We present a simple theory of the dynamics of force generation by RecA during homologous strand exchange and a continuous, deterministic mathematical model of the proposed process. Calculations show that force generation is possible in this model for certain reasonable values of the parameters. We predict the shape of the force-velocity curve for the Holliday junction, which exhibits a distinctive kink at large retarding force, and suggest experiments which should distinguish between the proposed model and other models in the literature.

RecA protein or one of its homologs has been found in every species where it has been sought (1). RecA plays a key role both in DNA repair and in the exchange of genetic material through its ability to promote DNA strand exchange. This activity is of great importance in the maintenance of the genome, and is essential for sexual reproduction. Indeed, RecA- mutants have a 10,000-fold lower frequency of recombination than wild type. RecA strand exchange activity has been conserved in species so diverse as to have taken different evolutionary paths in Precambrian times. In the SOS response, RecA also has a coprotease function in the autocatalytic cleavage of the LexA repressor and the λ repressor (2).

The strand exchange activity of RecA requires an ability to generate a “rotary torque” on DNA strands, as further discussed below. Even though RecA protein has been extensively investigated, the mechanism by which RecA generates torque is poorly understood. A variety of reasonable models have been proposed, as reviewed below, based on various analogies with well known forms of mechano-chemical force transduction such as by “motor proteins” like myosin or through polymerization or depolymerization as by actin and microtubules. In each case, however, experimental evidence is available that appears to be in disagreement with the basic assumptions underlying the model. In this paper we propose, on the basis of an analysis of the existing experimental evidence, that force transduction by RecA proceeds through a mechanism unique to RecA, namely force generation by hydrolysis waves.

The paper is organized as follows. We briefly review important experimental information concerning RecA. Under “Theory,” we discuss the existing theoretical models together with the experimental facts that appear to contradict them, and we present our “hydrolysis wave model.” Under “Results and Discussion,” we provide a quantitative analysis of the model and a number of predictions which should make it possible to clearly distinguish between the hydrolysis wave model and earlier models.

### EXPERIMENTAL PROCEDURES

#### RecA: Polymerization and ATP Hydrolysis

RecA is a 38-kDa protein which polymerizes in solution to form a right-handed helix with a diameter of ~100 Å (3) and a repeat length of 6 monomers per helical turn (4).

Both single- and double-stranded DNA (ssDNA1 and dsDNA) molecules act as nucleation sites for the RecA polymerization process, producing a stiff nucleoprotein filament. Filament formation on ssDNA may occur in the absence of any cofactor (4, 5) or with either a nucleoside diphosphate (NDP) cofactor (3), usually ADP, or a nucleoside triphosphate (NTP) cofactor (3, 4), usually ATP. Filament formation on dsDNA requires an NTP cofactor (3, 4), usually ATP (5).

Nucleation is the rate-limiting step for filament formation in vitro (6). Nucleation is fast on ssDNA but very slow on dsDNA (7). Once nucleation has occurred, however, the filament extends rapidly (6). RecA monomers associate preferentially with one end of the nucleoprotein filament so that the polymer extends in a polar manner in the 5′ → 3′ direction (8) relative to the DNA strand bound at site 1, which will be discussed later. As discussed below, the rapid adsorption of RecA onto ssDNA should not be interpreted as evidence for a more favorable binding of RecA on ssDNA than on dsDNA (8). Formation of nucleoprotein filaments on dsDNA is usually initiated in a preceding region of ssDNA (8, 9). Nucleation occurs in the single-stranded region, and extension of the polymer then continues into the double-stranded region.

Hydrolysis of the ATP cofactor is the energy source which allows for the mechano-chemical activity of RecA (10). RecA in solution is a slow but detectable ATPase, but the ATP hydrolysis rate strongly increases following binding to DNA (11). When RecA is bound to ssDNA, the hydrolysis rate is $k_{\text{cat}} = 29/\text{min}$ at $37^\circ \text{C}$, and $\ln(k_{\text{cat}})$ varies as $1/T$.

When bound to dsDNA, hydrolysis is about 30% lower ($k_{\text{cat}} = 21/\text{min}$ (6). Although hydrolysis is stimulated by filament formation, the reverse is not true; RecA does not depend on the ATPase activity to form nucleoprotein filaments. In fact, non-hydrolyzable ATP analogs such as ATPγS can serve to produce filaments (13).

The RecA/ssDNA filaments in the ADP-bound form and in the ATP-bound forms have quite different structures, referred to as the “collapsed” and “extended” forms, respectively. The ADP-bound form is a helix with a 64 Å pitch and a DNA base spacing of 2 Å per base (only ssDNA is bound in collapsed filaments) (3), while the ATP-bound form of the filament has a 95 Å pitch and the DNA is extended by ~50% to 5 Å per base or base pair (3). Only the extended filament participates in DNA strand exchange reactions (14). There is thus a considerable amount of elastic stretching energy stored in the ATP-bound form. The collapsed and extended forms of the filament, however, are not directly interconvertable (4, 15). Transition between the two forms can occur only by disassembly and depolymerization followed by reassembly.

ATP-driven disassembly of the highly stable dsDNA/RecA nucleoprotein filament is vital for the functional design of RecA (14, 16). Strand exchange between RecA-covered ssDNA and bare dsDNA leads to final

1 The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; bp, base pair(s); N, newton(s); ATPγS, adenosine 5′-O-(thiotriphosphate).
products of bare ssDNA and dsDNA covered by RecA (17, 18). Removal of the RecA from the product is required to allow functioning of the dsDNA and recycling of the RecA (16).

RecA filament dissociation proceeds from the end of the nucleoprotein filament opposite that on which it associates, so depolymerization proceeds 5′→3′ (8, 9). Each monomer in the RecA filament turns over 3′ to 5′ as rapidly as the average time between hydrolysis events on any given monomer (20). The most natural interpretation is that ATP hydrolysis on dsDNA is a cooperative process (20): the hydrolysis of ATP by one monomer induces the hydrolysis of ATP by its neighbor on the 3′ side. Cooperative hydrolysis will produce “waves” of hydrolysis propagating along the nucleoprotein filament in the 5′→3′ direction (20). Recently rapid disassembly of dsDNA filaments has been monitored on individual nucleoprotein filaments (21).

Strand Exchange

The central function of RecA is to promote strand exchange. Two types ofstrand exchange reactions are encountered: three-stranded reactions and four-stranded reactions.

