Motoring along with the bacterial RecA protein

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Abstract | The recombinases of the RecA family are often viewed only as DNA-pairing proteins — they bind to one DNA segment, align it with homologous sequences in another DNA segment, promote an exchange of DNA strands and then dissociate. To a first approximation, this description seems to fit the eukaryotic (Rad51 and Dmc1) and archaeal (RadA) RecA homologues. However, the bacterial RecA protein does much more, coupling ATP hydrolysis with DNA-strand exchange in a manner that greatly expands its repertoire of activities. This article explores the protein activities and experimental results that have identified RecA as a motor protein.

Recombinational DNA repair
A DNA-repair process that involves the strand-displacement and strand-exchange reactions of homologous recombination mechanisms.

Conjugation
A process by which plasmid or chromosomal DNA is actively transferred from one bacterium to another. Recombination that follows the DNA transfer provides the basis for genetic exchanges.

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The RecA-family recombinases promote the exchange of genetic information between two homologous DNA molecules. Their functional form in recombination reactions is a right-handed helical filament bound to DNA. This filament is usually formed on single-stranded (ss)DNA as the first reaction step. The bound ssDNA is then aligned with a homologous double-stranded (ds)DNA, and a strand-exchange reaction ensues in which the complementary strand of the DNA duplex is transferred to the originally bound ssDNA. These enzymes are also DNA-dependent ATPases, and ATP is hydrolysed during strand-exchange reactions.

RecA and its homologues therefore have a unique functional structure to complement their unique role in DNA metabolism. They do not simply bind DNA; they encompass it, burying the DNA in a helical nucleoprotein filament that can extend over many thousands of base pairs (bp). A deep helical groove provides access to the interior of the filament. Bound within the filament, DNA is stretched and underwound, thereby preparing each strand for the task of exchanging its pairing partner. The filament provides binding sites for three DNA strands. For DNA-strand exchange that involves a single strand and a homologous duplex, two of these strands are identical (or nearly so, as some heterology is tolerated; see below) and the other is a potential complement of either. Therefore, no more than three strands can participate in the partner exchange orchestrated in the interior of the filament. What is known about RecA and its filament structure is briefly described in BOX 1.

DNA-strand exchanges are important for long-term survival, effecting the recombinational DNA repair of replication forks and facilitating recombination in other contexts. The recombinational DNA repair of replication forks is the ultimate reason RecA homologues are present in the genomes and proteomes of almost every organism. When replication forks stall or collapse at DNA damage, or even if DNA damage is bypassed, recombination is required to promote repair and to allow replication to restart. Recombination has other important functions, including conjugation in bacteria and meiotic recombination in eukaryotes. However, the repair of replication forks is almost certainly why recombination systems evolved and why they are now a ubiquitous feature of living systems[1–4].

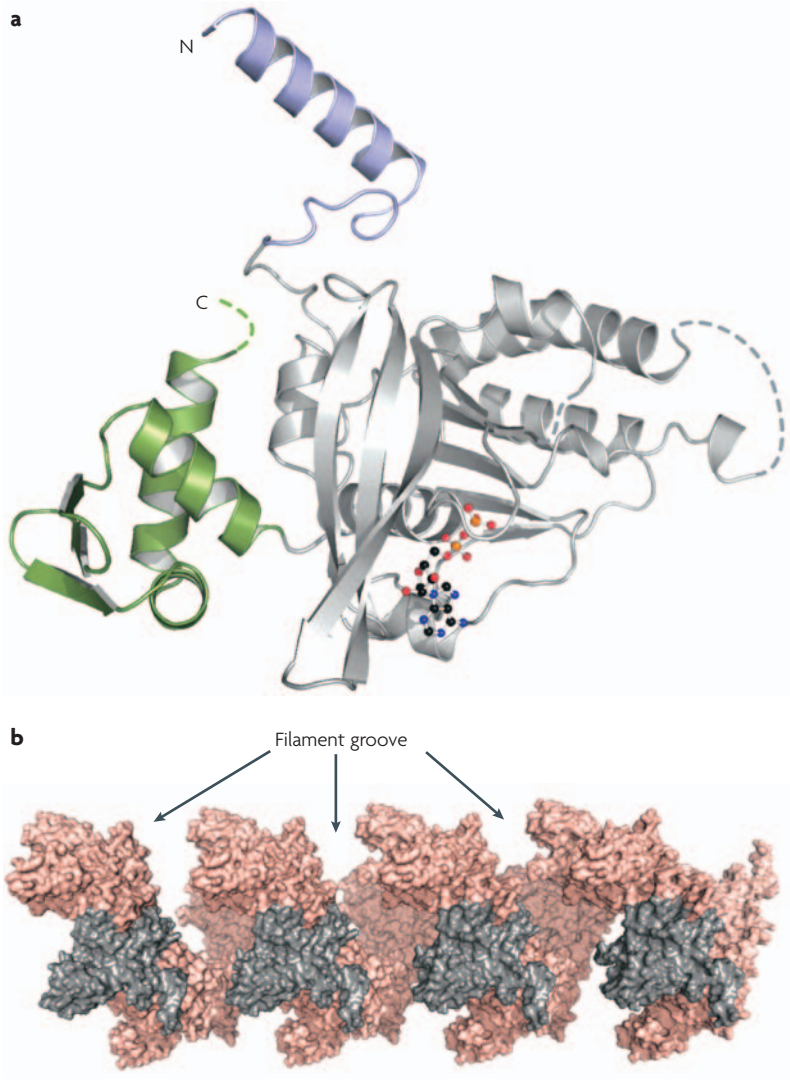
Recombination in inappropriate contexts is potentially deleterious. For example, recombination between two repeated genomic sequences has the potential to delete all the genomic DNA between the repeats. So, RecA and its homologues are regulated at multiple levels. As outlined in BOX 2, the RecA protein of *Escherichia coli* is subject to regulation of its gene expression, autoregulation of its activity by its C terminus and regulation by other proteins in the cell.

RecA-family recombinase functions

The nucleoprotein filaments made by RecA-family recombinases are scaffolds on which recombinase functions are executed. DNA-pairing and strand-exchange reactions are their forte and are the only documented activities for the non-bacterial RecA homologues. The *E. coli* RecA filament forms most readily on ssDNA and promotes the reactions that are typical for this class of protein. If the filament forms on linear ssDNA, DNA-strand invasion is promoted in which a 3′ end of the ssDNA invades a homologous duplex. This reaction

Box 1 | **The structure of RecA and its filaments**

Escherichia coli RecA is a 352-residue polypeptide chain (molecular mass of 37,842 D). The structure of RecA was elucidated by Story and Steitz in 1992 (REFS 76,77), and multiple structures of RecA and its homologues have since been determined[78–88]. Unfortunately, none of these structures include bound DNA, and much remains to be elucidated about the DNA-binding sites on this protein. The structure of a single filament subunit (part **a**) has a large core domain (shown in grey), and two smaller domains at the N and C termini (shown in purple and green, respectively). The core domain is the prototype of a motif known as the RecA fold, which is found to be common to a range of other proteins including motor proteins such as the F1 ATPase, multiple helicases, DNA-transport proteins and certain other transporters[89–95]. Bound ADP is shown in a stick-and-ball structure. In the active RecA filament, ATP is bound at the subunit–subunit interface[96,97]. A depiction of the RecA filament, based on the Story and Steitz structure, is shown in part **b**. There are six RecA subunits per helical turn of the filament, which corresponds to the extended (18 base pairs per turn) conformation of the DNA that is bound within the filament. The helical groove that provides access to the filament interior is indicated. Four individual subunits are shown in grey.



