is complemented by evidence that a similar structure is utilized in vivo [55] and that sufficient recA protein exists in the average cell to form a long filament [56]. To rationalize this, it is necessary to assume that extensive branch migration is an essential cellular process and that the filament is required to bring it about.

The importance of strand exchange can be made apparent by examining one role of homologous recombination in *E. coli*—i.e., postreplication repair. Evidence suggests that the replication apparatus is halted by unrepaired DNA lesions such as pyrimidine dimers [57,58]. If replication continues further downstream, the pyrimidine dimer is left in a region of ssDNA as illustrated in Figure 3.5, inaccessible to the normal cellular DNA repair systems. Cleavage of this single strand is a potentially lethal event. Repair of this lesion requires conversion of this DNA to duplex, and this is a recA-dependent process in *E. coli* [59]. It can be accomplished by utilizing recA protein to recruit a complementary strand from the other side of the replication fork. This is paired over a long region of

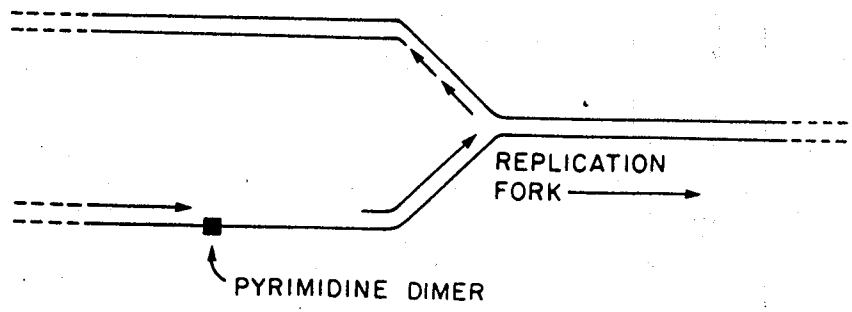


Figure 3.5. Postreplication repair. A pyrimidine dimer is positioned in a region of ssDNA after the replication fork has passed. Repair of this lesion requires recA protein as described in the text.

the damaged strand employing the pairing and branch migration activities of recA protein, as described in a model proposed by Howard-Flanders and colleagues [59]. RecA protein-promoted branch migration in vitro proceeds efficiently past pyrimidine dimers [60] and short mismatches [61]. This suggests that extensive branch migration is essential under some conditions for cell survival.

Once the importance of strand exchange is accepted, employing a filament can be rationalized as a logical cellular strategy and potentially the only strategy that can ensure that extensive branch migration will occur. A homologous interaction between two DNA molecules is likely to involve an unusual and unstable DNA structure (see "Paranemic Joints," below). A filament may be required to stabilize this structure. Also, in the cell there are a large number of barriers to branch migration that do not exist in the test tube. These include (1) nucleases, which may resolve crossover or Holliday junctions before branch migration has proceeded for any distance; (2) a variety of DNA-binding proteins that would represent impassable obstacles to a reaction of this kind; and (3) the DNA lesions themselves, which could represent a significant kinetic barrier to uncatalyzed branch migration. If extended branch migration is to occur, a mechanism must exist to protect the branch point, exclude other proteins from the path of the migrating branch, and provide the energy required to overcome short regions of heterology and DNA lesions. A filament meets these requirements.

Paranemic Joints

Before an exchange of strands begins, recA protein aligns two DNA molecules homologously without Watson-Crick interwinding of strands from the two DNA molecules. The resulting structure is very unstable, dissociating immediately if recA protein is removed [23]. Little is known about the architecture of this paranemic complex. The structure of paranemic joints is a critical parameter for understanding strand exchange. This can be seen by the fact that different models for strand exchange generally envision different types of paranemic joints. The structure also has interesting implications as a precedent for structures formed in synaptonemal complexes in eukaryotic cells during meiosis.

There are several different proposals for paranemic joints (Fig. 3.6). It is important to remember in each case that the DNA within the nucleoprotein filament is extended and underwound by 39.6% when ATP is present. The second DNA must be equally underwound over the region in which it is homologously aligned. The first model, proposed by Howard-Flanders and colleagues [62], envisions a three-stranded (or four-stranded in the case of four-strand exchanges) DNA structure such as that proposed by McGavin [63] and Wilson [64], with homology-dependent hydrogen-bonding between duplexes in the major groove. In a second