Three-stranded Reaction—In the three-stranded reaction, RecA first forms nucleoprotein filaments with ssDNA (22). A region of homology is then found on a dsDNA molecule, most likely by formation of a transient triplex structure (14, 22). The RecA-coated ssDNA replaces the identical strand from the original dsDNA, requiring a relative rotation between the two substrates (23). In terms of chemical reactions, the substrates are a RecA-coated ssDNA molecule and a bare dsDNA molecule (1), while the products are a RecA-coated dsDNA molecule and a bare ssDNA molecule (17, 18).

Neither the homology search nor a limited (1–2 kilobase pairs) three-stranded exchange reaction in homologous regions require hydrolysis of the NTP (24). Hydrolysis of the cofactor is required for exchange of longer homologous regions, for bypass of heterologous inserts, and for the four-stranded reaction (24). It follows from basic thermodynamic principles that, because limited three-stranded exchange reactions proceed spontaneously, the dsDNA/RecA nucleoprotein filament has a lower Gibbs free energy than the ssDNA/RecA nucleoprotein filament. Thus, even though the RecA binding kinetics is more rapid on ssDNA, the double-stranded variant must provide the larger binding energy. It is this clever “design strategy” that permits spontaneous three-stranded strand exchange; the ssDNA filament is a rapidly forming "transition state" that converts more slowly to the dsDNA filament ground state when presented with a homologous dsDNA partner. Hydrolysis plays the role of an auxiliary “engine,” which stimulates an "anti-binding" site which will tend to expel any DNA strand occupying that position.

From the fact that the three-stranded exchange proceeds spontaneously (13, 14, 24), we conclude that the strongest DNA binding site of dsDNA/RecA is site I, that a weaker binding occurs at site II, and that site III is the weakest binding site. We will denote the binding energies by $E_1$, $E_{II}$, and $E_{III}$ with $E_{III} < E_{II} < E_1$. We take the state of free DNA exterior to the RecA filament and not associated with RecA to have a binding energy of 0. It is not necessarily the case that $E_{III} < 0$. So long as $(E_{III} + E_{II}) < 0$, the double-stranded DNA in the three-stranded reaction will be brought into the filament by the net binding energy there. If $E_{II} > 0$, it is a repulsive "anti-binding" site which will tend to expel any strand occupying that position.

We will discuss this further under “Three-stranded Reaction.” In a four-stranded exchange, the displaced strand is assumed to vacate site II when it leaves the nucleoprotein filament at the junction. Site II is then occupied by the incoming strand (22). Site I is always occupied by the strand complimentary to that at site II. Whether site III plays any role is not clear.

Recall that the dsDNA nucleoprotein filament extends DNA by 50% (3). Molecular mechanics experiments on naked DNA show that stretching DNA by that amount requires an amount of work W roughly equal to $10k_BT$ per base pair (28). The free energy gain per base pair obtained in transforming a ssDNA filament into a dsDNA filament is $E_{III} + W$. It follows that $E_{III} > W$, since the reaction proceeds spontaneously.

THEORY

Force Generation Models—How does ATP hydrolysis generate the torque that produces the counter-rotation of the DNA strands during exchange? Mechanochemical force transduction has been considerably discussed in the biophysical literature. Two classes of force transduction have received attention. Motor proteins like myosin and dynein contain flexible sections. Motion of these sections driven by ATP hydrolysis generates mechanical forces in the 10 pN range (29). Force generation is also possible by the polymerization reaction. Actin and microtubule filaments are able to generate mechanical force by polymerization and depolymerization reactions fueled by ATP or GTP (30). RNA polymerase is a motor protein that also produces mechanical forces in the 20 pN range (31) based on nucleotide polymerization. From our earlier discussion, it would seem very reasonable to expect that RecA generates force by polymerization and/or depolymerization.

Polymerization/Depolymerization—Polymerization can generate mechanical force through the “Brownian ratchet” mechanism (32). Thermal fluctuations allow a polymer to make energetically unfavorable steps. The steps are then “locked in place” by the next monomer added to the polymer, preventing relaxation to the previous state. If we apply the Brownian ratchet mechanism to strand exchange, we could imagine that, during four-stranded exchange, RecA covers dsDNA from the 5′ end up to the Holliday junction. The junction now moves for-
ward one step in the 3’ direction by a thermal fluctuation, and the next RecA monomer snaps in place, preventing the return step. The Holliday junction would now move in a unidirectional way.

However, during in vitro experiments, the strand exchange reaction occurs under conditions where one of the substrates is known to be completely coated with RecA prior to the initiation of the reaction, while the RecA remains bound to the end product when the reaction is completed (17, 33). This indicates that force transduction must be able to proceed without net polymerization or depolymerization.

**Treadmilling**—Treadmilling is a variant of the previous model in which there is no net polymerization or depolymerization (8). The treadmilling process occurs when monomers are removed from one end of a polymer segment and are added to the other end at the same rate. Treadmilling has been observed, for instance, in actin fibers.

The net effect would be that a RecA segment of roughly constant length travels along the DNA in the 5’ to 3’ direction. The Holliday junction could be imagined to be either at the front of the RecA-coated segment and propelled forward by it or behind the segment and dragged along with it (8). The segment could exert force on a junction by the Brownian ratchet mechanism in the same manner as before.

The fact that RecA monomers preferentially associate on one end of the nucleoprotein filament and preferentially dissociate from the other would be in support of treadmilling. The direction of motion of the junction is indeed consistent with that implied by the direction of RecA polymerization (8, 25).

However, evidence of treadmilling in RecA has been sought for, but with no success (8). In particular, electron microscopy studies on nucleoprotein filaments do not exhibit the segmented structure expected from the treadmilling scenario (34). Additionally, changes in reaction conditions which are known to greatly affect the rate of RecA monomer association/dissociation have little or no effect on the rate of strand exchange, contrary to what would be expected if treadmilling were the propulsion mechanism for strand exchange (8).

**Depolymerization/Depolymerization**—The idea here is that RecA monomers sequentially dissociate from the invading strand in front of the exchange point, and re-associate behind the junction on the displaced strand as the exchange point moves forward by one monomer (35). The incoming monomer locks the Holliday junction in the new position, and the process begins again. Force transduction could proceed by the Brownian ratchet mechanism.

This model has several advantages. As in treadmilling, the total number of RecA monomers bound to the polymer remains constant. It also is consistent with the easier nucleation of RecA onto ssDNA, and it does not require monomer exchange with the bulk. However, the model is in disagreement with experiments on three-stranded reactions, in which the displaced strand is observed to be covered with single-stranded binding protein after the reaction is completed (instead of RecA), while the dsDNA product remains coated with RecA (17, 33). The model also couples the strand exchange to depolymerization of RecA monomers from ssDNA. Although directional disassembly of RecA from ssDNA has recently been observed (36, 37), it occurs at a rate of 105–120 bases/min. The observed strand exchange rate of about 380 ± 20 bp/min (10) is more than 3 times this, and the depolymerization is therefore insufficient to explain it. Finally, the rate of strand exchange is insensitive to changes in reaction conditions which greatly affect the rates of polymerization and depolymerization (8).