Collapsed replication fork
A replication fork that has encountered a DNA lesion, often a break in a template strand, that results in the disassembly of replication enzymes.

creates a D-loop structure — an intermediate in the repair of collapsed replication forks and double-strand breaks[1,5] (FIG. 1a). Once DNA-strand invasion occurs, DNA-strand exchange can be extended over thousands of bp if the structures of the participating DNA molecules permit[6,7] (FIG. 1b). These basic reactions occur at varying levels of efficiency even when ATP hydrolysis is prevented by the addition of unhydrolysable ATP analogues or the use of recombinase mutants that can bind, but not hydrolyse, ATP.

The role of bacterial RecA proteins does not stop there, as they promote various reactions that can be considered special functions. Once DNA-strand exchange is initiated in a DNA gap, the bacterial recombinases extend the reaction into regions where the exchange involves four DNA strands[8] (FIG. 1c). When bound at a stalled replication fork, the fork branch can be propelled in either direction, depending on the disposition of the RecA filament, resulting in fork regression in one case (FIG. 1d) and DNA unwinding in another (see below). Bacterial RecA proteins also promote DNA-strand exchange through structural barriers such as heterologous insertions in one of the DNA molecules undergoing exchange. All of these reactions require ATP hydrolysis and are described in more detail below.

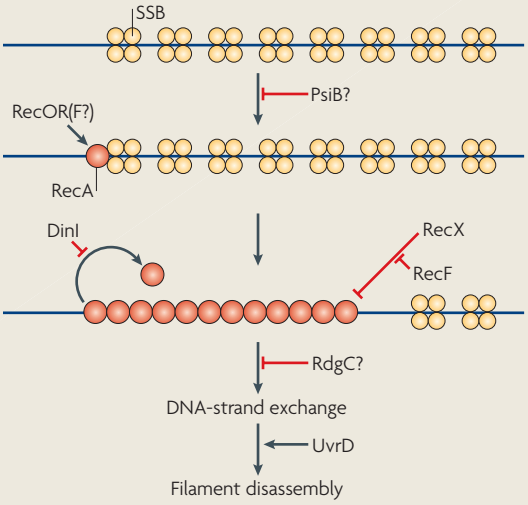
Not all RecA reactions result in the alteration of bound DNA. Many proteins bind to RecA, generally at the ends or in the helical groove of the filament. Several proteins undergo autocatalytic cleavage, leading to activation or inactivation, when bound within the RecA-filament groove (FIG. 1e). In this mode, the *E. coli* RecA is said to function as a coprotease, with a crucial but indirect role in the cleavage[9]. Last, *E. coli* RecA activates translesion DNA polymerase V[10,11] (FIG. 1f) and functions *in trans* in this reaction[12]. The *E. coli* RecA has an important but less well understood role in DNA-crosslink repair[13,14]. Further functions have been found for the RecA proteins in other bacteria, including a role in pilin antigenic variation in *Neisseria gonorrhoeae*[15].

The full spectrum of RecA functions in bacteria has not been reviewed here. The structural features of RecA and RecA-mediated DNA pairing have recently been reviewed in some detail[16,17], as have the functions of the related Rad51 protein[18]. This article focuses on special functions of *E. coli* RecA — that is, DNA transactions that are not promoted by the archaeal (RadA) and eukaryotic (Rad51 and Dmc1) members of this family.

RecA filament

The special DNA functions of RecA cannot be properly described without relating a few basics that are covered in much more detail in recent reviews[16,17]. *Escherichia coli* RecA-filament assembly occurs in a 5′→3′ direction (FIG. 2). Once a slow nucleation step is overcome (often with the aid of regulatory proteins, as described in BOX 2), filament growth occurs primarily on the 3′-proximal end. RecA binding here is a simple reversible association of ATP-liganded RecA protomers. The reported rates of 5′→3′ filament growth range from 2 to 20 subunits per second[19–22]. Dissociation occurs primarily on the 5′-proximal end. The release of RecA subunits at this end is not part of a simple reversible binding process, as dissociation is coupled to ATP hydrolysis (which is not entirely accounted for in recent studies that used single-molecule approaches to measure dissociation

Box 2 | **Regulating the RecA recombinase**



Recombinases are regulated at multiple levels to ensure that RecA filaments are assembled only where recombinational DNA repair is required and to eliminate the filaments when repair is completed. The first level is provided by the regulation of *recA* gene expression in the SOS regulon[98]. A second level is provided by autoregulation by the C terminus of RecA. Truncation of the RecA polypeptide by removing 17 C-terminal residues enhances almost every aspect of RecA function[99–105].

A third level of regulation is provided by other regulatory proteins (see figure). The list of such proteins is growing. RecA-filament assembly (red circles) is facilitated by the RecF, RecO and RecR (RecFOR) proteins[67–70]. The RecOR proteins, in particular, mediate the nucleation of RecA filaments on single-stranded (ss)DNA that is coated with ssDNA-binding protein (SSB). The RecX protein binds along the RecA filament and specifically to the growing filament end, where it limits filament growth[71]. RecF antagonizes the activity of RecX[67]. Another protein that is induced early in the SOS response, DinI, can stabilize RecA filaments while subtly altering their coprotease functions[72]. When RecA function is no longer needed, RecA filaments can be removed from DNA by certain helicases, such as UvrD[73,74]. The RdgC protein competes well with RecA for DNA-binding sites, particularly on double-stranded DNA. RdgC will block DNA-strand exchange if it is bound to the duplex DNA that is homologous to the ssDNA in a RecA nucleoprotein filament[75].

The PsiB protein is encoded by conjugational F plasmids and blocks RecA function in recipient cells during conjugation.

It is likely that additional proteins involved in RecA regulation remain to be discovered.

constants and/or filament growth at the disassembly end in the presence of ATP[21,22]). Dissociation from ssDNA occurs at a rate of ~70 RecA monomers per min[23], rising to 120 monomers per min when the filament is bound to dsDNA[19,24]. The subunit–subunit interface should be identical on both filament ends. When ATP analogues that cannot be hydrolysed are substituted for ATP, the filaments can in principle grow in both directions (albeit not necessarily at the same rate), as has been observed[22].

