Chapter 7

BINDING TWO DNA MOLECULES AT ONCE: THE *REC*A PROTEIN

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I. INTRODUCTION

The DNA of every organism is subject to rearrangements via a multitude of reactions collectively termed genetic recombination. Some of these reactions result in a net loss or gain of DNA; others are conservative. The physiological functions of genetic rearrangements are as diverse as their mechanisms. Genetic recombination events play important roles in gene regulation, development, pathogenicity, genetic diversification, DNA repair, viral infections, and many other processes.

Homologous, or general, recombination is the best-studied and most common type of genetic rearrangement. It is one of several broad classes of recombination, the others being site-specific recombination, illegitimate recombination, and transposition. Site-specific recombination occurs only at a specific sequence recognized by a given site-specific recombinase. Illegitimate recombination requires neither a specific site nor homologous sequences. Transposition involves the movement of defined genetic elements (transposons) from one location in the genome to another. The exchange of genetic information in homologous recombination involves two regions of DNA that share sequence similarities, but the process is largely nonspecific with respect to the sequences themselves. The functions of homologous recombination include several types of recombinational DNA repair and genetic diversification during meiosis in eukaryotes.

The requirement for homology implies that the two DNAs are homologously paired and aligned at some stage in the process. Two types of homologous interaction have been observed, and these have been described by Radding and co-workers.[1,2] An exchange of strands between two chromosomes to produce heteroduplex regions, where a strand from one is interwound and base paired (Watson-Crick) with its complementary strand from the other, produces what is defined as a *plectonemic* joint. The classic Holliday intermediate is an obvious example of a plectonemic joint. Homologous interaction between two chromosomes can also occur without actual strand exchange to produce what has been termed a *paranemic* joint.

The formation of paranemic and plectonemic joints is mediated by an unusual class of DNA binding proteins. The function of these proteins is to promote first pairing between homologous DNA molecules, and then strand exchange, the events at the heart of any homologous recombination event. These proteins bind to two DNA molecules, the first nonspecifically, and the second with a specificity defined by the sequence of the first. These proteins have been found in a wide range of organisms. Some require ATP and hydrolyze ATP in the course of their DNA strand exchange activities. Others appear not to require a nucleotide cofactor. In many cases the active species is a novel nucleoprotein complex that can contain thousands of identical protein monomers arranged in a filament on the DNA.

A. *REC*A PROTEIN-MEDIATED DNA STRAND EXCHANGE

The best characterized of these proteins is the *rec*A protein of *Escherichia coli*.[3-9] This protein promotes DNA strand exchange reactions *in vitro* that mimic key steps in homologous genetic recombination *in vivo* (Figure 1). This reaction requires ATP and can be broken down into several stages: (1) the formation of a *rec*A-DNA nucleoprotein filament, with one *rec*A monomer per every three to four nucleotides or base pairs of DNA; (2) the pairing of the DNA in this complex with a second homologous DNA molecule to form a paranemic joint; (3) conversion of the paranemic joint to a plectonemic joint; and (4) branch migration to complete the exchange.

Ten years of intensive investigation in many laboratories has defined many properties of *rec*A protein and the reactions it promotes. Several of the most important questions, however, remain to be answered. In particular, we do not know the structure of the paranemic joint formed in step 2, or the mechanism of the reactions in steps 3 and 4. This chapter concentrates

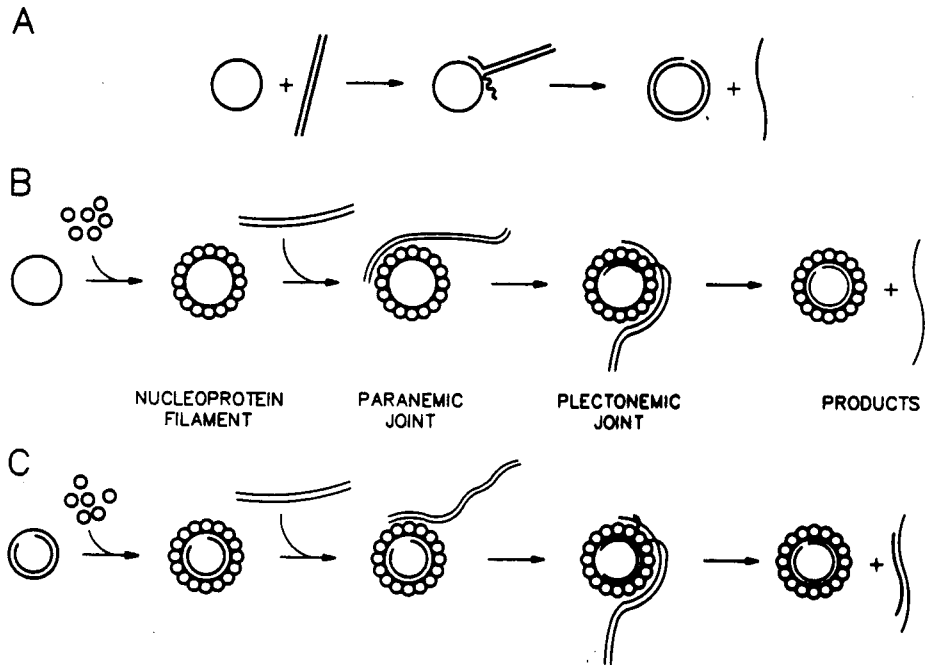


FIGURE 1. *rec*A mediated strand exchange. The steps illustrated (from left to right) are (A) formation of a *rec*A-DNA nucleoprotein filament (primary DNA binding), (B) homologous alignment with a second duplex DNA to form a paranemic joint (secondary DNA binding), (C) conversion of the paranemic joint to a plectonemic joint, and (D) unidirectional branch migration to yield products. (A) Strand exchange showing the DNA only. (B) A three-strand reaction. (C) A four-strand reaction.

on a summary of experimental data accumulated to date on basic *rec*A activities (DNA binding, ATP hydrolysis, etc.) that are relevant to the understanding of DNA strand exchange. Several models for the DNA strand exchange reaction are also considered. This discussion is not intended to be a comprehensive review, and the reader is urged to consult References 3 to 9 for more detailed accounts of experimental results, proposed models, and other mechanistic perspectives.

An overview of the general properties of the strand exchange reaction is useful to provide perspective in the following discussion. ATP is required in steps 2 through 4, although ATP hydrolysis is required only in steps 3 and 4.[10] Paranemic joints can be formed in the presence of ATP[γS], which is hydrolyzed very slowly by *rec*A protein.[11] The branch migration reaction in step 4 is unidirectional, proceeding 5′ to 3′ relative to the single-stranded DNA bound in step 1.[12-15] The reaction, however, is apparently inefficient with respect to its use of chemical energy.[5,16] The rate of branch migration is generally reported to be 3 to 6 bp s^{-1},[5,8,10,17] but may approach 20 bp s^{-1} under optimal conditions.[5,18,19] ATP is hydrolyzed throughout the filamentous complex with a k_{cat} (*rec*A monomer) of about 20-25 min^{-1}.[20-23] Since ATP is being hydrolyzed everywhere but the branch is moving at only one point, the reaction typically consumes 100 or more ATPs for every base pair of heteroduplex DNA formed *in vitro*.[5,16,24,25]

One final property concerns the role of single-stranded DNA. At pHs above neutrality, there is a significant kinetic barrier to the binding of *rec*A protein to duplex DNA.[23,26,27] The *rec*A complex of step 1 is formed on the single-stranded DNA in a three-strand exchange (Figure 1A), or on the gapped duplex in a four-strand exchange (Figure 1B). A single-strand gap of 50 nucleotides or more circumvents the slow step in duplex DNA binding, providing a nucleation site that permits rapid formation of a complete nucleoprotein filament as shown in Figure 1B. The single-stranded DNA is not simply required for *rec*A protein binding. The

four-strand exchange in Figure 1B occurs only if the second DNA (the linear duplex) has a homologous end that overlaps the single-stranded region in the gapped duplex. This and other related results suggest that single-stranded DNA may govern steps 2 and 3 as well as the initial formation of the nucleoprotein filament.

B. THE BIOLOGICAL PROBLEM

The system outlined above has a structural and mechanistic complexity that can be rationalized only in the context of its cellular function. This is somewhat problematic in the case of *rec*A protein because the function of homologous genetic recombination in bacteria is not clearly defined.[28] On the surface, DNA strand exchange is a simple reaction. Branch migration itself is a spontaneous reaction in solution under physiological conditions, and the rates of this uncatalyzed process in model reactions can quantitatively account for the observed formation of heteroduplex DNA in some cellular recombination events.[29] One could imagine an enzyme that could form crossover junctions between homologous DNA molecules at random locations (a nonspecific version of the integrase class of site-specific recombinases, for example),[30] with branch migration proceeding spontaneously. The extended helical filament of *rec*A protein, and the apparent wasteful investment of ATP, seems like biological overkill.