**Defect Removal**—It has been proposed that ATP hydrolysis is only necessary to allow RecA monomers to dissociate and re-associate sufficiently in order to remove discontinuities in the RecA coating of the DNA (38). Strand exchange would derive the necessary energy for the reaction from the binding energy of the DNA within the nucleoprotein filament, i.e. by the lowering of the Gibbs free energy of the strands with no need for an additional energy supply.

Although possible for the three-stranded reaction, this model obviously cannot work for the case of the four-stranded reaction, where the reaction products are indistinguishable from the substrates, and where no net gain in free energy to drive the reaction is obtained from DNA binding. Having to invoke completely different force transduction mechanisms for three- and four-strand exchange reactions is not appealing.

**Facilitated Rotation**—Having ruled out force transduction by polymerization or depolymerization, we will now look for a force transduction mechanism that is more analogous to that of the motor proteins like myosin and dynein. The nucleoprotein filament is assumed static, insofar as monomer addition and removal are concerned, but the RecA monomer is able to generate force directly on DNA during ATP hydrolysis. The two DNA substrates are assumed to lie next to and parallel to each other during strand exchange. The DNA strand that is not covered by RecA binds to the exterior of the RecA helix. The RecA is assumed to act as a rotary motor that causes the two strands to be mechanically rotated around their axes, in an opposite sense, with the axes kept in place. Alternately, the two strands are rotated around each other (see Fig. 1). Strand transfer is then automatically accomplished by the rotary motion. Indeed, RecA has structural similarity with a well known linear rotary motor protein, F1 ATPase (39).

This “facilitated rotation” model (20, 25) avoids all the difficulties, mentioned above, that ruled out polymerization. Moreover, it is consistent with an elegant experiment by MacFarland et al. (40) discussed later, which shows that RecA is able to produce a rotary torque on DNA and transmit it along naked dsDNA over a considerable distance from the point where it is applied. However, the model also makes the following predictions. 1) The two substrates must lie parallel to each during strand exchange. 2) There must be DNA binding sites on the exterior of the RecA.

Little is known about the actual conformation of the Holliday junction when RecA coats one of the substrates. There is no incontrovertible experimental refutation of prediction (1), but there is much evidence which makes it seem very unlikely.

Work on Holliday junctions in which the DNA substrates are bare (41, 42) shows that, in the presence of Mg2+ (which is necessary for RecA-mediated strand exchange; Ref. 5), the Holliday junction adopts the configuration shown in Fig. 2 (43) with the small angle being about 63° (44). Although the undefined or “helical” strands in Fig. 2 cross at an angle of 63°, the conformation is called “anti-parallel” because viewed from the side, the helical strands are parallel to each other, but with their 5’ to 3’ orientation reversed. A “parallel” conformation

![Image](image-url)
with the 5' to 3' orientation the same in both helical strands can also be seen in some circumstances (45). Rotating the near pair of diagonal arms in Fig. 2 counterclockwise by 60° produces the parallel arrangement.

The anti-parallel conformation shown in Fig. 2 is not in keeping with conventional models of recombination intermediates, because it implies that homologous regions of the substrates are separated from each other by significant distances (45). It would also make explaining any regions of triplex structure (22, 27, 46) formed during a strand exchange reaction very problematic. Finally, it would require the energetically expensive translation of the two substrates, roughly along their axes, with a relative velocity little less than double that with which the strand exchange occurs. This is in addition to the required rotation of the two substrates.

This makes it seem virtually certain that the anti-parallel conformation adopted by naked DNA Holliday junctions in the presence of Mg2+ is not the conformation adopted during RecA mediated strand exchange. The parallel conformation, however, does not have the problems listed above. In addition, it has been shown that this conformation can be induced by effects at the crossover point (45), which implies that RecA may force the Holliday junction into this or a similar conformation. Since the angle which the groove on the RecA helix makes with the helix axis is about 50°, it is possible that the bare dsDNA molecule is somewhat constrained to be aligned with this groove.

It is difficult to judge how far the results from work on bare DNA can be applied to the RecA case, but so far as it applies, it implies that the substrates cross at a large angle. Certainly, work on bare DNA provides no evidence to indicate that the substrates in the recombination process are parallel in a geometrical sense or in contact with each other for an extended length.

Prediction 1 is also in conflict with some experiments. Analysis by chemical and nuclease probes indicate that there are probably no unpaired bases inside a Holliday junction (47). Although it might be argued that the RecA shields unpaired bases from nuclease probes, the only certain way to avoid this is by having the two double helices in the stacked helix conformation, either as shown in Fig. 2, or in the parallel conformation (45).

In summation, while we cannot rule out prediction 1, there is a large body of related information which makes us feel such a geometry is very unlikely. We will therefore assume that a correct model must be able to accomplish strand exchange when contact between the substrates is restricted to the point of exchange.

Regarding prediction 2, no DNA binding sites have so far been identified on the surface of RecA. This does not mean that such sites are not present, but the lack of any report of them in the literature is surprising. Additionally, electron micrographs generally do not show the substrates parallel to each other near the Holliday junction (24, 27, 38). It is reasonable to argue that electron micrographs are known to induce distortions, but it also seems reasonable to expect DNA bound to the exterior of the filament to be somewhat held in place by such binding if it exists.

Although these objections do not disqualify the facilitated rotation model from consideration, they do argue strongly against it. Nevertheless, despite these objections, the experiment mentioned above on transmission by DNA of RecA generated torques indicates that the facilitated rotation model addresses an important feature of force generation by RecA.

In summary, we will draw the following conclusions. 1) ATP drives the reaction. Limited three-stranded exchange reaction occurs spontaneously without ATP hydrolysis, but ATP hydrolysis is necessary for exchange of long strands. Four-stranded exchange strictly requires ATP hydrolysis in order to proceed. 2) ATP hydrolysis is cooperative. Hydrolysis of a RecA monomer-bound ATP triggers hydrolysis of the adjacent RecA monomer-bound ATP on the 3' side producing hydrolysis waves traveling 5' → 3' along the filament. 3) Polymerization and depolymerization play no role during homologous strand exchange. 4) It is likely that torque is generated only at the strand exchange point. We assume that this is the case. This conclusion is based on our discussion above of the likely arrangement of the Holliday junction, and the lack of any evidence for DNA binding sites on the exterior of the RecA filament.