The DNA inside a RecA filament is in an unusual conformation[17,25]. It is extended by 50–60% and underwound to ~18 bp per turn. The resulting strain might facilitate both the exchange of DNA strands and the homology search that precedes it[17,26]. Each RecA subunit in the filament covers three nucleotides or DNA bp. Physical studies in which multiple approaches were used indicated the presence of binding sites for only three DNA strands in the interior of the RecA filament (reviewed in REFS 17,27,28). Simultaneous binding of two duplex DNAs was documented in one study, but the location of the binding sites was not determined[29]. Binding sites for duplex DNAs might be present on the exterior of the RecA filament (in addition to the sites buried deep in the filament groove), as discussed below.

Once formed, filaments that hydrolyse ATP are likely to be dynamic. When bound to a DNA circle, they might contain breaks at which dissociation at a disassembly end is rapidly compensated for by association to the trailing assembly end. Again, the filaments on ssDNA and dsDNA differ, with the ATP-hydrolysis turnover rate per individual subunit being ~30 ATP molecules per min on ssDNA and 20 per min on dsDNA.

The properties of RecA filaments on different DNA substrates are diagnostic of different filament states[17,30]. As RecA binds to ssDNA in the presence of ATP, it undergoes a transition between states, designated O (ordinary) and A (activated), respectively. The A state on ssDNA is converted to a so-called P (pairing) state on dsDNA. The capacity of RecA to pair two DNA molecules and to promote DNA-strand exchange improves from the O to the A to the P state, and many filament properties change.

Some RecA reactions depend on ATP hydrolysis

RecA and all of its homologues are efficient at DNA pairing, as reflected by the D-loop-formation and strand-exchange reactions (FIG. 1a,b). As already noted, these processes do not require ATP hydrolysis (although ATP hydrolysis greatly improves the efficiency of long-strand-exchange reactions when RecA is involved[31]). What distinguishes the bacterial RecA protein from its archaeal and eukaryotic homologues are certain special functions, such as those shown in FIG. 1c–f. Our focus here is the role of RecA-mediated ATP hydrolysis, particularly the coupling of ATP hydrolysis throughout the filament to a number of apparently RecA-exclusive reactions on DNA (see FIG. 1c,d and others described below). Some of the ATP that is hydrolysed by RecA is simply coupled to filament disassembly at one filament end. However, ATP hydrolysis occurs uniformly throughout a RecA filament[32], and disassembly usually accounts for only a vanishingly small fraction of it. Is the remaining ATP hydrolysis incidental, or does it contribute to the cellular role of RecA? The reactions described below, which are all completely dependent on RecA-mediated

Fork regression
A reaction in which a replication fork is reversed so as to re-pair the template strands and displace the newly synthesized strands.

Translesion DNA polymerase
A class of DNA polymerases that exhibit a reduced fidelity in DNA synthesis that allows them to replicate through a DNA lesion in the template strand. Translesion DNA synthesis often results in a higher than normal level of introduced mutations.

SOS regulon
A set of coordinately regulated genes that are all repressed by the LexA protein and that encode an organized cellular response to heavy DNA damage.

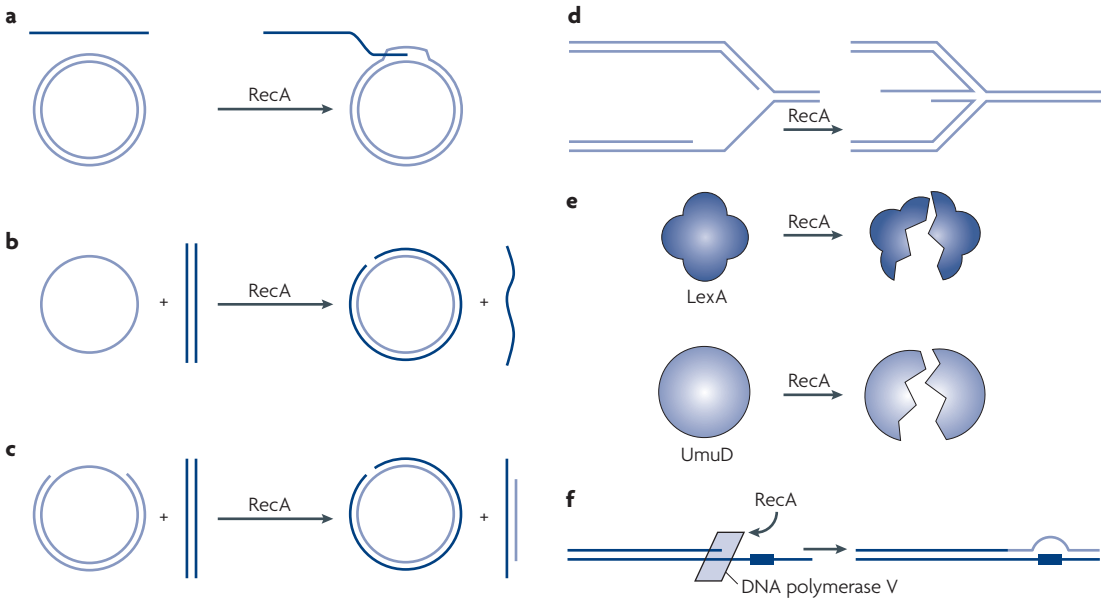


Figure 1 | **Activities of *Escherichia coli* RecA.** RecA is involved in a surprising range of reactions in an *Escherichia coli* cell. Some of these involve DNA transactions, and some do not. **a** | A strand-invasion reaction causing D-loop formation. **b** | A three-strand-exchange reaction. **c** | A four-strand-exchange reaction. **d** | The replication-fork-regression reaction. **e** | The RecA coprotease function. RecA facilitates the autocatalytic cleavage of several proteins, including LexA and UmuD. **f** | RecA stimulation of the translesion-DNA-replication activity of DNA polymerase V. The dark-blue rectangle represents a DNA lesion, and the light-blue line represents newly synthesized DNA.

ATP hydrolysis, reveal a RecA filament that promotes a range of unusual DNA transactions with implications for RecA function at replication forks and elsewhere. They illustrate how chemical energy might work in this system, generating patterns that allow the prediction of how RecA will affect a DNA structure given a particular binding orientation of a RecA filament on the DNA. Last, they identify RecA as a motor protein.

Unidirectional DNA-strand exchange. In a three-strand-exchange reaction, RecA promotes DNA-strand exchange in either direction when ATP is not hydrolysed[31,33,34]. ATP hydrolysis renders the reaction unidirectional, in the 5′→3′ direction relative to the strand that is first bound by the RecA filament[31,33]. The directionality of the strand-exchange reactions that are promoted by eukaryotic Rad51 is in some dispute, so it is not clear whether this feature of DNA-strand exchange is unique to bacterial RecA proteins. However, multiple studies indicate that the Rad51-promoted strand-exchange reaction moves readily in both directions[35–38].