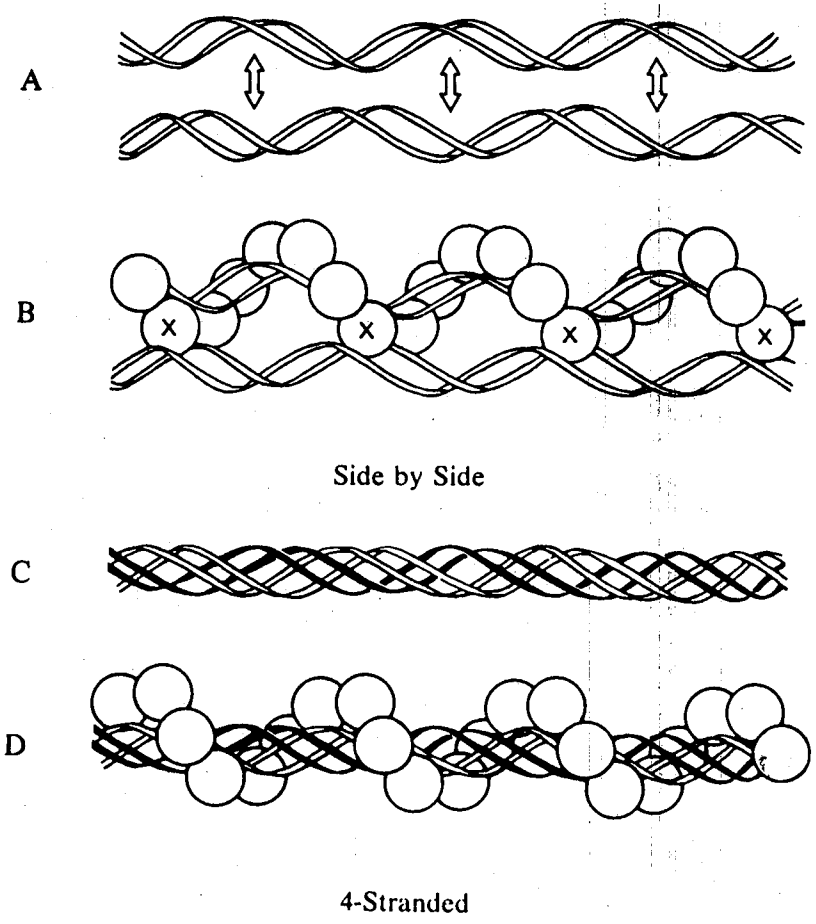


Figure 3.6. Some possible structures for paranemic joints. The side-by-side and four-stranded structures are shown with and without recA protein. In the side-by-side configuration, open arrows (panel A) indicate points where periodic homologous interactions between the two DNAs might occur. In panel B the second DNA molecule is shown bound to every sixth recA monomer (denoted by the symbol "X"). In the four-stranded structure, homologous contacts between the two DNAs in their major grooves are continuous. The two DNAs are still bound asymmetrically, however. The dark strands denote the second DNA to be bound in the paranemic joint. This is the DNA that remains DNase sensitive in studies of paranemic joining.

model, the two DNAs are aligned side by side [24,65]. Periodic homologous contacts would occur in the major grooves of the DNAs, with perhaps 2 or 3 bp homologously paired for each 18-bp turn of the underwound helices. A third model, in which the two DNAs are aligned with alternating right- and left-handed helical regions (not shown), seems

unlikely in view of the fact that recA protein always forms regular right-handed helical structures on DNA, even when the bound DNA is in the left-handed Z conformation prior to binding.

Because of the inherent instability of the paranemic joint, available evidence concerning the structure of these complexes is indirect. RecA protein in recA-ssDNA complexes hydrolyzes ATP with a turnover number of approximately 25 min^{-1}. Upon addition of homologous dsDNA, a 33% drop in the rate of ATP hydrolysis is observed that has been shown to be associated with the formation of paranemic joints [24]. The decrease is complete in less than 2 minutes, is completely homology dependent, and occurs even when homologous pairing is topologically restricted to paranemic complexes. More important, the extent of the decrease is linearly proportional to the *length* of available homology between the two DNA molecules [24]. These and other results imply that paranemic joints are formed in this reaction that are much longer than had been previously suspected, possibly extending for as much as 7,000 bp.

Extensive homologous pairing was detected in this study between circular DNA molecules in which interwinding over the entire homologous region was topologically prohibited [24]. At the moment this extended paranemic alignment is completed, the ssDNA is found to be completely protected from DNase digestion, while the incoming duplex DNA is still almost completely sensitive to digestion [66]. This indicates that in the paranemic complex the ssDNA is bound within the recA filament and the incoming duplex is bound in a position accessible to nuclease.

As expected, the incoming duplex DNA is underwound as a result of alignment with the DNA in the nucleoprotein filament [25]. This work employed nicked circular duplexes, and underwinding that occurred as a result of paranemic joint formation was trapped by sealing the nick with DNA ligase. As with the drop in ATP hydrolysis, the underwinding is homology dependent and kinetically competent to reflect formation of paranemic complexes. In addition, the underwinding occurs in circular duplexes that do not permit plectonemic joint formation, and the degree of underwinding exhibits a linear dependence on the fraction of the duplex homologous to the DNA within the filament [25]. This suggests again that all available homology is detected in paranemic joints.

The observed degree of underwinding, however, is insufficient for complete alignment throughout the homologous region. There is a broad distribution in the observed degree of underwinding, consistent with an average of 30% and a maximum of 45% of the available homologous duplex DNA aligned at any instant [25]. This indicates that in a 7,200-bp homologous duplex up to 2,900 base pairs can be paired simultaneously in a paranemic complex. Since the relationship between underwinding and length of homology suggests that all homology is detected, the paranemic complex may be noncontiguous, or it may be dynamic and move back and forth rapidly within the region of homology.

Pairing two circular DNA molecules, as in the experiment above, precludes net interwinding of the two DNAs. This topological constraint is removed if the duplex DNA is linear. The ends of the linear DNA must be heterologous to prevent plectonemic joint formation. Underwinding of the duplex resulting from paranemic joint formation can be trapped by ligation or by using a site-specific recombination reaction. The underwinding of duplex DNA in such an experiment is similar to that observed with nicked circular DNAs. More important, the duplex, after it is circularized, is interwound (catenated) with the circular single strand [B.S. Schutte and M.M. Cox, unpublished results]. This result is consistent with the formation of a three-stranded DNA helix over at least a short distance in the paranemic complex. Alternatively, the interwinding could be a fortuitous outcome of the formation of long paranemic joints. Determining whether this catenane reflects a three-stranded paranemic DNA structure will require a quantitative comparison of the degree of DNA interwinding and the degree of duplex underwinding.