Hydrolysis Wave Model

Introduction—We have seen that a number of natural explanations of RecA force generation contradict one or more of the experimental observations. In this section, we will develop an alternative phenomenological model for RecA force generation, the hydrolysis wave model, based on the conclusions listed at the end of the preceding section. In addition, the hydrolysis wave model will work regardless of what the precise geometry is at the Holliday junction, and will generate torque only at the strand exchange point.

As in the facilitated rotation model, we assume that the RecA polymer always remains bound to DNA during strand exchange, and that polymerization and depolymerization play no role. Our model is based on the fact, discussed previously, that the RecA/DNA filaments exist in extended and collapsed forms (3), depending on whether the cofactor used is an NTP or an NDP. It follows that the RecA monomer must be able to adopt two quite distinct equilibrium conformations. One conforma-
RecA Force Generation by Hydrolysis Waves

RESULTS AND DISCUSSION

Quantitative Analysis

Rotary Force Generation—In this section, we will treat the physical aspects of force generation by the hydrolysis wave model in more detail. Our aim is first to show that the proposed mechanism really does act as a rotary motor and to compute its force-velocity relation. We will see that the model predicts that RecA differs substantially from conventional motor proteins. The second aim is to make quantitative predictions that should allow the model to be tested experimentally. Because some mathematical analysis is required here, it is suggested that readers not interested in the mathematical details skip to the concluding paragraphs of the section.

Fig. 2 shows the geometry of a four-strand exchange reaction. A naked dsDNA strand makes a fixed angle with the RecA/dsDNA filament. If the angle is close to 60°, as in the anti-parallel arrangement shown in Fig. 2 or in the similar parallel arrangement, it will permit strand exchange without broken hydrogen bonds (47), as discussed earlier. Although the hydrolysis wave model will work with any angle of intersection, we will assume that the parallel conformation with an angle of

three strands. Both of these things would tend to expel the strand at site III from the filament interior. In both cases, the strand which occupies site III would originally have had to be dragged along into the filament by its base pair bonds to the strand which occupies site II, and the net process would need to be favorable, meaning that we require $E_{III} + E_{II} < 0$.

It is interesting to consider the effect of polyvalent salts, such as Mg$^{2+}$, on the strand exchange reaction. It is well known that polyvalent counterions facilitate DNA bundling (48). It appears reasonable to assume that polyvalent ions will stabilize triplex DNA/RecA complexes. Indeed, it is also well known that strand exchange reactions require Mg$^{2+}$ salt (38). It has also been established that Mg$^{2+}$ ions affect the angle of Holliday junctions on bare DNA, allowing for smaller angles (43). High salt concentration eases the assembly of the three-stranded structure and makes the eventual expulsion of the strand at site III more difficult.

Four-stranded Reaction—In the four-stranded reaction, the DNA initially in the filament is double-stranded and bound to sites I and II. The incoming DNA molecule intersects the filament, and the strand initially at site II is exchanged with the identical strand from the external DNA molecule. Our model can accommodate any angle of intersection between the incoming DNA molecule and the filament, but an angle of about 60° seems likely, as this will allow strand exchange with no net breaking of Watson-Crick base pairs (47) (see Fig. 2). The incoming strand now occupies site II, from which it has just displaced the former strand. The point of strand exchange, the Holliday junction, interacts with a tense segment in a similar manner as the simple exchange point in the three-stranded reaction. The point of strand exchange is again propelled forward by the hydrolysis waves, and the strand exchange proceeds unidirectionally.

Like the facilitated rotation model, the hydrolysis wave model requires neither polymerization nor depolymerization. Both are essentially rotary motors and allow for the transmission of torque. The key mechanical difference with facilitated rotation is that, in the present case, the torque is generated at the exchange point and requires no extended region of substrate-substrate contact. There is thus no need in our model for the two substrates to run parallel for any length, nor for the presence of DNA binding sites on the filament exterior. Indeed, our model of the four-stranded exchange reaction predicts that a 60° intersection of the two substrates would be mechanically very favorable.

RecA Force Generation by Hydrolysis Waves

Suppose a RecA monomer is part of an extended filament and that the cofactor has just been hydrolyzed with no depolymerization taking place. Since it is known that it is not possible to directly interconvert between the extended and collapsed forms of the filament without depolymerization (4), the RecA monomer cannot adopt its equilibrium configuration; hence, it must be under some form of elastic stress: the “tense” state.

We know that hydrolysis is cooperative, with waves traveling from 5° to 3° (13). The front of this hydrolysis wave is expected to move with a velocity, $\nu$, comparable to the depolymerization rate. Behind the wave front, there will be a region in which the RecA is in the tense configuration and either bound to an ADP cofactor or with no cofactor. It is known that RecA monomers spend only a small fraction of their hydrolytic cycle bound to ADP (1), indicating that the ADP region is likely to be rather short. The replacement of the ADP cofactor by an ATP cofactor from the bulk will allow the RecA monomer to revert to the starting configuration. Since strand exchange and torque generation depend on ATP hydrolysis (10), and assuming that contact is restricted to the strand exchange points, we conclude that the hydrolysis waves directly exert a force on the strand exchange point.

So far, we have not made assumptions that are not supported in some form by previous experiments. Our primary assumption is that the point of entry of the naked dsDNA into the nucleoprotein filament during three-stranded and four-stranded strand exchange, the exchange point, interacts attractively with the extended tense region behind the hydrolysis wave front. In other words, we assume that the free energy cost of producing an exchange point is less if the exchange point is inside the stressed region. In practice, the calculations presented below show that a repulsive interaction can have the same net effect as an attractive potential.

The result of such an attractive interaction between hydrolyzed sectors of the filament and the exchange point is that, for certain reasonable values of the physical parameters, the exchange point tries to bind to the hydrolyzed sector and is consequently dragged along by the traveling hydrolysis wave. For the case of a repulsive potential, the exchange point is pushed along in front of the hydrolysis wave. In both cases, the effect is to force the naked dsDNA into the nucleoprotein filament.

Three-stranded Reaction—The strand exchange process proceeds from here on the same as assumed by earlier workers. For the case of the three-stranded reaction, the dsDNA is brought into the filament and there is a region of the filament in which there are three strands of DNA inside the RecA helix. The newly introduced strands occupy sites II (for the strand complementary to the strand at site I) and III (for the strand identical to the strand at site I).

Recall that the binding of DNA to the different sites occurs with per monomer binding energies $E_I$, $E_{II}$, and $E_{III}$ with $E_{III} > E_{II} > E_I$, so that the binding to site III is the weakest.