DNA-strand exchange through a barrier. ATP hydrolysis contributes one clearly unique feature to the RecA reaction — it allows the bypass of significant barriers in one of the DNA substrates. An insertion of 100 bp of heterologous sequence in the duplex DNA substrate is bypassed with good efficiency when ATP is hydrolysed (FIG. 3a). The reaction is blocked if ATP cannot be hydrolysed[39–41]. Similar bypasses are limited to a few (<9) bp for the yeast Rad51 protein, regardless of whether ATP is hydrolysed or not[37]. RecA-mediated bypass involves the unwinding of the entire DNA insertion (FIG. 3a).

Four-strand exchanges. Neither RecA, nor any of its homologues, can align two duplex DNAs *de novo*. However, once a RecA-mediated DNA-strand exchange is initiated in a single-strand gap, it does not halt at the boundary of the gap. Instead, it transitions from a three-strand to a four-strand reaction and continues unimpeded (FIG. 3b). Unlike the initial stages of pairing and three-strand exchanges in general, the transition to a four-strand exchange is dependent on ATP hydrolysis[40,42,43]. Four-strand exchange is entirely unidirectional, proceeding in the 5′→3′ direction relative to the ssDNA in the gap where the reaction was initiated[44]. There are no reports of this reaction being promoted by RecA homologues outside the bacterial kingdom. The three- and four-strand-exchange reactions are further discussed in BOX 3.

An indirect helicase reaction. Given the capacity of RecA to underwind the DNA to which it is bound and to hydrolyse ATP, several efforts have been made to detect helicase functions. Only one such activity has been reported[43,45] (FIG. 3C). The observed DNA unwinding seems to be an indirect result of torsional stress applied to the DNA, as it occurs only when the two strands to be unwound separate into duplex branches immediately upstream[43,45]. This indirect helicase function requires ATP hydrolysis[43,45] and can lead to the unwinding of up to 400 bp. The complete unwinding of duplex DNA to which RecA is directly bound (without the DNA branches) is limited to ~30 bp[46,47], reinforcing the novelty of the indirect activity. A nick that detaches one of the two DNA branches eliminates the unwinding. If RecA assembles in a gap on the lagging-strand template of a stalled replication

Indirect helicase
DNA-strand separation brought about by the activity of RecA functioning on DNA segments that are adjacent to the region being unwound.

Lagging strand
The template strand that directs DNA synthesis in the direction that is opposite to that in which the fork is proceeding.

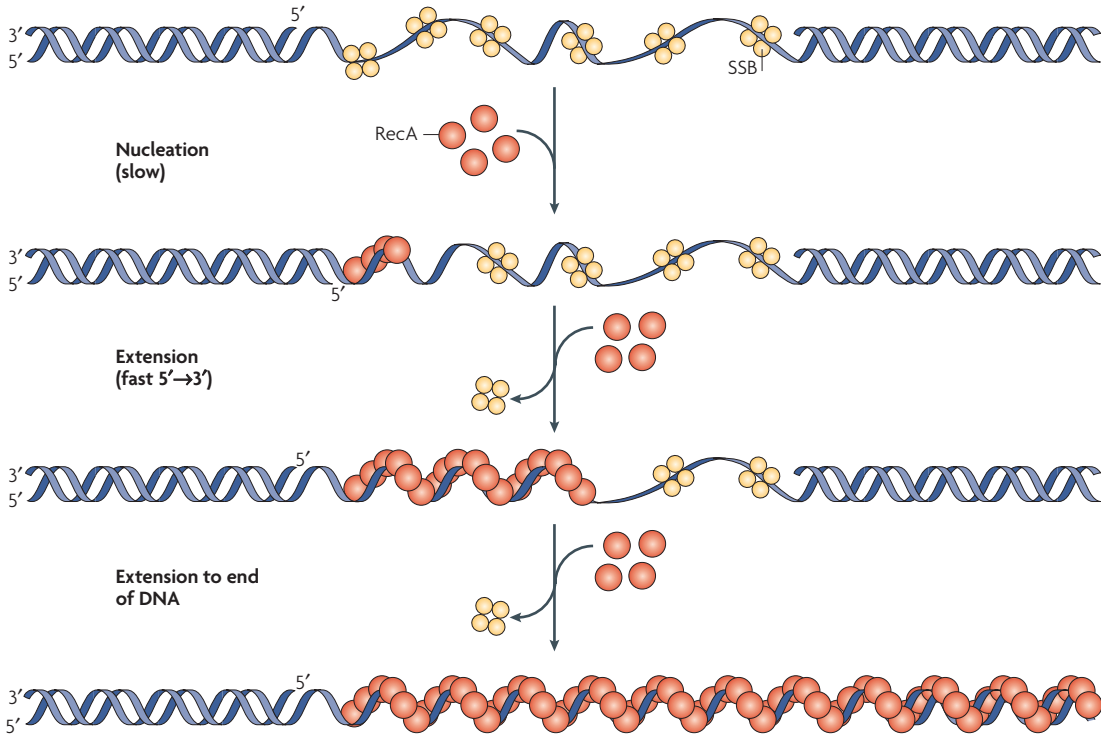


Figure 2 | **Assembly of RecA filaments.** RecA binds to DNA and forms a nucleoprotein filament in several steps. First, nucleation occurs slowly, with the single-stranded DNA-binding protein (SSB) functioning as a barrier to nucleation. Next, the filament extends rapidly, adding new subunits in the 5′→3′ direction and extends to the end of the DNA. When ATP is hydrolysed, disassembly also occurs, but more slowly than assembly (not shown). Disassembly also proceeds in the 5′→3′ direction at the end that is opposite to that where assembly occurs.

fork, this reaction could lead to downstream unwinding of the replication fork that would progress (as opposed to regress) the branch. The indirect helicase activity also explains how RecA can bypass double-strand breaks during four-strand exchanges (BOX 3).

Fork regression. If RecA assembles on a gap in the leading-strand template, a different reaction occurs. The fork is regressed in a systematic DNA-strand exchange[48,49]. Given that the DNA is already branched, the reaction is not preceded by a homology search. The reaction can encompass thousands of bp of DNA, and can lead to the formation of a Holliday junction (FIG. 3d). This reaction, even the part that technically involves only three DNA strands, is also dependent on ATP hydrolysis[48,49].