Measurements of paranemic joints observed in the electron microscope by Griffith and colleagues [26] also indicate they can extend for more than 1,000 bp. Notably, the incoming duplex disappears into the filament in these complexes without significantly altering the ultrastructure of the complex [26,67].

Most of these results can be rationalized by either of the models in Figure 3.6. The two are not necessarily mutually exclusive. A paranemic joint could, for example, involve a region of four-stranded DNA flanked on one or both sides by DNA aligned side by side.

Properties and Kinetics of the DNA Strand Exchange Reaction

Whereas formation of paranemic joints is rapid, the subsequent branch migration phase is slow and rate limiting. About 10 to 20 minutes is required to cover 7,000 bp [7], yielding an optimal rate of at least 350 bp min^{-1}. As it proceeds, one strand of the duplex is progressively paired with the ssDNA in the complex and thus gradually becomes partially resistant to DNase treatment [66].

A successful model for this reaction must explain a number of observations related to this process:

1. As demonstrated by Brenner et al. [39], ATP hydrolysis occurs throughout the filament, rather than being restricted to filament ends or to monomers at the branch point. This is true prior to and during strand exchange [39].
2. ATP hydrolysis is *not* tightly coupled to association and/or dissociation of recA monomers with or from complexes [46,68,69].

3. *After the 33% decrease in the rate of ATP hydrolysis that follows rapidly after the addition of homologous duplex DNA, ATP hydrolysis then continues at a constant level throughout and subsequent to the branch migration phase of strand exchange [24].*
4. RecA protein remains on the heteroduplex product after the reaction is complete as determined by measuring underwinding of the nicked circular DNA products. Note that observation 3 also requires continued recA protein binding [66].
5. As seen in the electron microscope, the ultrastructure of the complex is unaffected by strand exchange, although dissociation of recA protein is sometimes observed in these studies well after strand exchange has occurred [18,26,67].
6. ATP hydrolysis in this system is apparently inefficient, with over 100 ATPs hydrolyzed per base pair of heteroduplex DNA formed [42].
7. In a three-strand exchange, branch migration can proceed not only past mismatches and pyrimidine dimers, but also past insertions or deletions involving hundreds of base pairs at a significant frequency [70]. This does not occur as readily in four-strand exchanges [71].
8. Both three- and four-strand exchange reactions can traverse a double-strand break in the incoming duplex, but not in the DNA within the nucleoprotein filament [10,24,71; B.C. Schutte and M.M. Cox, unpublished].

Models for Strand Exchange: Constraints

Models for strand exchange can be considered on several levels. The first obvious question involves the branch point where exchange is taking place. Is the recA protein filament stable and contiguous across the branch point, or is branch migration linked to association/dissociation of recA protein at this point? An obvious possibility is a treadmilling reaction such as that observed with actin and tubulin filaments [72]. A variation of treadmilling, called polar polymerization, has also been suggested in which strand exchange is coupled to addition of recA protein at a growing filament end [19]. Both assembly and disassembly of recA protein filaments proceed in the same direction as branch migration is driven [37,46,47]. Assembly, however, occurs at least 3- to 10-fold faster than branch migrations [44,46]. Dissociation occurs under some conditions at rates comparable to strand exchange [46], although strand exchange proceeds efficiently under conditions in which no net dissociation is observed (observations 3 and 4 above). Nuclease protection experiments and observations in the electron microscope provide additional evidence that the recA filament spans the branch point [26,50,51] without a structural alteration or interruption.

If branch migration were linked to a treadmilling process, ATP hydrolysis would be restricted to the ends of the filament. For this reason, observations 1 and 2 effectively eliminate a classical treadmilling reaction as a mechanism in this system. Observation 1 echoes the structural data above, indicating that there is nothing special about the branch point with respect to ATP hydrolysis. Since ATP hydrolysis is required to drive branch migration, we infer that the important molecular events are not restricted to filament ends. Observations 1 through 5 together suggest that the nucleoprotein filament remains essentially intact throughout the reaction. This indicates that models must be considered that do not require association or dissociation of recA protein to drive branch migration. Griffith and Harris [18] refer to these as stable filament models.

To develop models for strand exchange in which the nucleoprotein filament is static, it is necessary to consider energetics, structure, and DNA topology. Observation 6 is especially problematic. ATP hydrolysis clearly occurs even before duplex DNA is introduced. This and other results may indicate that much of this ATP hydrolysis is uncoupled. This view has most recently been advanced by Roman and Kowalczykowski [73]. At first glance, an efficiency of 1 ATP per base pair appears logical. This estimate can be reduced by considering the fact that one recA monomer binds three to four base pairs, and reduced further by considering the fact that the activation energy for branch migration is negligible. Strand exchange itself, in fact, may not require ATP hydrolysis, as suggested by numerous reports of eukaryotic proteins that promote strand exchange without an ATP requirement [74,75]. The apparent excess of ATP hydrolysis, however, is an experimental fact that may be an important mechanistic clue. The reaction as a whole accomplishes something of importance to the cell, and an understanding of the use of ATP in this system requires that a complete assessment of the biological and physical properties of the reaction be added to the chemical equation. The use of ATP can, in fact, be readily explained in a simple model (see below). Notably, on a cellular level, the expense is not great. The turnover number for ATP hydrolysis per recA monomer is about 25 min^{-1}. The 1,000 recA monomers present in the average cell [56] are therefore capable of hydrolyzing about 25,000 ATPs per minute. This is less than 0.1% of the potential energy consumption of the 10,000 to 20,000 ribosomes present in the same cell. In spite of the apparent inefficiency, the price may not be exorbitant when the alternative is cell death. The potential return on this investment will be described below.