When the strand at site II switches its base pairing from the strand at site III to the strand at site I, the strand at site III is expelled from the filament interior (41, 42, 47). This means that it must be energetically unfavorable for the strand to remain in site III. There are two things which might contribute to this.

First, we speculated earlier that site III may be a repulsive anti-binding site with $E_{III} > 0$. Second, DNA is negatively charged, and there will be electrostatic repulsion between all

RecA Force Generation by Hydrolysis Waves

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RecA Force Generation by Hydrolysis Waves

The DNA strands are shown in red and violet, regions of RecA bound to ATP are shown in blue, and regions of RecA bound to ADP are shown in black. The point of strand exchange is $y(t)$, the front of the hydrolysis wave is $x(t)$, and the distance between them is $s(t)$. As shown, $s(t)$ is positive.

approximately 60° is adopted. The RecA helical repeat length will be denoted by $P$. Strand exchange requires the naked dsDNA to slide along the groove of the RecA helix.

Geometrically, the strand exchange can be achieved in two different ways. 1) If the filaments are geometrically parallel and in the parallel conformation, the axes of the filament and the naked dsDNA strand can rotate around each other in space, maintaining a fixed relative angle; or 2) the direction of the strands remains fixed in space, but both strands rotate around their respective axes (like speedometer cables).

If the ends of the strands are constrained in space then process 1 is not possible. Likewise, process 1 will not work if the substrates are in the anti-parallel conformation. Process 2 requires that strands should be free to rotate to avoid build-up of torsional stress that eventually will stop strand exchange. We will focus on case 2, which is known to be the actual motion (23).

The dynamical parameters of the model are shown in Fig. 3. The front of an incoming hydrolysis wave is indicated by a time-dependent function $x(t)$. Behind $x(t)$, there are one or more tense RecA monomers that have just hydrolyzed their cofactor but have not yet exchanged their ADP cofactor with bulk ATP. We will assume that the bulk concentration of ATP is sufficiently high that this tense region is short (a few monomers).

Position $y(t)$ denotes the strand exchange point along the RecA filament. As the RecA filament rotates, the exchange point moves forward. If $\Omega$ is the rotary angular velocity of the filament and $P$ the pitch of the RecA helix, then the velocity $\dot{s}$ of the exchange point must equal $P\Omega/2\pi$. The distance between wave-front and exchange point is

$$s(t) = x(t) - y(t) \quad (\text{Eq. 1})$$

The key assumption of the model is that the free energy cost of the strand exchange point is significantly reduced if the exchange point occupies the stressed region behind the wave front. The free energy cost, $U$, of introducing an exchange point thus must depend on $s(t)$ and we will denote it by $U(s)$.

If $s(t)$ is large compared with the typical size $S$ of the tense region (of the order of a few RecA monomers), then $U(s)$ must be a constant (denoted by $U_0$) independent of $s(t)$. For distances $s$ small compared with $S$, the exchange point free energy is reduced below $U_0$ by an amount $\Delta U$. It is not possible to compute $\Delta U$ without further detailed molecular modeling. Removing a RecA monomer from the filament right at the exchange point, however, would allow the two DNA strands to adopt an optimal configuration, so we may assume that $\Delta U$ must be less than the free energy cost of removing a RecA monomer from the filament. Since ATP hydrolysis is known to produce spontaneous depolymerization, it is reasonable to conclude that $\Delta U$ must be less than the ATP hydrolysis energy (about $12k_BT$).

The force generation mechanism relies on Newton’s Third Law, according to which the exchange point and the wave front should exert equal but opposite forces on each other given by

$$F(x) = -\partial_s U(s) \quad (\text{Eq. 2})$$

and

$$F(y) = \partial_s U(s) \quad (\text{Eq. 3})$$

We now will construct the equations of motion obeyed by the exchange point and the wave front, starting with the latter case.

The wave front is propagated forward along the filament through the cooperative nature of ATP hydrolysis. Let the activation barrier against hydrolysis of a single monomer inside a RecA/ATP filament be $W$. From the known cooperativity of the hydrolysis reaction, we know that this activation barrier must be significantly lowered once the neighboring RecA monomer on the 5’ side has hydrolyzed its cofactor. Let $W'$ be the reduced activation barrier for that case and let $k' = e^{W'/k_BT}$ be the associated cooperative rate constant. The hydrolysis wave front has a “natural” velocity.

$$V_n = k'\alpha \quad (\text{Eq. 4})$$

We may identify $V_n$ with the depolymerization velocity.

The reaction rate will be altered if the wave front approaches a strand exchange point. We saw that the strand exchange free energy $U(s)$ depends on the distance between wave front and exchange point, and this will alter the free energy gain of hydrolysis.

Suppose the wave front moves forward by one RecA monomer. The change in strand exchange free energy $U(s)$ following a change of the separation $s$ by one monomer length is $\delta W = a(s\Delta U/\bar{a})$. This change in the free energy between initial and final states of the hydrolysis chemical reaction may or may not be reflected in the activation barrier.

There are two extreme cases. If the activation barrier is not affected by the change in the final state energy, then the reaction rate is just $k'$. If the change in final state free energy applies as well to the kinetic barrier, then we must replace $W'$ in $k' = e^{(W'/k_BT)}$ by $W' + \delta W$. In general, the velocity of the wave front can be expressed as

$$\dot{s} = V_n e^{(s/\bar{a})} \quad (\text{Eq. 5})$$

The length $a_0$ varies between 0 and $a$, the length of one RecA monomer, depending on the extent that this change in reaction free energy on altering $s$ is reflected in a change of the activation barrier $W'$. Next, we must construct the equation of motion for the exchange point. We saw already that the exchange point is subjected to a force $F_\perp(s)$ coming from the wave front. The exchange point will move, and the RecA filament will rotate around its axis, under this force. The resulting velocity of the exchange point is controlled by the rate of free energy dissipation. There are two contributions to dissipative losses: chemical dissipation due to base pair unstacking and breakage and reconstruction of pairs of hydrogen bonds right at the exchange point, and distributed hydrodynamic viscous losses due to rotary motion of the two strands.

It follows from the general principles of non-equilibrium thermodynamics that, at sufficiently low velocities of the exchange point, the free energy dissipation rate must be proportional to the square of the velocity $v$ of the exchange point and that the resulting drag force $F(v)$ must be proportional to $v$. We
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where we defined here an “effective” potential energy of interaction.

\[ U_{\text{eff}}(s) = \frac{1}{\mu} \left[ U(s) - s F_{\text{Ext}} \right] - \int V e^{-\frac{m}{kT} s} ds \]  

(Eq. 10)

Note that \( U_{\text{eff}}(s) \) does not have the dimension of energy. The motion of an exchange point under the impact of a hydrolysis wave front is now fully determined by the two preceding equations.