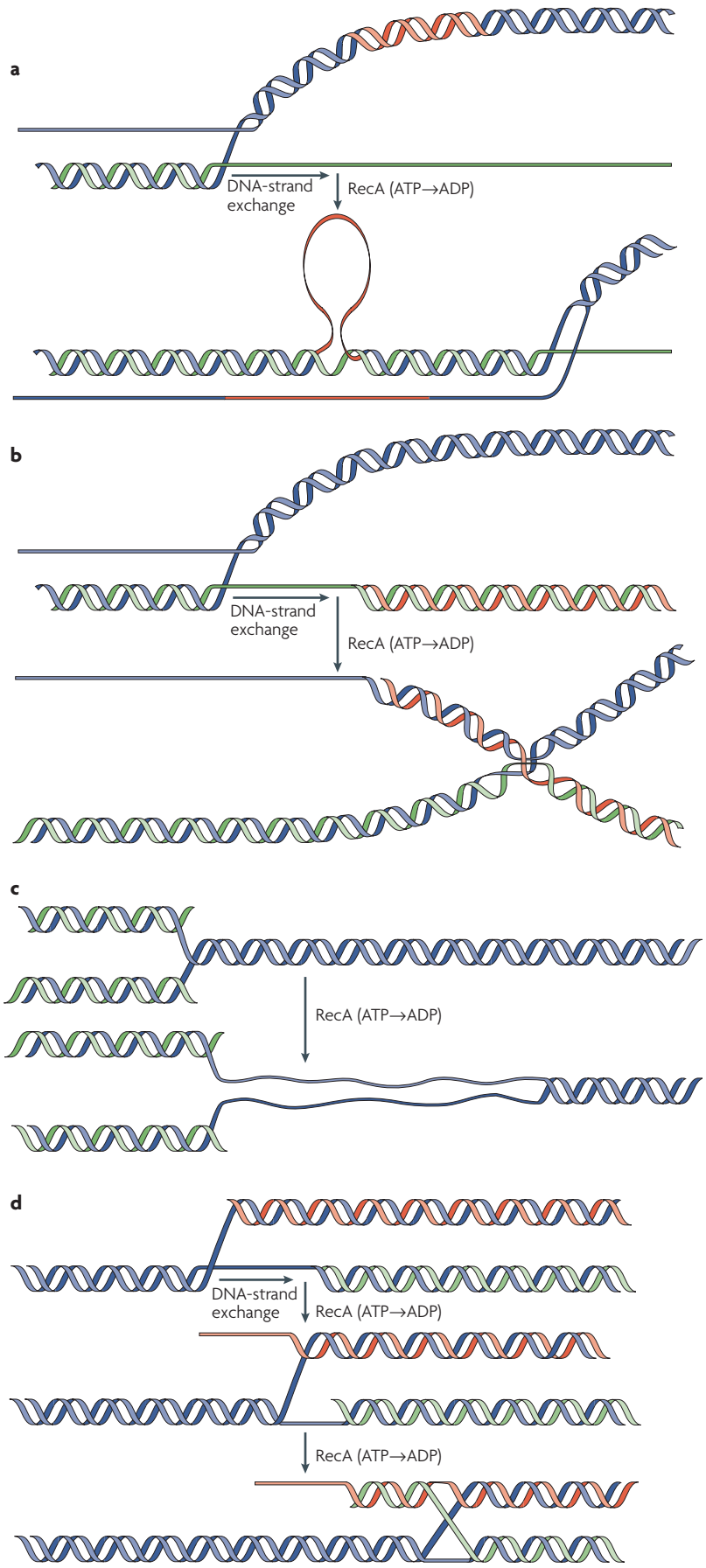
ATP hydrolysis and DNA-strand exchange

The DNA reactions mentioned above feature the separation of two strands of a duplex DNA over significant distances and/or an efficient movement of a DNA branch over even larger distances. For all of these reactions, the dependence on ATP hydrolysis implies a coupling between ATP hydrolysis and DNA-strand exchange. None of these reactions have been observed with any RecA-family recombinase other than bacterial RecA, and most have been explored only for the *E. coli* RecA. Over the past two decades, a wide range of models have been proposed to explain the properties of RecA-mediated DNA-strand exchange (for reviews, see REFS 50,51). There are three general mechanisms for the coupling of ATP hydrolysis to DNA-strand exchange that still sustain active discussion, and these are described here.

Facilitated-DNA-rotation model. This model couples ATP hydrolysis to DNA-strand exchange in a manner that requires a motor function[16,50–52] (FIG. 4a). In this model, RecA rotates the axes of two DNA molecules about each other such that any DNA branches that interconnect the two are compelled to migrate. All of the ATP-hydrolysis-dependent reactions, and all of the observations described below, are readily explained if RecA can rotate one DNA molecule about another (one inside the filament and one on the exterior of the filament). This requires the presence of DNA-binding sites on the exterior of the filament, arranged longitudinally along the filament, as well as the DNA-binding sites inside the filament. Rotation could generate the torsional stress needed to unwind heterologous insertions so as to bypass them. The capacity to rotate one duplex about another explains the capacity of RecA to promote four-strand exchanges with a filament that can only bind three strands in its interior, and it also explains the properties of the four-strand-exchange reactions outlined in BOX 3. Once DNA-strand exchange is initiated in a gap, it can be propagated beyond the gap and can transition to a four-strand reaction by rotating the two duplexes about one another.

Leading strand
The template strand that directs DNA synthesis in the same direction as the fork is proceeding.

Holliday junction
A DNA structure, often a recombination intermediate, in which two duplex DNAs are linked by a two-strand DNA crossover.

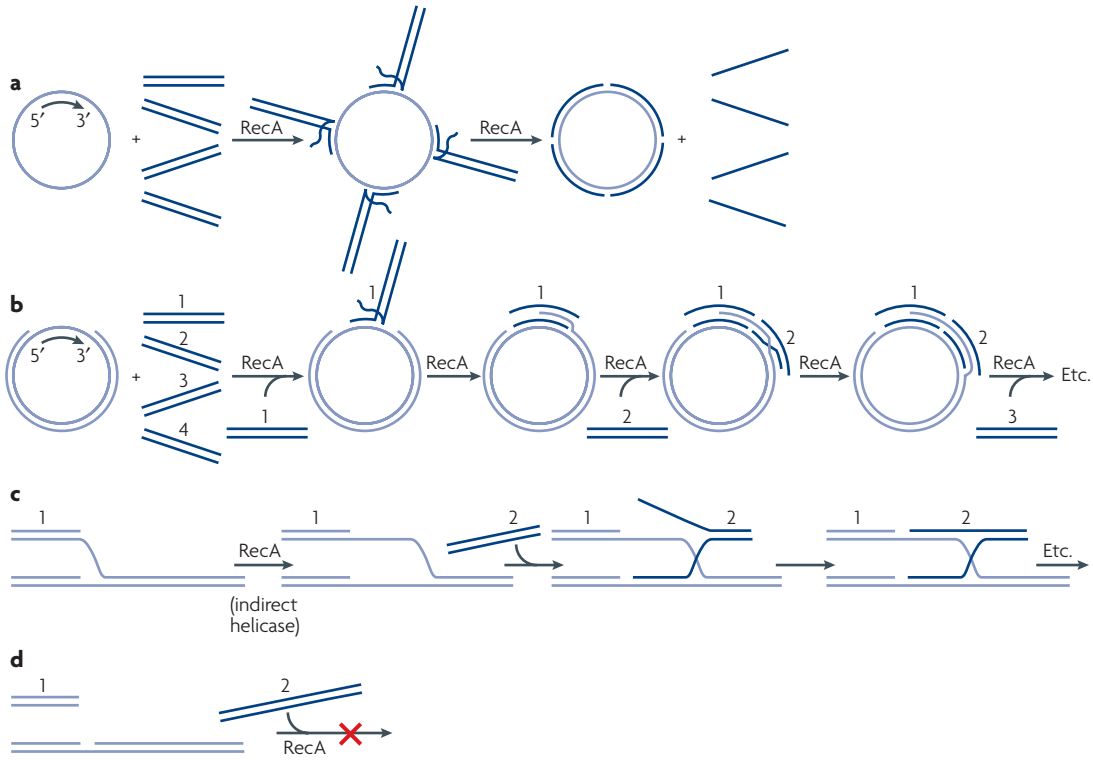


This model was also used to predict the indirect helicase and fork-regression activities[45,48], and both are readily explained by a RecA filament that has the capacity to rotate one DNA molecule about another. At a replication fork, RecA filaments formed on the lagging-strand template will promote the unwinding of the fork through the indirect helicase activity. If the filament forms instead on the leading-strand template, the rotation will result in fork regression.