Observation 7 is also difficult to explain. Bianchi and Radding [70] observed branch migration through long insertions at varying efficiencies. When the insertion was in the duplex DNA, the efficiency dropped off rapidly with increasing insertion size. When the insertion was in the ssDNA, these workers observed a low, but constant and significant, reaction efficiency that was relatively independent of insertion size. Insertions of hundreds of base pairs were bypassed in both cases.

Observation 7 suggests that an asymmetry exists in the recA nucleoprotein filament with respect to the binding sites of the ssDNA and the duplex DNA. This asymmetry is also reflected in observation 8 and in the nuclease sensitivity of the two DNAs in the paranemic joint. As suggested by West and Howard-Flanders [10], this is evidence for two nonidentical binding sites for DNA on each recA monomer.

In addition to these observations, a successful model must provide a viable role for a recA filament and a mechanism for coupling ATP hydrolysis to a molecular event that would result in unidirectional branch migration. If complete coupling is assumed, a direct relationship between the rate of ATP hydrolysis and the observed rate of strand exchange in vitro should be evident. The right-handed DNA helix also imposes topological constraints on a workable model that must be considered.

Models

Only two models have been outlined in the literature in sufficient detail to consider at length. For some additional mechanistic suggestions and viewpoints, the reader is encouraged to consult two recent articles [18,19]. The first model is that proposed by Howard-Flanders and colleagues [62]. This model develops directly from the four-stranded DNA paranemic joint illustrated in Figure 3.6. At one end of this joint the two homoduplexes enter and become intertwined. Within the complex there is a rotation of bases to effect strand exchange, and two heteroduplexes exit at the other end of the complex. As originally proposed, the model links strand exchange with recA dissociation, although this is not

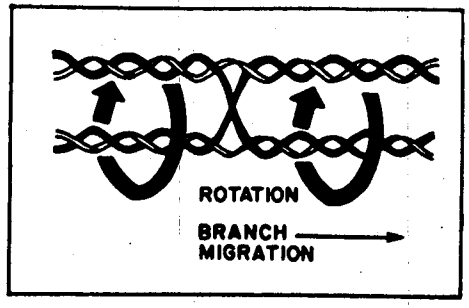
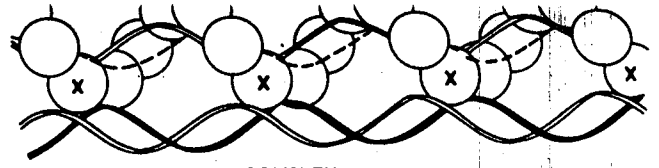
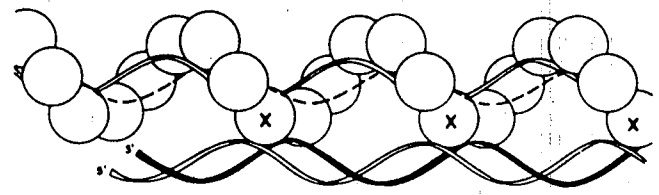
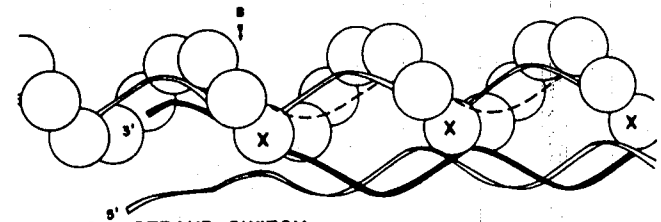
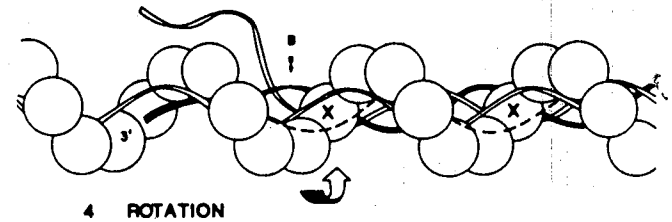


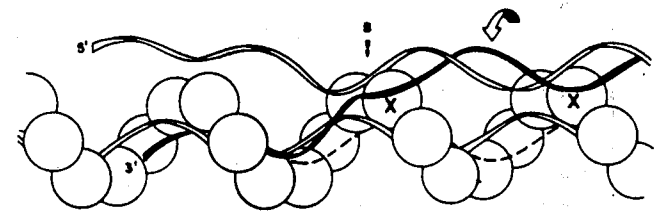
Figure 3.7. Model for DNA strand exchange. The proposed rotation of DNA molecules about the other is illustrated above. In each of the succeeding panels (next page), the dark strand corresponds to the "−" strand as described in the text. The arrow labeled "B" denotes the position of the branch point in panels 3 to 6. The balls represent individual recA monomers in a helical filament. The monomers to which the outer duplex DNA molecule is bound are denoted by "X" symbols in each panel. See text for details.