Assume that a hydrolysis wave approaches a strand exchange point from the 5’ side. Mathematical analysis shows that two scenarios are possible: either the exchange point is “captured” by the hydrolysis wave front, and the two points move with a common velocity of the order of that of a hydrolysis wave front; or the hydrolysis wave front overtakes the exchange point, giving it a “kick” in the process, and continues on. In the first case, the motion of the exchange point will be smooth. In the second case, the exchange point will move in a “jerky” manner under the repeated impact of hydrolysis waves moving from the 5’ to the 3’ end.

**Trapping**—The first case requires that the effective potential energy of interaction \( U_{\text{eff}}(s) \) has a minimum, denoted by \( s^* \). The reason is that for the wave front to capture the exchange point, we must demand that \( \alpha_s = 0 \), and since \( \alpha_s = -\alpha \cdot U_{\text{eff}}(s) \), the first derivative of the potential must vanish. This is the condition that the effective potential has an extremum. Only a minimum of the effective free energy would correspond to a physically stable configuration. A necessary (although not sufficient) condition for the existence of a minimum is thus that the equation \( \alpha U_{\text{eff}}(s) = 0 \) has a solution. We can write this condition as the requirement that

\[ \tilde{F}_{\text{Ext}} = \tilde{F}(s^*) - \tilde{V} e^{-\tilde{F}_{\text{rec}}} \]  

(Eq. 11)

must have a solution.

Physically this is just Newton’s First Law: the external retarding force on the strand exchange point must equal the driving force exerted by the wave front on the exchange point minus the drag force on the exchange point (which is also the dimensionless velocity of the exchange point).

We have introduced here three dimensionless quantities. The dimensionless external force is given by \( \tilde{F}_{\text{Ext}} = \alpha_0 F_{\text{Ext}}/k_B T \), the dimensionless hydrolysis velocity is defined by \( \tilde{V} = \alpha_0 k_B T \), and the dimensionless force exerted by the wave front on the exchange point is given by \( \tilde{F}(s^*) = a_0(dU/ds)|_{s=0} k_B T \). For conventional motor protein systems, these dimensionless parameters are usually of the order of 1 (32).

For a potential profile consisting of a simple well with a depth \( \Delta U \) and a width \( S \), there can be two solutions, one physical, and no solution for equation (11). In the first case, the first solution (i.e. with the smallest value of \( s^* \)) is a minimum of the effective potential while the second solution is a maximum. Assuming that a solution exists, we can use Equation 11 to compute the relation between the dimensionless velocity of the exchange point

\[ \tilde{V}_{\text{exch}} = \tilde{V} e^{-\tilde{F}_{\text{rec}}} \]  

(Eq. 12)

and the external force, producing a force-velocity curve

\[ \tilde{F}_{\text{Ext}}(\tilde{V}_{\text{exch}}) = \ln \left( \frac{\tilde{V}}{\tilde{V}_{\text{exch}}} \right) - \tilde{V}_{\text{exch}} \]  

(Eq. 13)

In Fig. 4, we have plotted the dimensionless force-velocity curve for a dimensionless hydrolysis velocity of 4.

**Maximum Retarding Force and Disruption**—It would seem that we could apply larger and larger external forces with the...
The maximum hydrolysis velocity of the trapped state is equal to

$$V_{\text{max}}(\text{max}) = F_{\text{hyd}}(\text{max}) e^{a_S/a_{\mu}}$$

(Eq. 17)

If the maximum hydrolysis force exerted on the exchange point is in the piconewton range (as we estimated earlier), then the right-hand side is on the order of 1. Unfortunately, because the mobility is hard to estimate, we can not tell whether or not the hydrolysis wave front velocity of RecA filaments exceed the maximum velocity for trapping. For our earlier estimate of the mobility ($\sim 10^6$), the maximum velocity is in the range of 10 Å/s. Note, however, that if the force level is significantly increased beyond the piconewton range, then the threshold velocity would be much higher. Merely doubling the force level gives 14 times the maximum velocity. Measured values are about 6–7 bps, or about 100 Å/s (10).

**Maximum Exchange Point Velocity**—If the retarding force is set to zero, then the exchange point assumes its maximum velocity. This velocity can be computed by setting $F_{\text{Ext}} = (a_S/k_B T)$ equal to 0 in Equation 13

$$\ln \left( \frac{V}{V_{\text{exch}}(\text{max})} \right) = \frac{a_S}{k_B T}$$

(Eq. 18)

Solving for the exchange point velocity, we find the following result

$$V_{\text{exch}}(\text{max}) = \begin{cases} V^2 & \text{if } V < 1 \\ \ln(V) & \text{if } V > 1 \end{cases}$$

(Eq. 19)

At low hydrolysis rates, the maximum velocity is thus equal to that of the hydrolysis wave front. At higher hydrolysis rates, the exchange point moves slower than the natural velocity of the wave front. In fact, the maximum velocity of the exchange point in dimensionless units cannot be large compared with 1, since $\ln(V)$ is of the order of 1 even if $V >> 1$. In standard units, we can conclude that $V_{\text{exch}} \leq k_B T a_{\mu}$. This is interesting because it means that the maximum velocity of the exchange point is directly determined by the mobility $\mu$ of the exchange point. Note also that the force $F_{\text{Ext}}$ exerted on the exchange point is of the order of $k_B T a_{\mu}$ in this case since $V_{\text{exch}} \leq k_B T a_{\mu}$. Interestingly this is just the characteristic force of a Brownian ratchet (30), even though the hydrolysis wave model is not a Brownian ratchet.

**Stochastic Motion**—If the external force exceeds $F_{\text{Ext}}(\text{max})$, then the exchange point can no longer be trapped. If there is no wave front in the neighborhood, then the exchange point drifts with a velocity $F_{\text{Ext}} a_{\mu}$ along the negative $x$ direction. When a wave front does pass by, it tries to drag the exchange point along in the positive $x$ direction, but eventually the exchange point is left behind. The cycle is repeated as the next wave front passes by from the 5’ to the 3’ direction. The net result is a stochastic “back-and-forth” motion.