Filament-dissociation model. A second model put forward in various forms proposes that DNA-strand exchange is coupled to RecA dissociation at the disassembly end of the filament (FIG. 4b). A current iteration would involve a modified version of a proposal first put forward by Howard-Flanders and colleagues[53]. A conformational change that occurs as RecA-filament subunits dissociate would serve to rearrange the strands so as to effect the required exchange. This model could explain the observed rates of DNA-strand exchange, as the measured rates of dissociation are very similar to the progress of the DNA branch during a three-strand exchange[24,54] (see also below). Following a DNA-strand exchange with circular DNA molecules (Fig. 1a), RecA is not dissociated but is instead bound to the heteroduplex DNA product[55]. This could reflect filament growth behind the dissociating end, replacing the RecA as the reaction progresses. The coupling of DNA-strand exchange to filament dissociation need not lead to net filament dissociation at the end of

◀ Figure 3 | **RecA reactions that depend on ATP hydrolysis.** The reactions shown are those that best illustrate the tight coupling that exists between RecA-mediated ATP hydrolysis and DNA-strand exchange. In addition, ATP hydrolysis is required for RecA-filament dissociation at a disassembly end, and also renders the strand-exchange reaction unidirectional. **a** | A strand-exchange reaction through a heterologous insertion in the duplex DNA substrate[39,40,42,107–109]. The DNA in the insertion (red) is unwound during the bypass. **b** | A four-strand-exchange reaction. These reactions must be initiated in a single-strand gap (as in three-strand exchanges), but the exchange is readily propagated beyond the gap[8]. The gap is not simply a loading point for RecA-filament formation, but the DNA-strand exchange must be initiated in the gap as a three-strand reaction[64,65]. **c** | The indirect helicase activity. In a DNA molecule that bifurcates into two duplex branches as shown, RecA will unwind the duplex DNA where the branches come together. Up to 400 base pairs of DNA can be unwound in this manner. Such a reaction occurs when a four-strand exchange is designed so that the duplex region of the gapped DNA substrate (to which RecA is initially bound) is longer than the second duplex DNA substrate[45], or possibly at a stalled replication fork where the RecA filament binds to a gap in the lagging-strand template. **d** | Replication-fork regression. If RecA binds to a gap in the leading-strand template of a stalled replication fork, the fork structure is driven backwards in a reaction that is dependent on ATP hydrolysis[48]. None of the reactions depicted in panels **a**–**d** have yet been observed for any archaeal or eukaryotic homologues of RecA.

Box 3 | **DNA-strand exchange with three and four DNA strands**



Four-strand exchange requires ATP hydrolysis, whereas three-strand exchange does not. But there are additional distinctions between these reactions. The two reactions behave differently on multiple levels, and these differences are best explained if the DNA pairing that is independent of ATP hydrolysis is mechanistically distinct from the subsequent DNA-strand exchange that is dependent on ATP hydrolysis.

Let us consider a variation of the three-strand exchange shown in FIG. 1b. If a duplex DNA substrate is cut into multiple fragments, all of these fragments can independently pair with the RecA nucleoprotein filament and undergo DNA-strand exchange with single-stranded circular DNA simultaneously[54] (part **a**). This reflects the inherent capacity of a RecA nucleoprotein filament to promote reactions between a bound single-stranded (ss)DNA and a homologous duplex.

Four-strand exchange, using a circular duplex with a short single-strand gap, is different. If the second DNA substrate is again presented in fragments, the duplex fragment that aligns with DNA in the single-strand gap will undergo exchange first. Additional fragments will then undergo exchange, but they will do so strictly one at a time[43,63] (part **b**). The order of reaction of the DNA fragments is dictated by the 5′→3′ orientation of the DNA strand in the gap, which, in turn, dictates the orientation of the RecA filament. None of these fragments initiates exchange until the fragment in front of it has completed its exchange. If a fragment of any significant length is missing, the one downstream of it does not undergo exchange at all.

The capacity of successive DNA fragments to undergo exchange, even though they must wait their turn, was initially considered to be evidence that RecA could pair two duplex DNAs[63]. However, there is another factor — RecA-applied torsional stress — that is required to allow these fragments to exchange[43], and this explains why each fragment must undergo DNA exchange in order. In effect, the indirect helicase must unwind the duplex DNA for a region beyond the point at which one fragment has completed its exchange, creating a short single-stranded region where the next fragment can initiate exchange as a transient three-strand reaction (part **c**)[43]. This is substantiated by the result shown in part **d**. If a nick is present in the gapped DNA at a position corresponding to the junction of the two fragments of the second DNA, the reaction of the second fragment does not occur[43]. Therefore, to bypass this double-strand break in the second DNA, torsional stress is needed to unwind the DNA of the gapped substrate and open up a region where the second DNA fragment can initiate DNA-strand exchange as a three-strand reaction. There might be double-strand-break repair scenarios in which this double-strand-break bypass activity of RecA, mediated by the indirect helicase function, is involved.

The three- and four-strand exchange reactions are also initiated differently. A three-strand exchange is readily initiated *de novo*. A four-strand exchange must be initiated as a three-strand exchange (in a single-strand gap in the RecA-bound DNA)[64,65]. When DNA pairing occurs, the incoming duplex DNA must be extended and underwound to bring it into register with the DNA that is already bound by RecA. When a RecA filament is bound to ssDNA, DNA pairing is readily detected as a robust underwinding of homologous circular duplex DNAs[106]. Assays that involve either supercoiled or relaxed duplex DNA circles give the same strong signal. When RecA is bound to duplex DNA, underwinding of homologous circular double-stranded DNA similarly indicates that a second duplex DNA can pair with the first[64,65] — an apparent four-strand pairing interaction. However, the signal is much reduced, and can be observed only when the second DNA circle is negatively supercoiled. The result can be explained by the rare incorporation of one strand of a supercoiled duplex into a RecA-filament groove to yield a three-strand rather than a four-strand pairing interaction[27,65].

Heteroduplex DNA
A duplex in which each strand is derived from different DNA molecules.