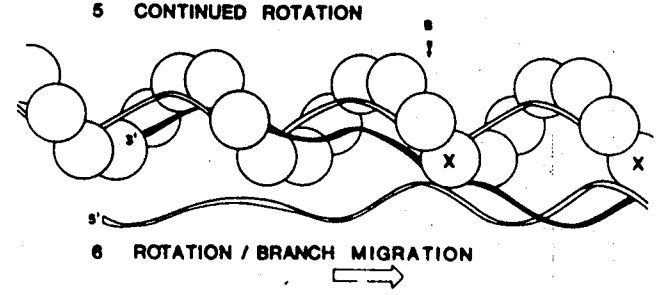












essential. This model has been outlined in much detail [62,71] and will not be reviewed extensively here, although a modified version will be illustrated below. The model is consistent with most of the data outlined above, and the four-stranded intermediate makes it easy to visualize homologous paranemic interactions. The model is less successful at explaining the energetics of the system, unless the assumption is made that much of the ATP hydrolysis is uncoupled.

The second model, the "rotation model" [65], will be expanded here and is presented in Figure 3.7. We begin with the paranemic complex, in which DNAs are aligned side by side. The paranemic complex includes a filament of recA protein enclosing the ssDNA. The protein is arranged in the right-handed helix with six recA monomers per turn as already described. The bases of the ssDNA (DNA 1) are exposed continuously in the helical groove of this filament. A similar complex can be formed on duplex DNA, and we infer that binding sites for two DNA strands exist within this complex.

The binding site for the second DNA strand is indicated by a dashed line. A partially underwound homologous duplex (DNA 2) is bound to the outside of this complex as described above, aligned side by side with the ssDNA. As drawn, this duplex is bound to every sixth recA monomer in the filament. We will arbitrarily designate the set of monomers to which DNA 2 is bound to be 6n. These recA monomers bind the outer duplex along the phosphate-deoxyribose backbone, and at these points the bases of the two DNA molecules are oriented away from each other. Between each periodic point of protein contact the duplex spans the groove of the filament. The twist of the DNA helices will cause the bases of the two separate DNA molecules to juxtapose in the groove, and at these points homologous contacts between the incoming duplex and the ssDNA are made.

This paranemic complex, with periodic homologous contacts, can span thousands of base pairs. The side-by-side alignment facilitates the formation of extended paranemic joints by minimizing topological constraints at this stage of the reaction. As shown in panels 2 and 3 (Fig. 3.7), strand exchange will be initiated readily if a free DNA end occurs anywhere within this paranemic complex. The melting of the end of this duplex and the pairing of the "−" strand with its complement within the complex to initiate the exchange will be facilitated by the intramolecular nature of the reaction, partial underwinding of the external duplex, base-pairing interactions, and possible favorable protein contacts within the unoccupied DNA strand binding site within the complex.

Once initiated, propagation of the exchange reaction encounters the topological requirement for rotation of the DNA molecules. This constraint can be met by rotating both DNA molecules in the same direction (longitudinal rotation [65]) or, similarly, by rotating one DNA molecule around the other as illustrated in the Figure 3.7 inset. The central feature of this model is the controlled rotation of the duplex DNA molecule relative to the nucleoprotein filament, as illustrated in Figure 3.8. The rotation does not result in movement of either DNA relative to the filament along its length, but it does result in movement of the branch point. Extensive paranemic contact between the two DNAs is maintained at all times. This is accomplished by having the set of recA monomers to which the duplex is bound transfer the duplex unidirectionally to the neighboring set of recA monomers. The duplex will again be bound to every sixth recA monomer in the filament, but now this involves the monomer set 6n + 1. This transfer is coupled to ATP hydrolysis via a set of conformation changes. These recA conformation changes could be analogous to the activity of the myosin ATPase during muscle contraction. The result is a unidirectional rotation of the duplex DNA around the ssDNA that leads directly to a unidirectional movement of the branch point.

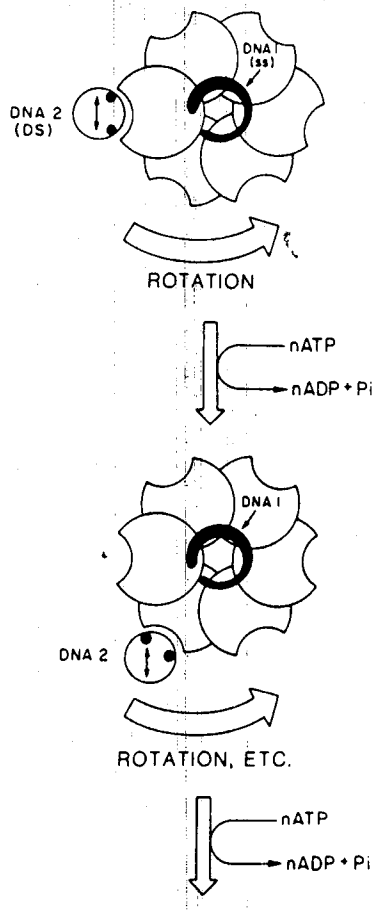


Figure 3.8. Proposed rotation of duplex DNA about the recA nucleoprotein filament. This is an end view of the recA nucleoprotein filament, as seen looking down the axis of the filament from right to left as illustrated in Figure 3.7. See text for details.

The rotation of the duplex DNA is better considered from the vantage point of a cross section of the complex shown in Figure 3.8. The complex may be considered fixed relative to an arbitrary plane bisecting it from top to bottom as drawn (not shown). The duplex DNA (DNA 2) is drawn as a cylinder viewed end-on and bound to one of the six recA monomers visible. ATP hydrolysis results in transfer of the duplex to the neighboring recA monomer, and repetition of this process causes rotation of the duplex around the outside of the complex in the direction indicated. The investment of chemical energy is used in part to ensure that this rotation, and the resulting branch migration, proceeds in only one direction. The system must therefore represent a coupled vectorial reaction as defined by Jencks [76]. The specific direction of branch movement will be predetermined and invariant relative to the asymmetry inherent in the filament.