We will consider the case that the external force is just a bit larger than the maximum force, $F_{\text{Ext}}(\text{max})$, and assume that wave fronts pass at a certain rate $\Gamma$ along the filament. As a wave front approaches an exchange point, the exchange point will start to move. The center of mass motion will be fastest at the point where the force exerted by the wave front on the exchange point is at a maximum but the relative motion is slowest at that point. We expand the effective potential at the point of maximum force $s_{\text{max}}$ as

$$F_{\text{ext}}(s) = F(\text{max}) - F_{\text{ext}} - C \left( \frac{\Delta F}{S} \right) (s - s_{\text{max}})^2 + \ldots$$

(Eq. 20)

(using conventional units). The dimensionless constant $C$ depends on the specific shape of the potential. During the collision, the equation of motion can be approximated as

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**Fig. 4.** Force-velocity curve for forces less than the trapping force beyond which trapping no longer occurs. Trapping only occurs to the right of the dotted line, and not in the shaded area to the left of the dotted line. $V_{\text{max}}$ is the velocity of the Halliday junction in the absence of any retarding force.

The exchange point merely reducing its velocity. That is of course not possible. The maximum value of the dimensionless force applied by the wave front is of the order of

$$F_{\text{ext}}(\text{max}) = \frac{a_S \Delta U}{k_B T S}$$

(Eq. 14)

where $S$ is the characteristic size of the potential trap (of the order of a few monomer lengths), and $\Delta U$ is the depth of the trap. If we use the earlier estimates, then this dimensionless maximum force is of the order of 1–10. It would seem reasonable that the maximum external retarding force beyond which trapping becomes impossible should equal $F_{\text{hyd}}(\text{max})$. According to Equation 11, however, the actual maximum retarding force is less than $F_{\text{hyd}}(\text{max})$ because of the drag force

$$F_{\text{ext}}(\text{max}) = F_{\text{hyd}}(\text{max}) - V_{\text{exch}}(\text{max})$$

(Eq. 15)

Mathematically, this is precisely the point where the two solutions of Equation 11 fuse and then disappear. Note that increasing the hydrolysis rate reduces the threshold force required to break the connection between the wave-front and the strand exchange point. The lower this threshold force, the easier force transduction by hydrolysis is disrupted by external forces. The velocity of the exchange point right at the point of disruption is

$$V_{\text{exch}}(\text{fracture}) = V_{\text{exch}}(\text{max})$$

(Eq. 16)
The exchange point that is dragged along the positive direction a distance $T(F_{\text{ext}})$ along the negative $x$ direction. The average velocity is

$$V(F_{\text{ext}}) = \frac{T(F_{\text{ext}})V_{\text{exch}}(\text{fracture}) - \mu F_{\text{ext}}}{T(F_{\text{ext}}) + \left(\frac{1}{T}\right)}$$

(Eq. 23)

and using the result for the trapping time gives a force-velocity curve,

$$V(F_{\text{ext}}) = \frac{\mu F_{\text{ext}} - F_{\text{ext}}(\text{max})}{S} \left(\frac{1}{T} + \frac{C\mu F_{\text{ext}} - F_{\text{ext}}(\text{max})}{S} \right)^{-1}$$

(Eq. 24)

The complete force-velocity curve has a sharp cusp at the fracture point. For large enough external force, the average velocity reverses sign. The critical external force where the velocity is equal to zero is known as the “stall-force.” We can now draw the full force-velocity curve (Fig. 5).

The exchange point velocity drops very rapidly when the external force exceeds the fracture force. For lower rates $T$ of hydrolysis wave production, we can identify the stall force and the fracture force, as follows from Equation 24. As the rate increases, the stall force will start to exceed the fracture force.

The well defined “kink” in the force-velocity curve is a feature that is absent from conventional motor protein models and should act as a clear indicator for the validity of the description proposed in this paper.

We expect that the exchange reaction will take place with a very constant and predictable velocity determined principally by the depolymerization velocity $v_{\text{depol}}$, but slowed in a predictable and calculable manner by the drag introduced as a result of having to push the exchange point forward, with the added necessity of rotating and translating the two substrates in order to do so.

Unwinding of an Unpaired Distal Segment of One Substrate—MacFarland et al. (40) have reported an experiment in which circular dsDNA with a single-stranded gap was coated with RecA and underwent a four-stranded exchange reaction with a linear dsDNA molecule not coated with RecA. They conducted several experiments in which the linear DNA substrate did not extend to the end of the double-stranded region of the circular DNA, and reported that separate hybrid DNA molecules were formed.

They interpreted this as indicating that the distal region of the circular dsDNA was unwound by the reaction as far as the single-stranded gap, allowing the products to separate. The presence of a nick in the gapped strand of the circular DNA substrate terminated the unwinding at that point, and the two products separated. The products in either case were a hybrid circular dsDNA molecule with a single-stranded gap and a linear duplex hybrid DNA molecule with a single-stranded tail at the distal end.

Although MacFarland et al. interpreted these results as support for the facilitated rotation model and a refutation of models requiring monomer rearrangement, it is clear that these results are what would be expected from the hydrolysis wave model as well. The process of expelling the displaced strand would continue, in the presence of RecA hydrolysis, even after there was no corresponding strand to replace it because the point at which a DNA strand leaves the nucleoprotein filament would be bound to the ADP region in the same manner as the point at which a DNA strand enters the filament. This continued, forced expulsion of the DNA would naturally unwind the remaining DNA.

A slight variation on this experiment, however, would distinguish between the hydrolysis wave model and the facilitated rotation model. This unwinding should occur for the facilitated rotation model, regardless of whether the extra distal extension is on the RecA-coated molecule or on the bare DNA molecule. In the hydrolysis wave model, however, the distal extension will only be unwound if it is on the RecA-coated molecule. If it is on the other molecule, it is not within a nucleoprotein filament, and since unwinding is accomplished by expulsion from the filament, it cannot then occur.

Consider then an experiment similar to that of MacFarland, where now the linear dsDNA molecule has the single-stranded primer at the proximal end, and the circular DNA substrate merely has a nick in one strand at the point where the unpaired
distal end extension meets the point at which the strand exchange begins. In the facilitated rotation model, the linear molecule should continue to be counter rotated around the RecA-coated molecule after the homologous pairing is completed, and the distal segment should be unwound. If the hydrolysis wave model is correct, however, there is no expulsion of the displaced strand since there is no RecA coating the unpaired distal segment, and so the products should not separate and will remain as a joint molecule.

CONCLUSION

Summary—We propose that the mechano-chemistry of the RecA force transduction mechanism is unique: it relies neither on polymerization/denpolymerization (as observed for actin and microtubule strands) nor on direct force transduction by the motion of flexible sections of the protein as for conventional motor proteins (like kinesin, dynein, and myosin). Instead, force is generated by localized, solitary hydrolysis waves propagating along the RecA helix, which impact the DNA exchange site. The proposed model is consistent with the experimental information on RecA force transduction available to date, including both the most likely structure of the exchange site as well as the fact that experimental conditions that alter polymerization behavior do not affect the rate of strand exchange.