It is always reasonable to presume, however, that form follows function. It seems unlikely that the primary function of this recombination system is the generation of genetic diversity. It can be argued intuitively that the advantages acquired from genetic exchange during rare bacterial conjugation or transformation events could not provide the selective pressure needed to maintain a multienzyme recombination system, or even a single component as complex as the *rec*A system. It is much more likely that some other selective pressure is operative, and an attractive hypothesis can be built around the role of these reactions in cellular DNA repair.[3,4,31] The most obvious phenotypic consequences of *rec*A null mutations is an extreme sensitivity to DNA damaging agents.[3,31] This is due at least in part to a lack of repair processes in which *rec*A protein participates directly.[9,31] One example of a *rec*A-dependent repair process is postreplication repair (Figure 2). The function of *rec*A protein in this case is to convert a region of single-stranded DNA containing a DNA lesion into duplex DNA so that it can be repaired via the normal repair pathways. This process is completely *rec*A-dependent in *E. coli*[31] and is probably accomplished by recruiting a complementary strand from the other side of the replication fork and pairing it with the lesion-containing strand in a strand exchange reaction as suggested by Howard-Flanders and co-workers[31] (Figure 2). The *rec*A protein-promoted branch migration *in vitro* proceeds readily past DNA lesions that would halt the progression of the replication apparatus.[15,32,33]

If reactions of this type represent the primary function of *rec*A protein, then the complexity of the *rec*A system can be rationalized. The simple imaginary system outlined above, in which a directed crossover is followed by spontaneous branch migration, could not do this job. The rapid, spontaneous branch migration observed *in vitro* would be impossible in a cellular environment. DNA binding proteins of many types would block the path of an undirected branch point long before it reached the lesion, and an exposed branch point would be susceptible to the action of cellular nucleases. Even the lesion would be a significant barrier to spontaneous branch movement. In order to have an optimal chance for success in postreplication repair, movement of the branch during strand exchange must be unidirectional and the branch itself must be shielded from nuclease action. It must also be driven with sufficient energy to overcome DNA lesions and other barriers, and its path must be cleared of other DNA binding proteins. Meeting these constraints poses a complex structural and functional challenge.

The architecture of the *rec*A system therefore makes sense if DNA repair is its primary function. The long nucleoprotein filament has the potential to exclude other DNA binding

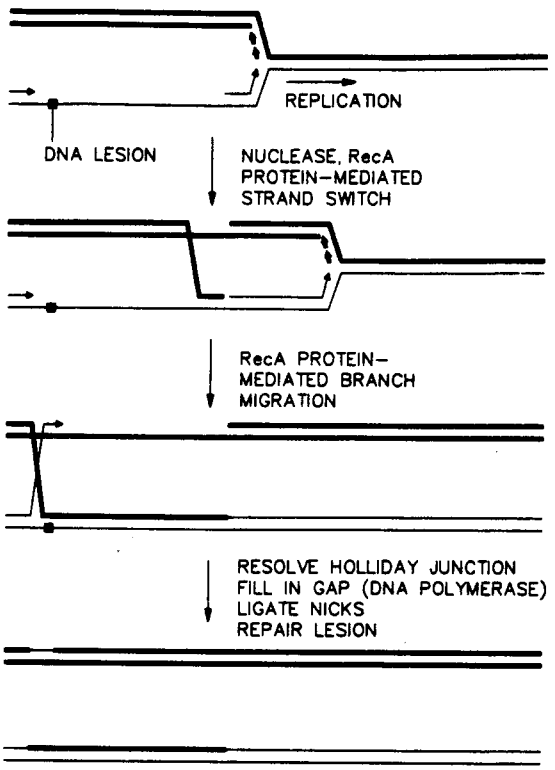


FIGURE 2. The role of *rec*A protein in postreplication repair. Repair of a DNA lesion left behind in single-stranded DNA after the replication fork has passed requires that this DNA be made duplex. This is accomplished by recruiting a complementary strand from the opposite side of the replication fork and pairing it with the lesion-containing strand via *rec*A-mediated strand exchange. Adapted from a model proposed by Howard-Flanders and colleagues.[31]

proteins over a large region, clearing a path for strand exchange, and might also protect the branch point. A unidirectional branch migration has a much greater chance of proceeding for a long distance than a spontaneous (and random) process.[12,24] ATP hydrolysis would supply the energy required to traverse structural barriers in the DNA, and to render the reaction unidirectional.

C. THE CHEMICAL PROBLEM

The most important problems in defining the mechanism of DNA strand exchange involve DNA structure, *rec*A protein and filament structure, and the use of energy in this reaction. Understanding strand exchange will require a knowledge of the structure of paranemic joints at the DNA level, as well as the structure of the *rec*A nucleoprotein filament that stabilizes them. Alterations in DNA structure during the reaction also need to be addressed in more detail, especially the motions needed to exchange strands between two molecules that are both right-handed helices.

The energetic problem is particularly complex, and a discussion beginning here can lead again to the conclusion that the primary function of the system must be DNA repair. *rec*A protein hydrolyzes ATP to drive a reaction that should probably not require the aid of chemical energy. No covalent bonds in the DNA are altered. In exchanging one complementary strand

for another, the products are essentially equivalent to the starting material and the reaction equilibrium should be 1.0. ATP, therefore, is not needed in a thermodynamic role, as it is in many anabolic processes. Separating the two strands of a duplex DNA over thousands of base pairs in order to effect strand exchange would be a reaction with a very high activation energy. In this reaction, however, exchange of strands occurs one or a few bases at a time. As already noted, branch migration is a facile reaction under physiological conditions. Once a crossover is formed, ATP hydrolysis should not be needed kinetically. The only aspect of the reaction that should require ATP hydrolysis is the unidirectionality of the branch migration,[12,34,35] a feature that greatly improves the yield of long heteroduplexes. The bottom line is that strand exchange should be possible without ATP hydrolysis, a prediction that seems to be borne out by the isolation of eukaryotic proteins that promote strand exchange in the absence of high energy cofactors.[36-38]

The use of ATP in the *rec*A system can nevertheless be rationalized if the normal cellular substrates for the *rec*A-mediated reaction are not completely homologous and undamaged DNA molecules. One or more DNA lesions or heterologies can make the strand exchange reaction thermodynamically untenable, and we arrive once again at the hypothesis that ATP hydrolysis is, at least in part, a contingency to push branch migration through any conceivable structural obstacle encountered in the course of a strand exchange reaction that is part of a DNA repair system.

The isolation of eukaryotic proteins that appear to promote strand exchange in the absence of high energy cofactors can be taken as evidence that ATP hydrolysis is actually irrelevant in the *rec*A system. These proteins, however, may represent systems with quite different functions, such as genetic rearrangements between undamaged DNA molecules during meiosis to generate genetic diversity. ATP hydrolysis may well be required only in a system with a primary repair mission. Given the facility with which *rec*A protein mediates strand exchange between DNAs containing lesions and even heterologous insertions, this chapter will proceed under the assumption that ATP hydrolysis is essential and relevant, at least when such barriers are encountered.

II. HISTORY

The *rec*A gene was discovered by Clark and Margulies[39] in the course of screening for mutations that had a strong effect on genetic recombination. Subsequent work in many laboratories showed that *rec*A protein has two distinct but related functions. It is a central component in the regulation of a cellular response to DNA damage called the SOS pathway. In this role it facilitates the cleavage of a small group of repressors that results in derepression of a large set of genes involved in DNA recombination, repair, mutagenesis, and cell division.[40-42] The repressor cleavage activity *in vitro* requires nucleoside triphosphates and DNA cofactors.[4,43] The first evidence for a second function, a direct role in recombination, came from the work of Kobayashi and Ikeda[44] on temperature-sensitive *rec*A mutants in 1978. The *rec*A gene was cloned in 1977 by McEntee and Epstein,[45] and 1 year later the first reports of an *in vitro* activity, single-strand DNA-dependent ATP hydrolysis, appeared.[46,47] This was quickly followed by the discovery of a variety of *rec*A-mediated DNA pairing activities.[48-51] The strand exchange reaction in Figure 1A was described in 1981.[10]

III. *REC*A PROTEIN

*rec*A protein monomers consist of 352 amino acids in a single polypeptide chain with a molecular weight of 37,842.[52,53] Although it is relatively small, *rec*A protein must have binding sites for ATP, other *rec*A monomers, and two DNA molecules. This protein readily

filaments in solution, and has proven difficult to crystallize. Its three-dimensional structure therefore remains unknown.[54-56]

In the absence of DNA, *rec*A protein has two activities: ATP hydrolysis and self-assembly into a variety of aggregation states. Both activities are highly sensitive to reaction conditions.