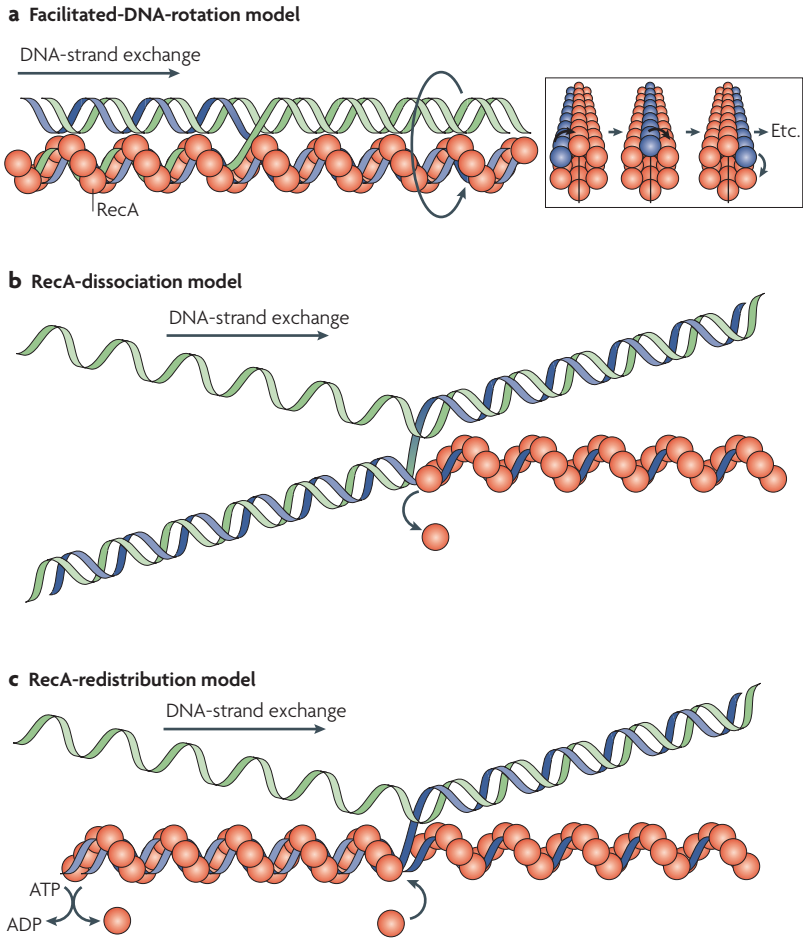


Figure 4 | **Models for RecA-mediated DNA-strand exchange coupled to ATP hydrolysis. a** | The facilitated-DNA-rotation model. Two DNA molecules, only one of which is within a RecA filament, are rotated about each other while maintaining an approximately parallel relationship. For this to occur, the ATP hydrolysis cycles of RecA subunits in the filament would have to be coordinated, with every sixth subunit hydrolysing ATP at a given moment (as shown in the inset and as has recently been shown[19,24]). **b** | The RecA-dissociation model has been proposed many times in a multitude of variations. Its main feature is the coupling of DNA-strand exchange to the dissociation of RecA monomers at the disassembling end. **c** | In the RecA-redistribution model, RecA has only a DNA-pairing function, and ATP hydrolysis serves only to facilitate RecA dissociation and redistribution. DNA-strand exchange should occur unimpeded wherever there is a contiguous RecA filament. For the models in parts **b** and **c**, only a three-strand-exchange reaction is shown. For all three models there would be a stage in which three strands would be wrapped in the filament groove (not shown). The actual DNA-strand exchange would occur in this three-stranded structure for at least the three-strand reactions and the three-strand portion of a four-strand reaction. For the models in parts **b** and **c**, the three-stranded region would extend into the filament for some distance to the right of the branch points in the reactions shown.

DNA-strand exchange. However, this model offers no clear mechanism for the DNA unwinding that is required for the bypass of a heterologous insertion in the duplex DNA, and is not obviously compatible with the indirect helicase activity. To explain the four-strand-exchange reaction, both duplexes would presumably have to be bound in the RecA-filament interior, where a site to accommodate a fourth DNA strand has proven difficult to detect.

RecA-redistribution model. The third proposed model can be described as RecA redistribution[56–58]. In this model (FIG. 4c), RecA does not have a motor function, but has a DNA-pairing activity. A contiguous RecA filament is sufficient to efficiently promote normal DNA-strand exchange. The strand-exchange process requires no ATP hydrolysis, and halts only when filament discontinuities are encountered. ATP hydrolysis occurs only to dissociate and redistribute the RecA to fill in filament discontinuities and allows the completion of DNA-strand exchange. ATP hydrolytic events in the interior of the filament are assigned no function. Some of the unusual reactions of RecA can be accommodated in this model by making use of the torsional stress that exists in DNA that is bound in a RecA filament. The model allows for the bypass of heterologous insertions in the duplex DNA substrate. If RecA dissociates from a tract of DNA, the highly underwound DNA that is released as a result could be translated into the strand separation that is needed to bypass insertions, assuming that the DNA strands were constrained so that the torsional stress could be applied to the needed DNA unwinding[59]. In principle, the indirect helicase activity might similarly reflect DNA unwinding that is mediated by the dissociation of RecA from its underwound DNA, although the requirement that the two DNA branches be contiguous with the unwound DNA is more difficult to explain.

In this model, ~70–80 RecA-filament subunits would have to dissociate without replacement in order to free sufficient underwound DNA to permit the unwinding and bypass of a 100-bp heterologous insertion in the duplex DNA substrate. Dissociation of ~300 RecA subunits would be needed to effect the unwinding of 400 bp of DNA by the indirect helicase. It is not clear if a gap of these lengths ever develops in RecA filaments on circular DNA molecules such as those used in typical RecA reactions *in vitro*. RecA assembly is demonstrably faster than disassembly, so opening up a large filament gap would require an insufficient supply of RecA or the presence of another protein that could block filament assembly. The addition of excess RecA (so as to fill in discontinuities) also does not improve the limited efficiency of DNA-strand exchange that is observed when ATP is not hydrolysed[40], nor does it block the bypass of heterologous insertions when ATP is hydrolysed. The heterologous insertions are bypassed as efficiently with linear DNA substrates — for which the DNA underwinding left behind by RecA dissociation could be dissipated by free rotation of the DNA ends — as they are when the DNA substrates are circular[45]. This model also offers no explanation for the coupling of ATP hydrolysis to four-strand exchanges or the observed properties of those exchanges (BOX 3).