Experimental Tests—The central features of the proposed model are open to experimental testing. The computed force-velocity curve shows a sharp kink at the point where the retarding force is strong enough to cause the strand exchange point to break away from the hydrolysis wave. Force-velocity curves of RNA polymerase, myosin, and other proteins have been determined experimentally by attaching the protein to a bead that could be trapped by a laser. The analog of these experiments would be to apply a rotary torque on the DNA strand that could either advance or retard the motion of the exchange site. Rotary torques have been applied to DNA by the magnetic bead method and could be employed for a study of strand exchange force transduction (52).

A second test would be an extension of the experiment of MacFarland et al. (40). The substrates would be a linear dsDNA molecule with a single-stranded primer at the proximal end, and a circular DNA substrate with a nick in one strand. This should result in the linear DNA molecule being the RecA-coated substrate, unlike the MacFarland experiment in which it was the circular DNA molecule which was coated with RecA. The circular DNA molecule should have a distal end which extends further than the end of the linear DNA substrate, and the nick should be between this unpaired distal end extension and the point at which the strand exchange begins.

The facilitated rotation and hydrolysis wave models make different predictions for what happens next. The facilitated rotation model predicts that the substrates should continue to be counter-rotated after the homologous pairing is completed, so the distal segment should be unwound to produce two separate molecules. The hydrolysis wave model predicts that the products should not separate but remain as a joint molecule.

Open Questions—We have examined in this paper only the mechanism of mechano-chemical force transduction for homologous DNA. We have not addressed one of the central functions of the force transduction mechanism: heterology bypass. Three-strand exchange proceeds spontaneously (in the absence of heterologues) since the exchange process lowers the free energy. Four-strand exchange does not lead to any net lowering of the free energy and requires ATP hydrolysis. DNA strand exchange between two organisms is only biologically relevant if there are differences in the base pair sequences of the two strands.

The length of heterology needed to interfere with successful strand exchange has been studied (53–56). There is some contradiction within the literature, but heterology bypass has been reported to have a dependence on both the length and the location of the heterology. It would be of great interest to develop a theoretical model which both describes how the exchange process solves topological problems encountered during heterologous exchange and is consistent with the observed effects which length and location of the heterology have on strand exchange.

It is also interesting to speculate how the proposed mechanism for strand exchange could have evolved. Force transduction is not crucial for three-strand exchange (14, 24). If the original function of RecA was to repair DNA through a three-strand exchange process, then the main function of ATP hydrolysis was to provide a means of removing the RecA helix from the repaired strand (9). Hydrolysis waves with no denpolymerization would assist the exchange process. When the RecA helix was recruited to promote four-strand exchange, the secondary function of force transduction took center stage and provided a means of overcoming the inevitable heterologous inserts.

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REFERENCES

1. Roca, A. I., and Cox, M. M. (1997) Prog. Nucleic Acids Res. Mol. Biol. 56, 129–223
2. Lodish, H., Baltimore, C., Berk, A., Zipursky, S. L., Matsudaira, P., and Darnell, J. (1995) Molecular Cell Biology, 3rd Ed., W. H. Freeman and Co., New York
3. Heuser, J., and Griffith, J. (1989) J. Mol. Biol. 210, 473–484
4. Yu, X., and Egelman, E. H. (1992) J. Mol. Biol. 227, 354–364
5. Takahashi, M., and Norden, B. (1994) Adv. Biophys. 30, 1–35
6. Pugh, B. F., and Cox, M. M. (1987) J. Biol. Chem. 262, 1326–1336
7. Kowalczykowski, S. C., Chow, J., and Krupp, R. A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3127–3131
8. Lindsey, J. E., and Cox, M. M. (1990) J. Biol. Chem. 265, 9043–9054
9. Lindsey, J. E., and Cox, M. M. (1989) J. Mol. Biol. 205, 685–711
10. Bedide, W. A., and Cox, M. M. (1996) J. Biol. Chem. 271, 5725–5732
11. Weinstock, G. M., McIntee, K., and Lehman, I. R. (1981) J. Biol. Chem. 256, 8845–8849
12. Schutte, B. C., and Cox, M. M. (1987) Biochemistry 26, 5616–5625
13. Menetski, J. P., Bear, D. G., and Kowalczykowski, S. C. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3127–3131
14. Kowalczykowski, S. C., and Krupp, R. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3478–3482
15. Lee, J. W., and Cox, M. M. (1990) Biochemistry 29, 7666–7676
16. Rehrauer, W. M., and Kowalczykowski, S. C. (1993) J. Biol. Chem. 268, 1299–1297
17. Ullesperger, C. J., and Cox, M. M. (1995) Biochemistry 34, 10859–10866
18. Pugh, B. F., and Cox, M. M. (1987) J. Biol. Chem. 262, 1337–1348
19. Brenner, S. L., Mitchell, R. S., Morral, S. W., Neuendorf S. K., Schutte, B. C., Bear, D. G., Cox, M. M. (1988) J. Biol. Chem. 263, 21644–21649
20. Cocks, M. M. (1994) Trends Biochem. Sci. 19, 217–221
21. Takahashi, M., Kubista, M., and Norden, B. (1991) Biochimie 73, 219–226
22. Jain, S. K., Cox, M. M., and Inman, R. B. (1995) J. Biol. Chem. 270, 4943–4949
23. Smith, S., Cui, Y., and Bustamante, C. (1996) Science 271, 795–799
24. Schnitzer, M., and Block, S. (1997) Nature 388, 386–390
25. Peskin, C., Odell, G., and Oster, G. (1993) Biophys. J. 65, 316–324
26. Ylin, H., Wang, M., Svodova, K., Landick, R., Block, S., and Gelis, J. (1995) Science 270, 1653–1656
27. Julicher, F., Ajdari, A., and Prost, J. (1997) Rev. Mod. Phys. 69, 1289–1281
28. Lavery, P. E., and Kowalczykowski, S. C. (1992) J. Biol. Chem. 267, 9315–9320
29. Flory, J., and Radding, C. M. (1982) J. Biol. Chem. 257, 591–540
30. Aisen, T., Tsodikov, O., and Cox, M. M. (1999) J. Mol. Biol. 288, 391–401
31. Menetski, J. P., Bear, D. G., and Kowalczykowski, S. C. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 21–25
32. Yu, X., and Egelman, E. H. (1992) J. Mol. Biol. 228, 401–4016
33. MacFarland, K., Shan, Q., Inman, R. B., and Cox, M. M. (1997) J. Biol. Chem. 272, 17675–17685
34. Lilley, D., and Clegg, R. (1995) Q. Rev. Biophys. 28, 131–175