DNA-independent ATP hydrolysis is very slow under conditions generally used for DNA pairing and strand exchange reactions (pH 7 to 8, 8 to 15 m*M* Mg^{2+}, very low salt concentrations), with an apparent k_{cat} of 0.015 min^{-1}.[57,58] The reaction exhibits a pH optimum at pH 6.0, with an apparent k_{cat} of 0.1 min^{-1}.[57,58] These rates are lower than those observed in the presence of DNA cofactors by factors of 200 to 2000. This reaction, however, is greatly stimulated by high (>1 *M*) concentrations of salt.[58] Rates of ATP hydrolysis similar to those observed with DNA are obtained with a variety of salts (e.g., sodium chloride, potassium and sodium acetate, sodium glutamate) at concentrations near 2 *M*. The properties of the salt stimulation suggest that three to four ions (anions?) are involved, possibly binding to the site that is occupied by the single-stranded DNA backbone.[58]

Self-assembly of *rec*A protein into large aggregates can, under some conditions, compete with the DNA binding reaction.[59] This process has been studied by light scattering,[59,60] sedimentation,[60] and electron microscopy.[60] Self-assembly has recently been examined by Brenner and colleagues[60] in sufficient detail to identify the prominent aggregation states. These include monomers, small rings (12 nm particles) that contain 5 to 8 (probably 6) monomers, rod-like filaments 10 nm in diameter and 50 to 200 nm in length, and much larger bundles of rods. An increase in *rec*A protein concentration or the addition of millimolar levels of $MgCl_2$ favors the formation of the larger rods and bundles. Under conditions generally used for strand exchange, *rec*A protein is present as a mixture of 12 nm particles and short rods, with few monomers evident.[60] The 12 nm particles may represent the unit that binds to DNA.

IV. PRIMARY DNA BINDING

Before considering the binding of *rec*A protein to DNA, it is necessary to define two distinct types of DNA binding. As outlined below, *rec*A protein binds to two DNA molecules, aligning homologous sequences in the process. The binding to the first, however, is very different from the binding to the second. This asymmetry in binding is readily apparent from DNAse protection and DNA underwinding studies outlined below. The first DNA is bound nonspecifically. This binding, which will be called primary binding, has been characterized in some detail and represents the formation of the nucleoprotein filament in step 1 in the scheme in Figure 1A. Binding of the second DNA is largely sequence-specific, with the sequence determined by the DNA within the nucleoprotein filament. This will be referred to as secondary binding and represents step 2 in Figure 1A.

A. PRIMARY BINDING TO SINGLE-STRANDED DNA

The central role of single-stranded DNA in *rec*A-mediated reactions showed up clearly in the earliest studies on this protein. Under conditions used for strand exchange (pH >7.0), *rec*A protein binds very slowly to native duplex DNA, and association with duplex (primary or secondary) is generally governed by a prior association with a single-stranded DNA or single-stranded region of a duplex. Binding to single-stranded DNA is rapid, highly cooperative, and results in a filament that covers the DNA. The binding is nonspecific, but the affinity of *rec*A protein for different homopolymers varies considerably.[61,62] Binding is impeded by secondary structure in the single-stranded DNA, an effect that is overcome by adding the single-stranded DNA binding protein of *E. coli* (SSB). The interaction of SSB and *rec*A protein is affected by the multiple DNA binding modes found for SSB (see Chapter 6). The mechanism by which SSB facilitates *rec*A protein binding is still controversial, and an excellent summary of the

relevant data can be found in a recent review by Radding.[8] The role of SSB is not considered further here, except to note that the final product of the binding reaction in the presence of SSB appears to be a uniform and contiguous filament of *rec*A protein.

The filament formed by *rec*A protein has a right-handed helical form with a deep helical groove evident in the beautiful image reconstructions of electron micrographs contributed by Egelman and Stasiak.[63,64] There are generally six *rec*A monomers per helical turn in these structures,[64] a fact that correlates well with a prominent aggregation state, the 12 nm particles containing 5 to 8 *rec*A monomers, observed in the absence of DNA.[60]

The binding to single stranded DNA is greatly affected by ATP and ATP analogs. In the absence of ATP a "collapsed" filament is formed, with a helical pitch reported to be 55 to 70 Å .[65-67] Addition of ATP or ATP[γS] leads to the formation of an extended filament with a pitch of 90 Å, and the complex is extended by 1.5- to 1.6-fold relative to duplex DNAs with the corresponding number of base pairs on the same electron microscope grids.[64-68] There are about 18 nucleotides of single stranded DNA per helical turn of this complex, with an axial separation of bases of 5.1 Å. The DNA is effectively held in a very open or activated state, and normal base stacking interactions are largely disrupted. ATP also governs the effect of SSB. The stimulatory effect of SSB on *rec*A filament formation on single stranded DNA occurs only when ATP is present.

Each *rec*A monomer binds to three to four nucleotides of DNA in the final complex. A variety of solution methods have yielded stoichiometries of 1 *rec*A per 4 nucleotides (see Reference 5). The observation that there are 6 *rec*A monomers per turn in the protein filament,[64,65] coupled with precise measurements that show 17 to 18 bases or base pairs of DNA per helical DNA turn in the same filament,[69,70] indicate that 1 *rec*A per 3 nucleotides is almost certainly the true binding stoichiometry.

*Rec*A-DNA contacts are concentrated along the ribose-phosphate backbone,[71-74] as might be expected for a protein that binds DNA nonspecifically. The bases are apparently arrayed in the large groove of the filament,[71-73] available to interact with a second DNA molecule.

The nucleoprotein filament exhibits a structural polarity as well as an asymmetry both in the binding of two DNA molecules and in the binding of individual strands of a single DNA molecule. Filament formation is unidirectional, with *rec*A units assembling in the 5′ to 3′ direction along a single stranded DNA (the same direction as branch migration during strand exchange).[75,76] This unidirectional assembly process, which has been observed only in the presence of ATP, is reflected in a clear structural polarity evident in computer-enhanced images of filament structure derived from electron microscopy.[76] This single strand of DNA, in a sense, directs the entire subsequent reaction. When SSB is present, it is almost completely protected from the action of nucleases once the filament is formed.[77] This strand is referred to below as the initiating strand, and its properties while within the filament suggest that it occupies a discrete binding site that does not change during a strand exchange reaction.

As outlined in more detail later, *rec*A protein can also bind directly to duplex DNA under some conditions. This binding is facilitated if there is a single-strand gap in the duplex.[21,78,79] Gaps of 50 nucleotides or more permit optimum binding rates. The gap provides a rapid nucleation site, and the filament is ordered and assembles in the 5′ to 3′ direction along the strand in the gap.[21,78,79] Assembly continues and invades the neighboring duplex, proceeding rapidly until the DNA is saturated. The strand in the gap is the initiating strand. The initiating strand and its complement are bound asymmetrically, a fact readily demonstrated in nuclease protection studies. The complementary strand is at least twice as accessible to DNAse I as the initiating strand.[79,80] The complementary strand, which is replaced during strand exchange, is referred to below as the "exchanging strand". These definitions are summarized in Figure 3.

B. PRIMARY BINDING TO DUPLEX DNA

Direct binding of *rec*A protein to duplex DNA, in the absence of a single strand gap, is very

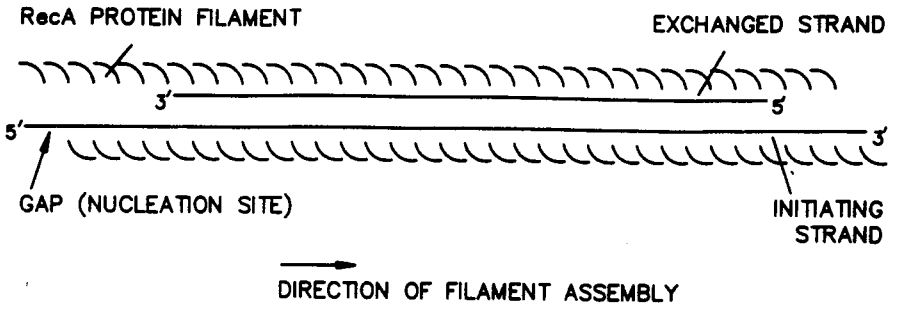


FIGURE 3. Asymmetric binding of *rec*A protein to the two strands of a duplex DNA in a *rec*A-duplex DNA nucleoprotein filament (primary binding of *rec*A protein to duplex DNA).

pH sensitive. At pH 7.5, the binding to B form DNA is negligible on the timescale of most experiments, and for many years this was taken as evidence that duplex DNA is not bound by *rec*A protein under these conditions. Recent reports have demonstrated that the barrier to duplex DNA binding is kinetic rather than thermodynamic.[23,26,27] The slow step is the nucleation of binding, and it is this step that is affected adversely by increasing pH.[27] Although the affinity of *rec*A protein for duplex DNA is not as great as that for single-stranded DNA,[77] filaments formed on circular nicked duplexes are demonstrably stable at pH 7.5 for 1 h or more.[26,27,77]

Direct or primary binding of *rec*A protein to duplex DNA exhibits an absolute requirement for ATP or an ATP analog such as ATP[γS].[26,27] The completed filament hydrolyzes ATP with a k_{cat} (*rec*A monomer) of 18 to 20 min^{-1}. Binding is also coupled to underwinding of the DNA. Duplex DNA is underwound by 39.6% in the presence of ATP,[70] and by 43% in the presence of ATP[γS].[69] The DNA is again extended by a factor of 1.5 in this complex, with 17 to 18 bp per helical turn.[66-68] Base-stacking interactions are disrupted by the 5.1 Å separation of base pairs. Although the DNA is extended and underwound, the two DNA strands are probably still linked by Watson-Crick base pairing in this complex.[72] As already discussed, the duplex DNA is being held in an activated state for strand exchange. The degree of DNA underwinding observed varies little from one complex to the next in the presence of ATP or ATP[γS],[69,70] suggesting a broad structural homogeneity in these complexes even when ATP is being hydrolyzed. ATP hydrolysis is not coupled to binding or DNA underwinding, but rather is a consequence of the formation of the complex.[26,27]

The slow nucleation step can be circumvented by a variety of DNA structural or physical perturbations that make the DNA more readily unwindable. These include an increase in DNA superhelicity, regions rich in A-T base pairs, or an increase in temperature.[23,26,27] *rec*A protein also binds faster to the left-handed Z form of DNA than to B form DNA.[81] The binding to Z DNA, however, does not reflect any special affinity of *rec*A protein for left-handed DNA. The final complex formed on Z DNA is a right-handed nucleoprotein filament,[82] structurally indistinguishable from the complex formed on B DNA. Even though binding is more rapid, the complexes formed on Z DNA are actually less stable than complexes formed on B DNA. When B and Z DNA are both present and compete for a limited pool of *rec*A protein, binding is ultimately restricted to the B DNA. The simplest interpretation of these results is that there is a single duplex DNA-binding pathway for *rec*A protein, and that the slow nucleation step is facilitated in some manner by Z form DNA.