As the rotation occurs, an arbitrary plane drawn through the axis of the duplex from top to bottom as shown in Figure 3.9 must be maintained parallel to the plane drawn through the complex. This ensures that each succeeding set of recA monomers will be bound in an identical fashion to the duplex and that homologous DNA base contacts in the complex groove occur similarly at each point in the rotation. Each monomer will

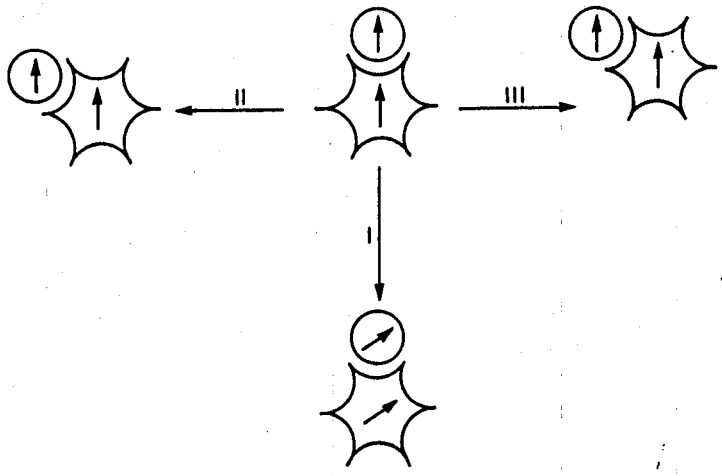


Figure 3.9. Three different types of rotation. The complex is viewed from one end, as in Figure 3.8. The planes bisecting the DNA within the complex (DNA 1) and the outer DNA (DNA 2—circle) are shown. Rotation I involves rotation of both DNAs about their longitudinal axis. In rotation II, DNA 2 rotates around DNA 1, and in rotation III, DNA 1 rotates around DNA 2. All three are compatible with the coupling mechanism outlined in Figures 3.7 and 3.8. The product of a single rotational step is structurally the same regardless of the rotation mechanism, although the positions in space of the two DNAs differ.

bind to the duplex DNA at the same point each time the duplex rotates 360° around the complex. Thus, each set of recA monomers will each have a unique and mutually exclusive set of DNA contact points for DNA 2.

Panels 4 through 6 in Figure 3.7 are related successively to each other by rotation through two of the transfer steps described above. The position of the branch point in each case is indicated by an arrow, and the rotation results in branch movement from left to right as drawn. The branch point is maintained within the helical groove of the filament at all times. In the cell, this rotation would cause topological strain elsewhere in the DNA that would require the action of topoisomerases. This is true, however, for any mechanism one might propose for branch migration.

The model is drawn in Figures 3.7 and 3.8 with the duplex rotating around the filament for ease of illustration. The same mechanism is compatible, however, with rotation of the filament around the duplex, or with the rotation of both the filament and the duplex around their rotational axes. The three possibilities are illustrated in Figure 3.9. The important feature is not the type of rotation itself, but the requirement that the planes through the two DNAs (shown with arrows) remain parallel at all points in the rotation. Any transfer mechanism that meets this condition is compatible with all three types of rotation. Strand exchange proceeds efficiently in this system when rotation is restricted to the rotational axes of the two DNAs [77].

This model satisfies all of the constraints listed above. A mechanistic role for the filament is provided that employs the important structural features of the filament and the paranemic complex. The filament groove becomes not only the site for homologous contacts between the two DNA molecules but an extended active site for migration of the branch point as well. There is no need for association or dissociation of recA protein. No special conformation or reaction of recA monomers at the branch point is required.

The model also provides a mechanistic role for ATP hydrolysis. The important molecular events that bring about branch migration are occurring throughout the filament rather than uniquely at the branch point. Each transfer event involves the making and breaking of hundreds of contacts between DNA 2 and recA monomers along the complex. It is not necessary, therefore, to invoke the idea of uncoupling to explain the observed reaction efficiency. In fact, both the *efficiency* and the *rate* of branch migration observed in vitro can be explained by assuming that ATP hydrolysis and branch migration are tightly coupled, with each recA monomer in the complex hydrolyzing one ATP to bring about a 360° rotation. Each rotation will result in an 18-bp (approximately) migration of the branch point. If the filament contains 2,000 monomers (as in a typical in vitro experiment), the expense will be nearly 100 ATPs per base pair—very close to the efficiency actually observed [42]. If the recA

turnover number for ATP hydrolysis during strand exchange (18 to 20 min^{-1}) is translated directly into 18 to 20 rotations min^{-1}, the rate of branch migration will be 320 to 360 bp min^{-1}—again very close to the rate observed.

The system is easy to rationalize in vivo. The filament would serve to protect the crossover junction from nucleases and to exclude other DNA-binding proteins from the region. In addition to driving the reaction, a role of ATP hydrolysis in this system would be to maintain homologous alignment throughout the filament at all times and to avoid topological complications in the region covered by the filament. The extent of branch migration in a given recombination event may even be defined or regulated by the length of the filament. Resolution of the branch would occur as soon as the branch migrated beyond the end of the filament.