Since binding to completely duplex DNA is not ordered by initiation in a single-stranded gap, orientation of the filaments is random. A presumption here is that each individual complex is asymmetric, and that one strand occupies the "initiating strand" binding site and is therefore better protected from nuclease digestion than its complement. This is impossible

to test, however, since the orientation of filaments in different complexes will vary, with either strand having an equal probability of being the initiating strand in any given complex. The observed partial nuclease sensitivity of either strand in the population reflects the average sensitivity of strands in the initiating or exchange positions.[77,79,80]

C. BINDING TO COMPLEMENTARY SINGLE DNA STRANDS: *rec*A-MEDIATED RENATURATION

*rec*A nucleoprotein filaments bound to single-stranded DNA can bind a second complementary DNA strand and pair it with the first. This renaturation reaction was among the first *rec*A protein activities detected,[48] and there is clear evidence that one *rec*A nucleoprotein filament binds to both strands. The mechanistic relationship between this reaction and three- and four-strand exchanges, however, is not clear. In particular, the complementary strand may or may not interact with the "second" DNA binding site alluded to above. Since the binding site allocated to the exchange strand of a duplex in primary binding (Figure 3) is initially unoccupied in this reaction, the incoming complementary strand is likely to simply bind directly in this site and interact with the initiating strand via Watson-Crick base pairing. No paranemic joints or complicated mechanisms to effect strand separation are required, and the reaction proceeds quite well without ATP hydrolysis.[83] This reaction is not necessarily a good model for any part of the strand exchange reaction, and binding to the second individual strands is not analogous to the binding of the second (duplex) DNA in strand exchange. The discussion of secondary DNA binding, below, is, therefore, focused exclusively on duplex DNAs.

V. SECONDARY DNA BINDING AND PARANEMIC COMPLEXES

A. SECONDARY BINDING WITHOUT HOMOLOGOUS ALIGNMENT

Binding of the *rec*A nucleoprotein filament to heterologous DNA has been detected under some conditions, and such binding is expected to take place as this *rec*A-DNA complex "searches" for homologous sequences in a second DNA.[84-87] Several studies have provided indirect evidence for the existence of a second DNA binding site in nucleoprotein filaments that can bind a naked, heterologous double-stranded DNA molecule.[84,85,88,89] Perhaps the best evidence is the coaggregation of duplex DNA by nucleoprotein filaments reported by Radding and colleagues.[88,89] To date, however, no changes have been observed in the structure or properties of either the *rec*A nucleoprotein filament or the second DNA that can be traced to heterologous interactions. This second binding site evidently interacts with duplex DNA without underwinding or otherwise altering it. The pairing of any homologous sequences present is nevertheless facilitated by this heterologous interaction, probably by means of proximity effects.

B. SECONDARY BINDING WITH HOMOLOGOUS ALIGNMENT

One important function of the *rec*A nucleoprotein filament is to align its DNA with a second, homologous DNA molecule. Much of the detectable binding to this second DNA molecule is therefore homology dependent.

A variety of observations have been linked to homologous interactions in paranemic joints that provide some indirect information about the structure of these complexes. Paranemic joints are formed rapidly upon addition of a homologous duplex DNA, with the pairing complete in 1 to 3 min in most studies. When the second DNA (duplex) is incorporated into a paranemic complex, ATP hydrolysis slows down and the second DNA is underwound.

The decrease in ATP hydrolysis has multiple properties that link it to paranemic joint formation. The decrease (~30%) is dependent on homology in the second DNA.[90] It occurs within 2 min, making it kinetically competent as an event associated with paranemic joint

formation. Also, the extent of the decrease is linearly dependent on the length of homology present in the second DNA. This suggests that paranemic joints can extend over thousands of base pairs, and that all available homology is sampled.

Paranemic joints extending for over 1000 bp have been observed directly in the electron microscope.[7,91] The structure of the filament is not detectably affected by the formation of these complexes. The second DNA simply disappears into the complex and re-emerges at another point.[91]

Secondary DNA binding also results in a homology-dependent underwinding of the second DNA. Since the first DNA is extended and (if duplex) underwound, the second DNA must be comparably extended and underwound if it is to be put in homologous register with the first. The underwinding of the second DNA occurs within 2 min, and its extent depends on the length of homology shared by the two DNAs.[92] The extent of underwinding, however, is insufficient to permit simultaneous alignment of all homologous regions. The second DNA is underwound an average of 12% over the length of homology. Since the first DNA (if duplex) is underwound by 39.6%,[70] this result indicates that about 30% of the available homologous sequences are paired at any instant in a paranemic complex. The linear relationship between the extent of underwinding (and the drop in ATP hydrolysis) and the length of homology suggests all the available homology is sampled somehow. The limited underwinding observed in the second DNA suggests that the homologous contacts in a paranemic joint are discontinuous, but that the probability of homologous contact is a constant throughout the region of homology.[92]

Secondary DNA binding involves a DNA binding site in the *rec*A complex that is distinct from the site(s) used in primary binding of single or double stranded DNA. This represents another level of asymmetry in DNA binding, and this asymmetry is evident in several pieces of data. Evidence for a separate binding site for a second heterologous or homologous DNA is provided by the observation that external duplex DNA is co-aggregated when *rec*A nucleoprotein filaments are present.[88,89] Asymmetry can also be seen in the subsequent strand exchange reaction, which can proceed through a break in the second DNA but is halted by a break in the first.[90,93,94] Finally, neither strand of the second DNA bound in a homologous paranemic complex is detectably protected from nuclease digestion.[77] Both strands of the first DNA are at least partially protected.[77,80]

C. POTENTIAL ARTIFACTS IN PARANEMIC JOINT FORMATION: DNA LOADING EFFECTS

The distinction between primary and secondary DNA binding is especially important when considering the structural consequences of paranemic joint formation on the second DNA. DNA underwinding can occur as a result of either primary or secondary DNA binding. An evaluation of the underwinding effects of secondary binding must be done under conditions in which primary binding to the second DNA is excluded. The potential for artifacts here is significant because of another phenomenon; single stranded DNA greatly facilitates the direct or primary binding of *rec*A protein to duplex DNA. This effect is homology dependent, and occurs under conditions in which plectonemic joint formation is topologically prohibited.[95] The effect, therefore, is related to paranemic joint formation. It could arise by direct transfer of *rec*A protein from single stranded DNA to the duplex or it could simply arise because the underwinding of the second DNA induced by paranemic joint formation produces good nucleation sites for primary binding to the same duplex by any free *rec*A monomers present. A relatively small amount of homologous single stranded DNA can reduce the observed lag in duplex DNA binding by 10- to 20-fold.[95] This loading effect is minimized if the levels of *rec*A protein in the experiment are sufficient to bind only the first (single stranded) DNA. What we are calling DNA loading is probably closely related to the "processive unwinding" phenomenon reported by Shibata and colleagues.[96-98]

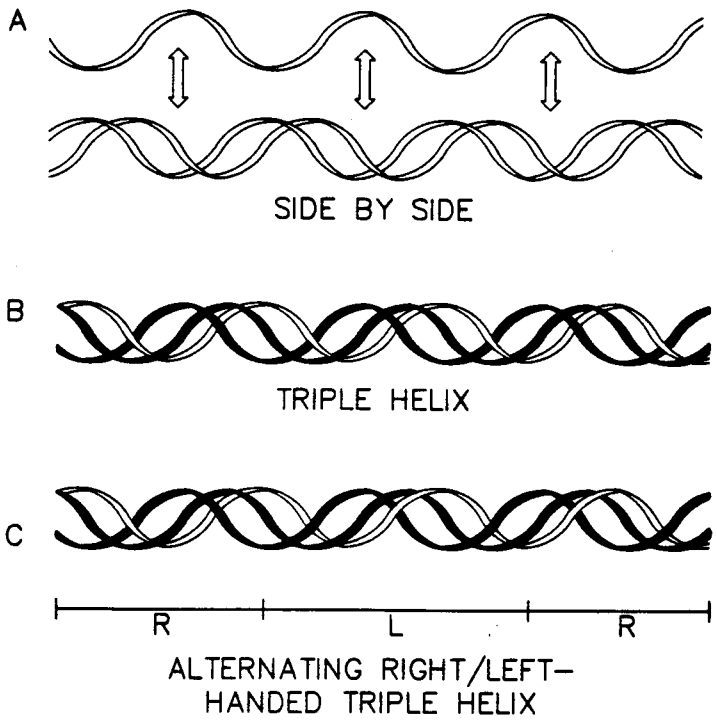


FIGURE 4. Proposed arrangements of DNA molecules paired in a paranemic joint. See References 7, 25, and 56 for more detailed descriptions of structures A, B, C, respectively.

D. THE DNA IN A PARANEMIC COMPLEX: THREE STRANDS VS. FOUR STRANDS

As indicated in Figure 1, strand exchange can involve either three or four strands. An assumption implicit in many studies is that the fundamental mechanism of these two reactions is the same. Hence, conclusions drawn from the three-strand system, which is easier to work with, are often extrapolated to the four-strand reaction. The two reactions are indeed similar with respect to the rates of ATP hydrolysis and branch migration,[5,8,99] the asymmetry in DNAse protection of different strands before and during strand exchange,[8,80,100] the polarity of the reaction relative to the initiating strand,[99] the apparent role of the initiating DNA strand,[99] and other properties.

It follows that there should be closely related three- and four-strand versions of plectonemic, and possibly paranemic joints as well. The published proposals for the structure of paranemic joints are illustrated in Figure 4. These include (1) side-by-side alignment, (2) interwound DNA helices, and (3) an alternating right- and left-handed structure. Only three-strand structures are shown, although each model can be envisioned in three- and four-strand versions.

Some data suggest, however, that paranemic joints are not readily formed with four strands. One key property of the four-strand reaction is that it must be initiated in a three-strand region; i.e., the end of the linear duplex (the second DNA) must overlap the single-stranded gap (see Figure 1B). When the homologous end of the linear duplex occurs at the border of the gap, the reaction does not occur, even though there are no obvious topological restrictions.[31,99] This suggests that the gap is not simply necessary for nucleating nucleoprotein filament formation, but may also mediate a pairing step required for strand exchange.

Two studies designed to detect duplex-duplex pairing have been reported in which heterologous DNA was present in the gap. Das Gupta et al.[101] did not observe pairing although it occurred readily when the single strand in the gap was homologous. Cassuto et al.,[102] in contrast, reported that some duplex-duplex pairing occurred.

More recent data tend to support the notion that four-strand paranemic joints either do not form or form much less readily than three-strand joints. One indirect manifestation of paranemic joint formation is the loading of *rec*A protein directly onto duplex DNA in a reaction that requires homologous single stranded DNA and excess *rec*A.[95] *rec*A nucleoprotein filaments formed on duplex DNA, in contrast, do not facilitate the loading of *rec*A protein onto other homologous, naked duplexes in the same reaction mixture.[95] Whatever pairing interaction is required for the three-strand loading reaction evidently does not take place with four strands. Another effect that more directly reveals the formation of a paranemic joint is the underwinding of the second DNA. This is readily detected under at least some conditions in a three-strand system.[92] When homology is restricted to the duplex region of a gapped molecule, underwinding is greatly reduced relative to that observed in the three-strand system.[103] Little underwinding of the second DNA is observed when only duplex-duplex pairing is permitted, other than the underwinding that results from direct (and slow) binding of any excess *rec*A protein. The degree of underwinding of the second DNA suggests discontinuous contacts, as already described. This is supported by data derived from attempts to crosslink the strands of DNA involved in a three-strand paranemic joint with psoralen.[104]

A triple helix is the most attractive model for producing homologous contacts between the two DNAs. A side-by-side alignment of DNAs in a paranemic joint has been proposed elsewhere by this laboratory,[25,92] but it has proven very difficult to generate a satisfactory molecular model for such a structure. In contrast, models already exist for homologous pairing between two intertwined DNAs.[105,106] In addition, several types of triple helical DNA structures have been observed experimentally.[107,108] All of the known triple helices involve poly purine or polypyrimidine sequences, and the polarities of the paired strands are not those expected in a recombination intermediate. The structures are therefore poor models for a paranemic joint that can be formed in any random sequence. The known structural parameters for DNA in the *rec*A nucleoprotein filament, 5.1 Å separation between base pairs and 18 bp per helical turn, should provide a good starting point for modeling a suitable triple helix. It seems likely that homologous alignment involves at least short triple helices. Between two separated triple helical regions the side-by-side DNAs can be held in alignment not by direct interaction, but by fixing their relative positions at either end.

Based on these observations, one more possibility for the structure of a paranemic joint is offered in Figure 5A. Only three strands are shown. The joint is made up of triple helices of unspecified length, separated by regions where the DNAs are side by side. Where it is not part of a triple helix, the second DNA is bound in the second DNA binding site (that accepts heterologous DNAs), which is drawn on the outside of the filament. Actual homologous contacts between the two DNAs exist only in the regions of triple helix.

It is interesting to consider variations of this structure. In the structure of Figure 5A, the second DNA (in the side-by-side region) is shown bound to every sixth *rec*A monomer in the filament, creating a complex in which the DNAs are completely parallel. If, instead, the second DNA is bound to every fourth *rec*A monomer in the filament, it will be twisted around the first DNA in a gentle left-handed spiral (Figure 5B). Such a structure could compensate topologically for the right-handed turns in the triple helix regions without introducing any left-handed DNA. This solution to the topological problem of aligning two duplex DNAs over extended regions is similar to one proposed by Wilson in 1979.[106] The structure in Figure 5B is only one of many possible variations.

One key difference between the triple helix in this model and other three- and four-stranded helices proposed previously[56,105,106] is in possible locations for the various strands (Figure 6).

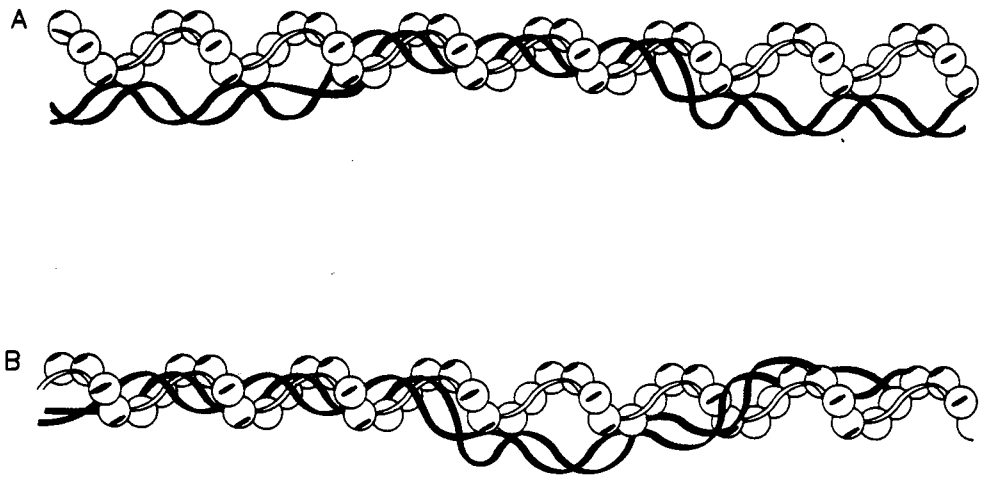


FIGURE 5. Proposed paranemic joint with triple helix alternating with side-by-side regions. (A) Second DNA (duplex) bound to every sixth *rec*A monomer in side-by-side regions. (B) Second DNA bound to every fourth *rec*A monomer in side-by-side regions.

In the nucleoprotein filament groove there are binding sites for two strands, the initiating strand and a complement (the exchanging strand in a *rec*A-duplex DNA complex). If paranemic joints can form only where the initiating strand has no complement, then the site occupied by the exchanging strand is vacant. It is often assumed that paranemic joints involve non-Watson-Crick base interactions, and that this DNA strand binding site is unoccupied in a paranemic joint, as drawn in Figure 6A. However, one of the two strands of the incoming duplex, the one complementary to the initiating strand, could occupy this position, and be paired in some manner (possibly Watson-Crick) with the initiating strand, as drawn in Figure 6B′. This would require significant unwinding of the second duplex over the region of the triple helix. The nuclease protection afforded the complementary strand by this arrangement would be minimal if triple helices were short and discontinuous, so this structure does not conflict with data that indicate little DNAse protection is afforded a duplex DNA by secondary DNA binding.[77]

VI. ATP HYDROLYSIS BY *REC*A PROTEIN

The final parameter that must be discussed before considering models for strand exchange is ATP hydrolysis by the nucleoprotein filaments. The most important observation here is that ATP is hydrolyzed by *rec*A monomers uniformly throughout the filament.[20-23] There is nothing unusual about a filament end in this respect, and there is no detectable change in the rate of ATP hydrolysis by monomers located near the branch point during strand exchange.[20] The amount of ATP that is hydrolyzed during strand exchange appears excessive, but it should be noted that the *rec*A system is not a serious drain on the cellular ATP pool. The 1000 *rec*A monomers present in a typical cell at saturation could hydrolyze less than 0.1% of the ATP that is used in cellular protein synthesis.[9]