The system, in a sense, reflects what is probably its primary day-to-day function—postreplication repair. The strand exchange reaction itself does not require a large infusion of chemical energy, as already noted. ATP hydrolysis may be required, however, for extensive, unidirectional strand exchange through barriers such as sequence heterologies and pyrimidine dimers. By making the reaction unidirectional and insensitive to small barriers in DNA structure, this expenditure of chemical energy can be viewed as a device to ensure that life-or-death repair events are promoted efficiently.

The maintenance of homologous alignment throughout the complex helps explain the capability to branch-migrate through mismatches and pyrimidine dimers. If homologous alignment were maintained only through a short region near the branch point, a pyrimidine dimer or a short stretch of heterology could destabilize these interactions and produce a significant barrier to branch migration. By stabilizing homologous contact through thousands of base pairs, however, the effect of pyrimidine dimers or short mismatches is rendered insignificant. The energy invested in rotating the duplex all along the filament will overcome most barriers of this kind.

The observation that branch migration proceeds through large insertions can also be explained. This is accomplished without a requirement for halting branch migration and reinitiation on the other side of the insertion. The solution to this problem will be different depending on whether the insertion is located in the ssDNA or the duplex. The key factor in both cases is the maintenance of homologous paranemic contacts on both sides of the insertion. An insertion in the ssDNA would probably require dissociation of recA monomers bound to the insertion sequences. We speculate that this might be induced by strain if the complex is bent enough to permit paranemic contacts on both sides of the insertion. In the case of an insertion in the duplex, paranemic contacts would cause a looping out of the insertion sequences. A possible structure for the final paranemic complex formed in both cases is illustrated in Figure 3.10.

For the ssDNA insertion, the problem of branch migration is then

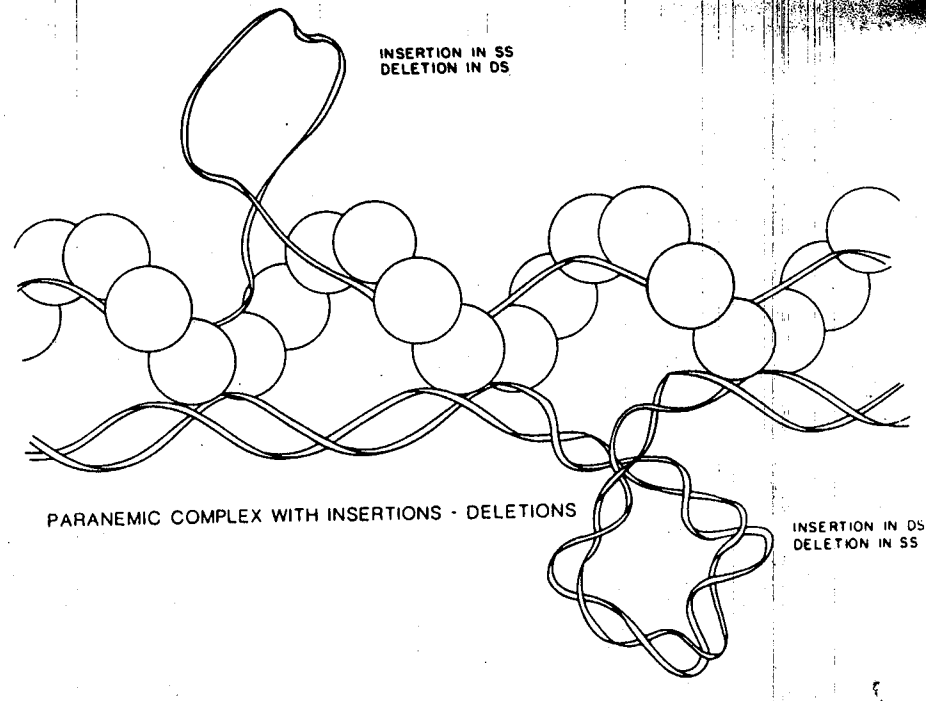


Figure 3.10. Paranemic complex with insertions in the ssDNA or the duplex DNA. DNA corresponding to insertion sequences is extruded from the complex, with homologous contacts maintained on both sides of each insertion. See text for details.

reduced to a steric restriction on the rotation of the duplex caused by the extruded single strand. This steric problem could be exacerbated by any protein that remains associated with this extruded DNA. Rotation of the duplex would require the extruded DNA to be wrapped tightly around the complex. The topological problem is different and possibly greater for an insertion in the duplex. As shown in Figure 3.11, the proposed rotation would result in the unwinding of the duplex DNA in the insertion. The "+" strand of the duplex will be extruded and displaced. The "−" strand will be wrapped around the incoming "+" strand in the complex without base pairing as shown. The number of non-base-paired wraps will be equivalent to the number of helical DNA turns within the heterologous region of the original duplex. The number of these wraps will increase with insertion size. Since the wrapping would be constrained to a small region of the complex, an inverse relationship between reaction efficiency and insertion size might be expected here, as observed. Upon dissociation of recA protein, this structure would relax spontaneously, as indicated in Figure 11.