What is *rec*A-mediated ATP hydrolysis coupled to? The models outlined below propose different consequences for the DNA that reflect, in part, different proposals for the structure of paranemic joints. However, one likely molecular consequence of ATP hydrolysis that is required in some manner in all models is a conformation change or set of conformation changes in *rec*A protein. Furthermore, in order to effect a unidirectional strand exchange, the ATP hydrolysis in adjacent monomers may be coordinated, perhaps taking the form of waves

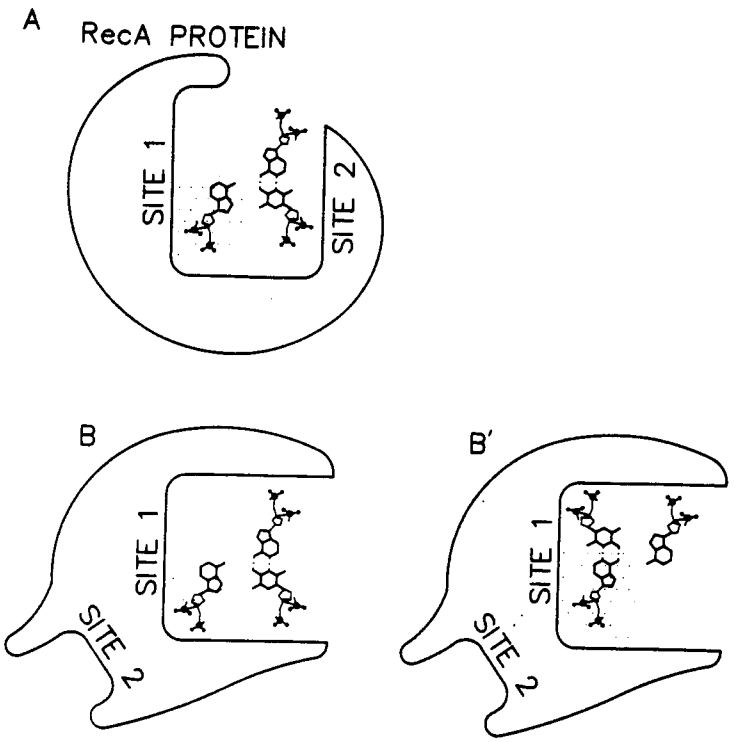


FIGURE 6. Some possible arrangements of three DNA strands in triple-helical paranemic joints. The nucleotide at lower left in each case (shaded) is that from the initiating strand. (A) Triple helix from model proposed by Howard-Flanders and colleagues.[55,56] The binding site for the second DNA (site 2) forms a contiguous pocket with the site occupied by the initiating strand. (B and B′) Alternative arrangements for DNA strands in a short triple helix formed in the paranemic joint of Figure 5. DNA site 2 in this case is the site occupied where the two DNAs are bound side-by-side. Structure B roughly corresponds to the triple helix in A. The structure in B′ is an alternative in which the second duplex has been unwound in the triple helix region, and the strand complementary to the initiating strand has been drawn into the site normally occupied by the exchange strand. In this structure the initiating strand is paired Watson-Crick with its complement in the duplex, so that a pseudo-exchange of strands has occurred over the triple helix region.

of ATP hydrolysis/conformation changes traveling unidirectionally through the polar filament.

There is no direct evidence for such waves of ATP hydrolysis, but one recent study may provide a clue.[79] When *rec*A filaments are formed on circular duplex DNA, they are stable for long periods at pH 7 to 8, but when a free end is available — as on linear duplex DNA — dissociation occurs. The dissociation occurs progressively from one end, in the 5′ 3′ direction relative to the initiating strand.[79] The properties of this reaction and of strand exchange do not implicate the former as a mechanism for the latter.[79] The dissociation reaction, however, could be related to the hypothetical waves of ATP hydrolysis. Starting from the considerations of Wegner,[34] it is reasonable (if not absolutely necessary) to conclude that this unidirectional dissociation is coupled in some manner to ATP hydrolysis. Since ATP hydrolysis is not restricted to ends, it must be assumed that complete dissociation is the result only when ATP hydrolysis occurs in the monomer at the 5′ end of the filament (relative to the initiating strand). Internal monomers are stabilized by protein-protein interactions on both sides. The maximum

rate of dissociation (and, presumably, the optimum coupling with ATP hydrolysis) occurs at pHs above 8.0,[79] and this rate corresponds to the dissociation of about 200 *rec*A monomers per minute, or one *rec*A monomer every 0.3 s. The monomer turnover number for ATP hydrolysis varies little with pH and is about 18 min^{-1} under these conditions, or one ATP every 2.4 s. If ATP hydrolysis occurred at random, the end *rec*A monomers would hydrolyze an ATP every 1.2 s, on average, after the previous *rec*A monomer dissociates. This is too slow for a coupled process. The data can be explained, however, by assuming that ATP hydrolysis occurs in highly coordinated waves so that adjacent *rec*A monomers hydrolyze ATP 0.3 s apart. If the waves were 8 *rec*A monomers apart, each individual *rec*A monomer would hydrolyze an ATP every 2.4 s to give the observed turnover number. The wave at the 5′ end would be coupled to dissociation. There is some uncertainty in these rate measurements and in certain key parameters such as the size of the *rec*A protein binding site. Allowing for this potential variation permits the waves to be anywhere from 5 to 11 *rec*A monomers apart.

VII. MODELS FOR DNA STRAND EXCHANGE

A. EXPERIMENTAL FACTS

In addition to the studies on the basic activities of *rec*A protein such as DNA binding and ATP hydrolysis, there is a wealth of data available on the properties of the complete strand exchange reaction. The reaction has three important general properties that were summarized in the Introduction and will be expanded upon here: it is relatively slow, it is unidirectional, and it proceeds past short regions of heterology or DNA lesions in one or both DNAs.

The rate of branch migration during strand exchange is generally reported to be 3 to 6 bp/s,[10,110] so that the entire process in a 6000-bp substrate DNA takes about 20 min. These rates, however, are almost certainly underestimates, in that they are based on calculations that assume the process is synchronized in all DNA molecules in the population. This synchrony is largely absent in many experiments, with products (the nicked duplexes of Figure 1A) appearing gradually over 10 to 30 min. There are a few examples of reported rates on the order of 20 bp/s,[5,18,19] and this probably more accurately reflects the true rate of branch migration in this system under optimal conditions. The branch moves progressively through the DNA, and the kinetics are incompatible with models that require the concerted exchange of strands or segments of strands hundreds or thousands of nucleotides long. *rec*A protein in any case does not possess a significant helicase activity, and is unable to promote complete strand separation over a region greater than 30 bp.[111] Virtually all of the DNA remains base paired throughout the strand exchange reaction, with the exchange itself occurring over a short region at the branch point.

The directionality of the reaction is a property of the last phase, i.e., branch migration.[12,15] The directionality is governed by, and proceeds 5′ 3′ relative to, the initiating strand. The formation of paranemic joints can occur virtually anywhere within homologous regions of two DNA molecules. Short plectonemic joints can be formed at either end of the linear duplex (Figure 1A), but those at the 5′ end of the strand complementary to the initiating strand are much less stable because they are subsequently eliminated by the unidirectional branch migration process.

The progression of strand exchange past regions of heterology and DNA lesions is one of the most difficult properties of the system to explain, but probably one of the most important properties with respect to the system's DNA repair function in the cell. In three-strand exchanges (Figure 1A), the reaction proceeds past thymine dimers,[32] all types of mismatches,[15] and short heterologies.[33] Heterologous insertions of 100 to 200 nucleotides in either the initiating single strand or the homologous duplex decrease the efficiency of product formation by only a factor of three or less. Strand exchange is even promoted in this system between M13 and fdDNA, which differ in sequence by about 3%.[8] The mechanism used in the strand

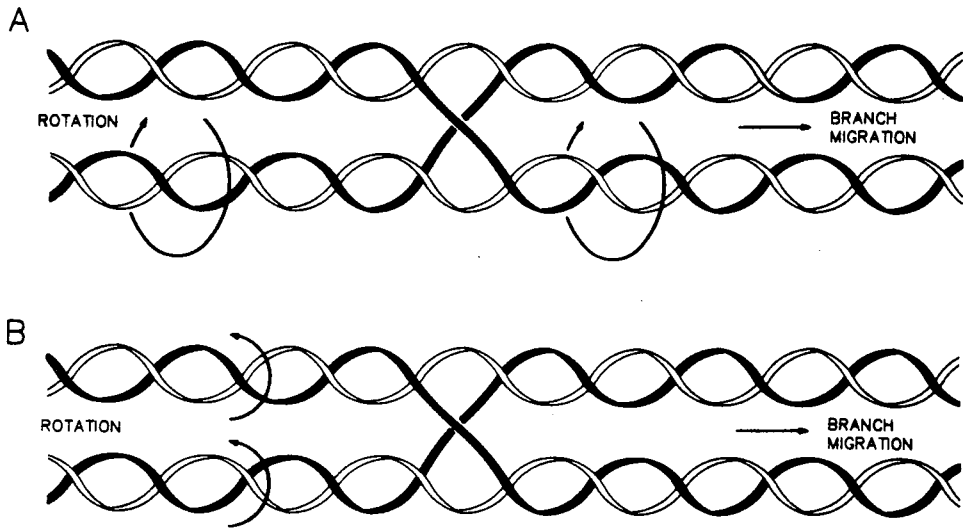


FIGURE 7. Rotation of DNA molecules required for branch migration. (A) Rotation of one DNA (upper) about the other. (B) Rotation of both DNAs about their helical axes.

exchange reaction must have only a limited sensitivity to homology. It is notable that the promotion of strand exchange past heterologous insertions does not occur in four-strand exchanges.[94] This is another key property that distinguishes the three- and four-strand reactions.

Several other properties of the reaction deserve note. The fact that ATP hydrolysis occurs throughout the filament effectively eliminates the simplest forms of a mechanism that was once quite attractive, i.e., treadmilling.[20,24,25] There is no evidence to indicate that branch migration is linked in any way to the association or dissociation of *rec*A monomers at the branch point. Under some conditions the filament remains intact throughout the reaction.[77] There is nothing, in fact, to suggest that there is anything special about the filament at the branch point. There is no relationship between the number of branch points and ATP hydrolysis,[20] and data from DNAse protection[80,100] and electron microscopy[91] indicate that the filament extends through and well beyond the branch point with no obvious changes in structure. A variety of conditions, or changes in conditions, that have demonstrable effects on *rec*A filament assembly or disassembly (dilution, pH changes, introduction of low concentrations of ATP[γS]) have little or no effect on the strand exchange reaction.[103] As noted by Griffith and colleagues,[7,91] the nucleoprotein filament must be considered a scaffold upon which strand exchange occurs. We have previously proposed that models for strand exchange be classified on the basis of a coupling to *rec*A association/dissociation (class I) or the involvement of an intact filament (class II).[24] Given the accumulating evidence against a direct role for association/dissociation in strand exchange, the former classification now has limited usefulness and the discussion below focuses on models that would fall into class II.

Another structural problem that must be overcome by this system is topological. The right-handed helical twist of the DNA helix imposes the constraint that the two DNA molecules taking part in a strand exchange reaction must be rotated to effect branch migration. The rotation can take several forms (Figure 7), although the forms shown are not necessarily mechanistically distinct.[9] Honigsberg and Radding[112] have shown that *rec*A protein can promote strand exchange even when rotation is restricted to rotation about the longitudinal axes of the two DNAs (Figure 7A), whereas restricting the rotation of DNA 2 about DNA 1 (Figure 7B) does not block the reaction.

One final aspect of the process is the conformation changes in *rec*A protein that appear to

occur during the ATP hydrolytic cycle. As noted above, conformation changes must be considered a required part of any complete model for this reaction. In other systems where ATP hydrolysis is coupled to a unidirectional process, such as muscle contraction and transmembrane ion transport, there are well-documented and required sets of conformation changes that are integrated into the coupling mechanism.[113-115] A similar set of conformation changes is being elucidated in the *rec*A system and it is likely that they are related to a mechanism that couples the expenditure of chemical energy in this system to unidirectional branch migration. Kinetic and electron microscopy (EM) studies provide evidence for at least two,[64,116,117] and possibly three, separate *rec*A protein conformations that correspond to different steps in ATP hydrolysis.

As already described, this coupling suggests that ATP hydrolysis and its accompanying conformation changes travel in coordinated waves unidirectionally through the *rec*A nucleoprotein filament. In the *rec*A system, it is necessary to explain the apparent inefficiency of the system as part of any model. The efficiency of the system will depend on how these waves or "power strokes" are used. Two general possibilities can be imagined. First, the branch point could travel in concert with one wave, just as a person might ride on one step of an escalator. In this case only the wave at the branch would be doing useful work. The other waves, like empty steps on an escalator, would be there simply to increase the probability that a reaction will occur when required. Models that link strand exchange to a *rec*A polymerization or depolymerization cycle fall into this class in a sense, although the weight of available evidence argues strongly that the filament remains intact through the branch point. The observed efficiency of the system would be low because of the many waves that are unproductive. Second, it is possible that most or all of the waves are coupled to a motion that brings about branch migration. As illustrated below, the motion would be DNA rotation. In this case the inefficiency would result from the hydrolysis of ATP at many points coupled to a motion that leads to branch migration at a single point.

B. MODELS

Numerous mechanistic suggestions have appeared in the literature pertaining to conformation changes or waves of conformation changes (or polymerization/depolymerization cycles) that might occur during these reactions.[6-9,24-25] Only two models have appeared to date, however, that propose specific motions to which these conformation changes could be linked to bring about strand exchange. One of these is an example of coupling branch migration to a single wave. The other illustrates how all of the waves might be coupled to this process.

The former is a model proposed by Howard-Flanders and colleagues.[56] The original version of this model, which coupled formation of a three- or four-stranded paranemic joint to unidirectional polymerization of the *rec*A filament and resolution or branch migration to depolymerization (dissociation) at the opposite end of the filament, is inconsistent with data that indicate the *rec*A filament is intact at the branch point. As illustrated in Figure 8, however, the model can be made largely consistent with available data with only slight modification.

In this model, the key intermediate is a triple helix[56] paranemic joint. First, the duplex DNA wraps and intertwines itself by rotary diffusion about the nucleoprotein filament to form a bound triple (or four-strand?) helix that can be hundreds or thousands of nucleotide residues in length. Binding interactions between the duplex DNA and the filament could be effective in driving the formation of this paranemic joint rather than the *rec*A association/polymerization reaction originally proposed. The actual exchange of strands occurs by a rotation of the bases within this paranemic joint as in Figure 8. This exchange is presumably coupled to ATP hydrolysis via one or more conformation changes, and this is the only process in the model that is linked directly to the expenditure of chemical energy. To make the exchange unidirectional along the helix axis, the movement of the branch point would be coupled to one of many available unidirectional waves of ATP hydrolysis, while ATP hydrolysis in other waves would

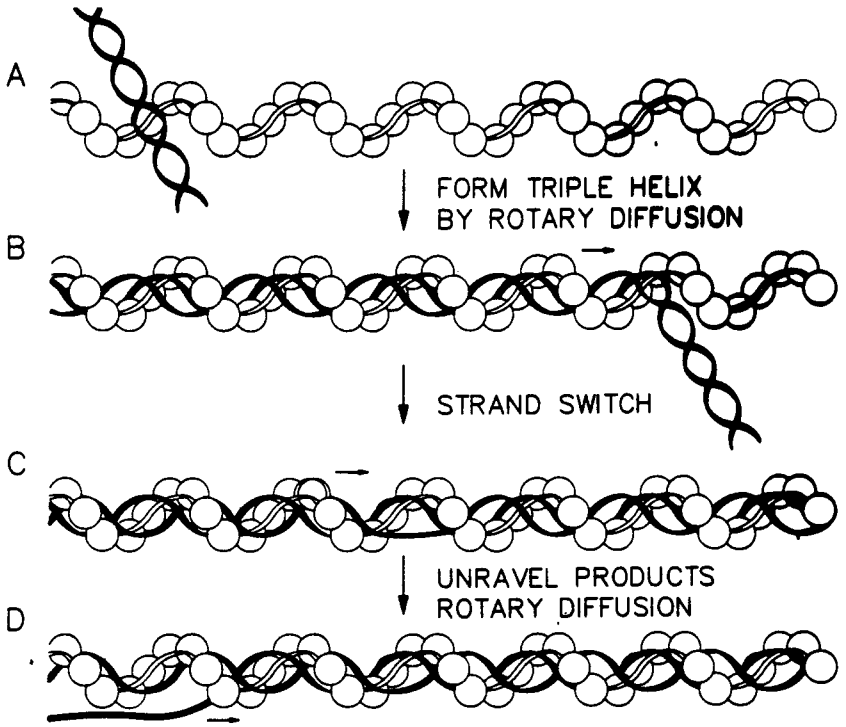


FIGURE 8. A model for DNA strand exchange in which branch movement is coupled to movement of one ATP hydrolytic wave. Model is adapted from that proposed by Howard-Flanders and colleagues.[56] See text for details.

be unproductive. The strand of the duplex that is separated and displaced in the reaction would undergo another stage of rotary diffusion to disentangle the products. In the cell, this passive disentanglement process might be facilitated by topoisomerases. The rotary diffusion required to form the paranemic complex and separate products takes care of the DNA rotation requirements for strand exchange. The model also provides an explanation for the observed energetics of the reaction, since much of the ATP hydrolysis of the filament would be uncoupled. One observation that is difficult to explain with this model is strand exchange through heterologous insertions in the duplex, since there is no provision for the separation of the strands in the duplex through the insertion. This model also requires the formation of four-stranded helical paranemic complexes to effect four-stranded exchange reactions, although these joints need not be very long.