The model as drawn requires that each recA monomer have two DNA

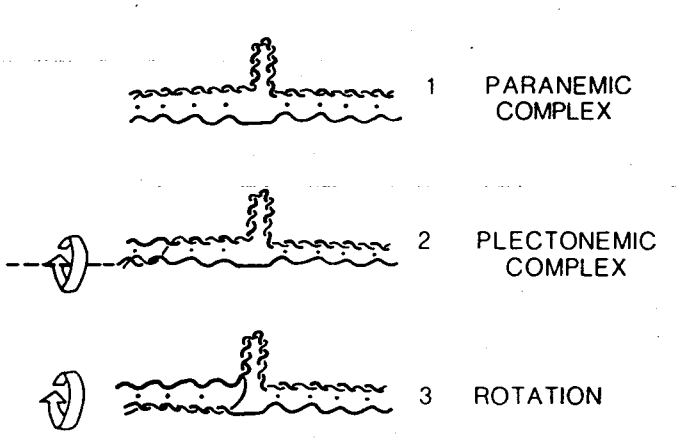


Figure 3.11. Topological consequences of branch migration through an insertion in the duplex DNA substrate. The lighter of the three DNA strands corresponds to the "−" strand as described in the text. Panel descriptions: (1) The paranemic complex is illustrated, with homologous contacts spanning the insertion in the duplex. The recA filament (not shown) encloses the ssDNA at the bottom of the panel. (2) A nascent plectonemic complex results from the first rotation of the duplex around the ssDNA as shown. (3) Continued rotation brings the branch to the boundary of the insertion sequences. (4) The next four rotations unwind the duplex within the insertion without advancing the branch. The "−" strand in this region becomes wrapped around the incoming "+" strand without base-pairing. Once the insertion is unwound, branch migration proceeds normally. (5) Strand exchange is complete. (6) The duplex DNA will relax spontaneously to this form if the wrapped structure is not maintained by recA protein.

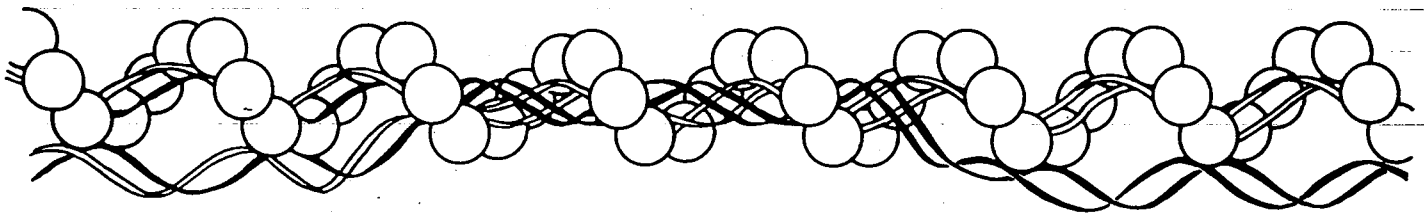


Figure 3.12. A composite model for recA protein-mediated DNA strand exchange. The four-stranded structure proposed by Howard-Flanders and colleagues is seen in the center of this complex. Rotation of DNA 2 in regions flanking the four-stranded region, in the direction and by the mechanism illustrated in Figure 3.7, will result in branch migration left to right.

binding sites as proposed by Howard-Flanders and co-workers [62], although here they must be positioned on opposite sides of the protein. The model predicts the pronounced asymmetry, observed in several studies, in the interaction of the nucleoprotein filament with the two DNA molecules participating in the exchange. The observation that strand exchange is blocked by a double strand break in a gapped duplex [10,71] is readily explained. In a four-strand exchange reaction, the gapped duplex will be within the filament, and a break in this duplex will result in a break in the entire complex. On the other hand, a break in the linear duplex (which will be outside the filament) will simply result in the formation of two contiguous paranemic complexes. Strand exchange should traverse the break in this outer duplex, as observed. In the case of a three-strand exchange, the model predicts that essentially any number of linear duplex fragments with nonoverlapping homology should be able to participate in strand exchange simultaneously on a single recA nucleoprotein filament. Results consistent with this prediction have already been obtained [24; B.C. Schutte, unpublished].

Several factors distinguish the rotation model from the four-strand model. The most important potential problem with the rotation model is the structure of the paranemic joint itself. The side-by-side alignment is difficult to envision, and no molecular models for it are available. The four-stranded structure proposed by Howard-Flanders, in contrast, is based on published models [63,64] and is accepted as a reasonable possibility. The interwinding of DNAs in paranemic complexes may be an experimental manifestation of this structure. A principal advantage of the rotation model is the rationalization it provides for energy use in this system. The two models differ in several other respects. For example, if the very long paranemic joints detected in several studies are completely three or four-stranded, considerable rotation of the two DNAs must occur during paranemic joint formation. For the model described above, rotation is not needed until branch migration commences.

For all of this, the two models are not necessarily incompatible. A composite model is illustrated in Figure 3.12. In this version, a rotation of the two DNAs as outlined in Figures 3.7 through 3.9 is coupled to formation and resolution of a four-stranded DNA intermediate, the four-stranded region has a significant length, and rotation leads to extension on one end and resolution on the other. The position of the four-stranded region would change as the reaction proceeded, and conversion of homoduplex to heteroduplex could occur at any point within it. This model is flexible in that the four-stranded region could be very short or extend for thousands of base pairs. The rotation at either end provides the same coupling and energetic advantages as the rotation model. Rotation could be driven with or without homologous interactions in the DNA flanking the four-stranded region.

Summary

Several models for recA-protein-mediated strand exchange are discussed in light of available evidence. A final resolution of the structure of paired DNA complexes bound by recA protein and the mechanism of DNA strand exchange continues to be elusive. Continued study of this problem should contribute to an understanding of recombination, cellular energetics, and chromosome-chromosome interactions.

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