The second model (Figure 9) is a variant of a model described previously in some detail.[5,9,25] It begins with the paranemic joint of Figure 5, with homologous interaction and alignment between the two DNAs mediated by periodic regions of triple helix. The triple helices are again of undetermined length, but periodic contacts of this kind would occur throughout the region of homology. Between the triple helical regions the two DNAs are aligned side by side, with the duplex possibly occupying the heterologous DNA binding site. The major difference between this model and the last is that the process coupled to ATP hydrolysis is *rotation* of the two DNAs relative to each other, while the actual exchange of strands is simply a by-product of this rotation. The "motor" in this reaction involves the duplex DNA that is not involved in triple helices, but is aligned side by side with the first DNA in the nucleoprotein filament. This DNA could have periodic homologous interactions within this side-by-side structure but, as already discussed, this kind of interaction is difficult to model and here it is

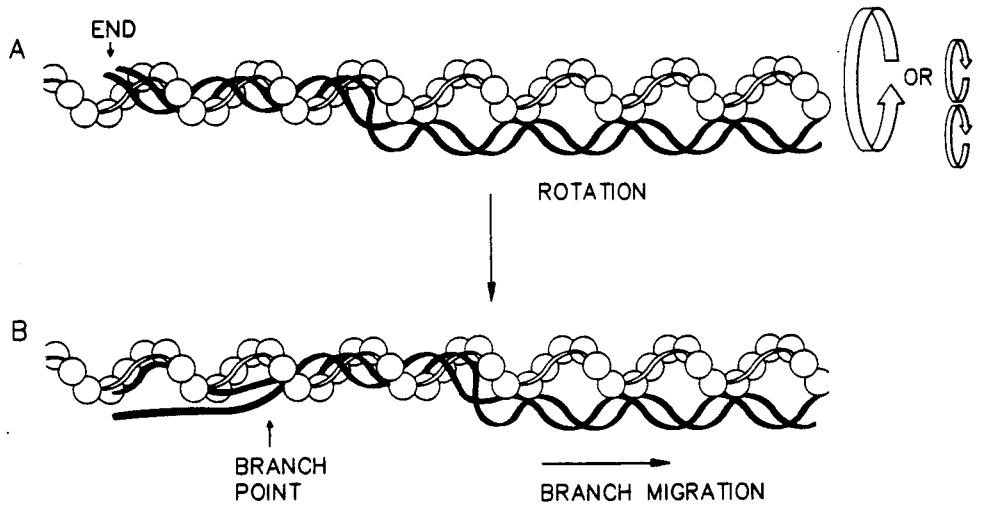


FIGURE 9. A model for DNA strand exchange in which branch movement is coupled to ATP hydrolysis throughout the *rec*A filament. Model is based on the paranemic joint of Figure 5. Rotation in either mode shown (see Figure 7) will move the branch point and the triple helix left to right. One complete rotation is shown. This rotation would presumably occur in six ATP-mediated steps. See text and Reference 9 for further details.

assumed that the duplex is simply bound in the heterologous DNA binding site. This second DNA binding site then assumes a dual role, first as a site to facilitate coaggregation and pairing, and second as the "piston" of this biochemical machine. When bound to the second DNA binding site, this DNA would actually contact only one of every six or so *rec*A monomers in the nucleoprotein filament (Figure 9, also see variation in Figure 5). ATP hydrolysis would be linked to conformation changes resulting in the passage of the duplex to the next monomer in the filament. This would happen throughout the filament in a coordinated process linked to the unidirectional waves of ATP hydrolysis. In this model, most or all of the waves would be coupled to useful work. The triple helical regions themselves would migrate along the filament keeping pace with the energetic waves, with exchange of strands occurring within one of them.

The reaction in Figure 9 is drawn as though the second DNA rotates about the first for illustrative purposes. The coupling mechanism envisioned, however, is compatible with either of the rotations in Figure 7.[9] The coupling can be thought of as a kind of ratchet that will result in different modes of rotation depending on which parts of the system are fixed.

The apparent inefficiency of the reaction is a direct consequence of hydrolyzing ATP in many places to bring about branch migration at one point. The rate of branch migration would be determined by the rate at which the waves of ATP hydrolysis traveled along the helix. It is assumed in this discussion that the observed k_{cat} for ATP hydrolysis (~20 min^{-1}) reflects the rate at which waves are moving through the filament, i.e., a wave reaches an individual *rec*A monomer every 3 s, and that each succeeding wave corresponds to one complete rotation of the DNA. Each 3-s rotation would move the branch 18 bp (the helical periodicity of DNA in a *rec*A nucleoprotein filament) giving an overall rate of branch movement of 6 bp/s. This is within the range of reported rates, although somewhat different assumptions are necessary to obtain the optimal rates of branch migration (up to 20 bp s^{-1}) seen *in vitro*.

Given a certain pattern of ATP hydrolytic waves, the energetic efficiency and the rate of branch migration will be the same whether only one wave of ATP hydrolysis or all of them are coupled to strand exchange, and the models described above cannot be distinguished in this respect. The second model has the advantage that it provides a mechanism for unraveling the

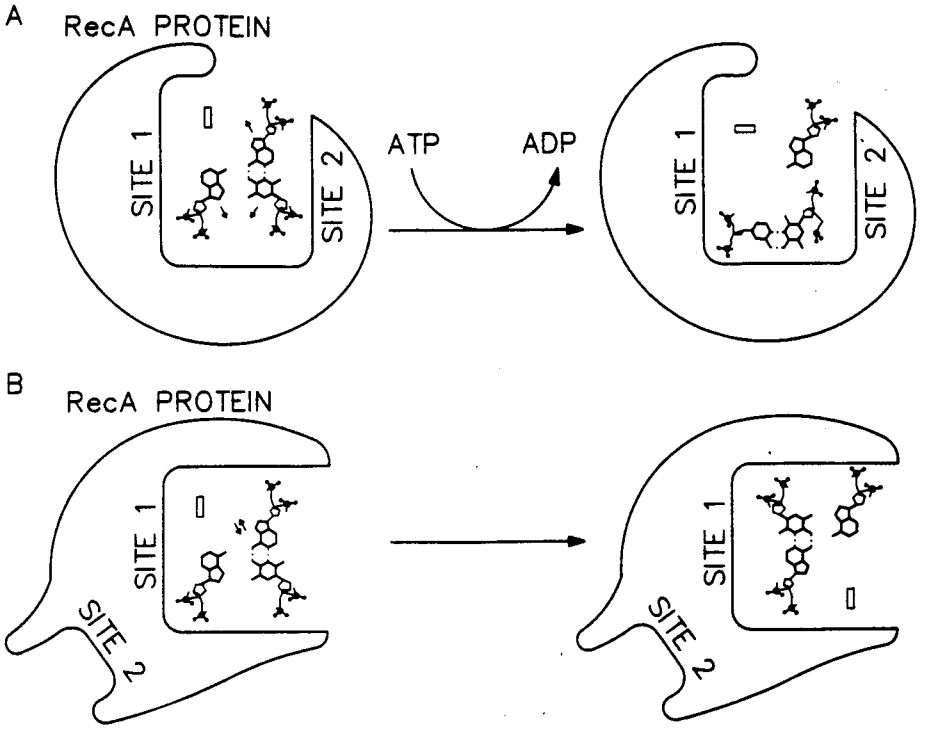


FIGURE 10. Fate of DNA strands in model of Figure 8 (A) vs. model in Figure 9 (B). Rectangle denotes position and movement of another DNA strand in a four-strand exchange.

two strands of a duplex within a heterologous insertion, allowing the system to bypass such barriers.[9] The two models also make somewhat different predictions with respect to the disposition of DNA strands (Figure 10). In the first model, all of the strands in the paranemic joint would occupy the same binding sites before and after the passage of the branch point, although each would rotate within its site. In the second model, the initiating strand is fixed, and its complement (if any, in a four-strand exchange) trades places with the incoming strand (from the second DNA) that replaces it. Finally, the second model provides a mechanism for a four-strand exchange that does not require homologous contacts in the four-strand region. Once alignment has occurred in a three-strand region and the reaction is initiated, the rotation mechanism will drive branch migration through all available contiguous homologous DNA without further need for direct interaction between the two DNAs.

VIII. CONCLUDING REMARKS

A detailed understanding of the mechanism of *rec*A-mediated DNA strand exchange has been elusive despite a decade of research in dozens of laboratories. This research, however, is gradually imposing constraints on potential mechanisms. When combined with constraints imposed by DNA structure and physical laws, there is every reason to expect that one model or another will soon emerge with clear experimental support. Some of the ideas presented here are speculative, but they fall well within the constraints now perceived by the author to exist.

Defining the kind of DNA motion that is coupled to ATP hydrolysis is a necessary first step in understanding *rec*A-mediated DNA strand exchange. Understanding the coupling mechanism will subsequently require a definition of the number and sequence of conformation changes relative to steps in ATP hydrolysis and specific changes in the position or structure

of DNA. Ultimately, methods must be found to elucidate the detailed structure of the *rec*A nucleoprotein filament at different stages in the reaction. The biochemical stakes are high: an improved understanding of DNA structure, protein-DNA interactions, genetic recombination, DNA-DNA interactions, and biochemical energetics await.

ACKNOWLEDGMENTS

Work cited in the author's laboratory was supported by N.I.H. grant GM 32335. The author is supported by N.I.H. Research Career Development Award A.I. 00599.

The author thanks his students and co-workers, past and present, who have made this chapter possible with contributions too numerous to list.

NOTE ADDED IN PROOF

Further work on the structure of paranemic joints generally supports the strucutre shown in Figure 5A.[104]

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