Models put to the test. The ATPase-dependent DNA transactions detailed above inherently provide some stringent tests of the models (FIG. 4). A range of additional observations are also relevant to the discussion of mechanisms of action. When RecA promotes a strand-exchange reaction that involves three strands, the

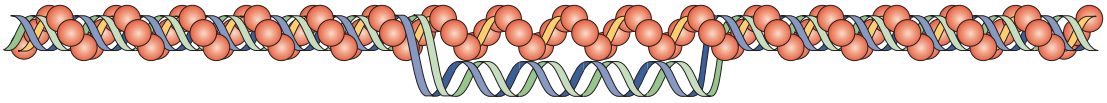


Figure 5 | **An intermittent DNA-pairing structure as an intermediate in DNA-strand exchange.** The formation of an intermittent DNA-pairing intermediate involves an interaction between two DNA molecules, one of which is extended and the other of which is not (at least initially). Once homologous alignment is achieved in one segment of the second DNA, additional homologous contacts can be made. The formation of such an intermediate can explain several observations described in the main text, but no detailed analysis of potential pathways for its formation has been carried out. In principle, interaction with the postulated DNA-binding sites on the exterior of the filament could facilitate the extension of this second DNA.

reaction unfolds in several steps. First, the DNA in the filament is aligned with a homologous partner through a mechanism that involves base flipping (that is, the bases rotate out of their stacked conformation to allow the formation of transient interactions with a new DNA strand) and can use the extension of the bound DNA as a facilitator[26,60,61]. This homology search has been reviewed in detail elsewhere[17], and it proceeds efficiently with ATP analogues that are not hydrolysed or with RecA mutants that can bind but not hydrolyse ATP. Once homology has been found, ATP hydrolysis positively affects all facets of the reaction.

When a three-strand-exchange reaction is initiated, all of the homology available to a given RecA filament seems to be sensed rapidly. The nucleoprotein filament on ssDNA hydrolyses ATP at the turnover rate of 30 per min, which is characteristic of the A state. When a homologous DNA is added that is the same length as the ssDNA and pairing begins, the rate of ATP hydrolysis shifts to 20 per min — which is characteristic of the P state — within two min[62], even though DNA-strand exchange between the same DNA molecules might not be completed for 15 min or more. If duplex DNA that is shorter than the ssDNA is present, the decrease in ATP hydrolysis is proportional to the length of the homologous duplex. If only part of the duplex DNA is homologous, the decrease is proportional to the homologous part[62]. This result indicates that all of the DNA that is homologous to the bound ssDNA is sensed quickly by the filament. The timing discrepancy between the sensing of homology and the completion of DNA-strand exchange might reflect an initial DNA-pairing complex in which the duplex was only intermittently in contact with the ssDNA — the contacts being separated by loops of DNA outside the filament[40] (FIG. 5). A single such loop would be sufficient to halt DNA-strand exchange and render it dependent on ATP hydrolysis. In any case, DNA-strand exchange proceeds across the available DNA at a rate of ~360 bp per min at 37°C (REF. 54). As one strand of the original duplex is displaced, it is bound by the ssDNA-binding protein (SSB).

The capacity of a RecA filament to bind three versus four interwound DNA strands in the filament interior is a key point of contention between the various models. The RecA-dissociation and RecA-redistribution models rely on a RecA-filament interior that can accommodate two interwound duplex DNAs to explain the four-strand-exchange reaction promoted by RecA. A range of physical and biochemical studies that have been reviewed elsewhere[27,28] all indicate that only three interwound strands are accommodated in the RecA-filament groove. Evidence for the homologous alignment and interwinding of two duplex DNAs (four strands) in the interior of the filament is limited to two observations. RecA can bypass double-strand breaks during a four-strand-exchange reaction[43,63], an observation that was initially considered to be support for a RecA-mediated duplex–duplex pairing interaction. Two other reports documented a homology-dependent underwinding of a circular duplex upon its interaction with a RecA-bound duplex[64,65]. These observations, however, are much less supportive of duplex–duplex pairing models when examined more closely (BOX 3).

The rates of several aspects of RecA-filament dynamics and DNA-strand exchange have been measured carefully to shed light on the strand-exchange mechanism. The facilitated-DNA-rotation model predicts that every RecA subunit in the filament will hydrolyse one ATP per 360° rotation. This in turn will move the DNA branch by 18 bp. The turnover number for RecA on dsDNA (in the P state, ~20 per min) is also the rate observed during DNA-strand exchange. The ratio of the rates of branch movement during DNA-strand exchange (measured in bp per min) and the turnover rate of ATP hydrolysis (measured as per min) is the predicted 18 bp[54]. This relationship holds over a wide range of temperatures[54]. On ssDNA, the rate of filament disassembly (~70 dissociated monomers per min[23]) reflects a minimal cooperativity or coupling between adjacent subunits. On dsDNA, in the P state (the filament state that is relevant to DNA-strand exchange), the rate increases to just over 120 dissociated monomers per min[19,24]. This increased rate is consistent with the existence of a tightly organized filament in which ATP hydrolysis cycles are coordinated over subunit multiples of six. Every sixth subunit is at the same point in the hydrolytic cycle, with waves of hydrolysis travelling through the filament in a manner that is also consistent with the facilitated-DNA-rotation model. A visualization of the organized waves of ATP hydrolysis in a RecA filament in the P state is shown in Supplementary information S1 (movie). The actual rate of filament dissociation in the P state is also consistent with a coupling of DNA-strand exchange to RecA dissociation, as RecA binds to 3 nucleotides so that a dissociation rate of 120 monomers per min translates into a 360 bp per min reduction in filament length.

ssDNA-binding protein (SSB). A class of protein, found in all cells, that binds to single-stranded (ss)DNA and interacts with many other proteins that are involved in DNA metabolism.

Topoisomerase
An enzyme that changes DNA topology by increasing or decreasing the twist of the DNA.

Conclusions

RecA couples the hydrolysis of ATP to DNA-strand exchange in a manner that gives RecA a robust capacity to alter the DNA around it. The reactions that are promoted by RecA when ATP is hydrolysed have implications for any proposed role of RecA in replication-fork or double-strand-break repair. If a RecA filament is assembled at the replication fork, and ATP is available, something will happen to the DNA at the fork. The fork will get regressed or unwound, depending on the orientation of the RecA filament. When RecA promotes DNA-strand invasion to repair a double-strand break, the RecA ATPase will tend to lengthen the region paired in the resulting D-loop. All of these reactions have topological consequences that must be modulated by topoisomerases in the cell. Genetic studies have not yet provided a complete *in vivo* context for these reactions[66].

RecA regulation, however, remains a wild card (BOX 2). RecA-filament assembly is facilitated by the RecF, RecO and RecR (RecFOR) proteins[67–70]. The RecX protein can limit filament growth[71] in a process that is blocked by the RecF protein[67]. The DinI protein can stabilize RecA filaments while subtly altering their function[72]. RecA filaments can be removed from DNA by certain helicases, particularly UvrD[73,74]. The RdgC protein has the potential to block all RecA-mediated DNA transactions at the fork, particularly fork regression or unwinding, as it binds to duplex DNA and denies the RecA filament access to this substrate[75]. However, it is unclear how or even whether RdgC is targeted to the replication fork. The understanding of RecA regulation is far from complete, and regulatory processes could have a major role in determining what RecA does where.

With respect to the coupling of DNA-strand exchange and ATP hydrolysis, most of the experimental results obtained so far are compatible to some degree with at least two of the models presented above. However, only the facilitated-DNA-rotation model is seamlessly compatible with all of them. Facilitated DNA rotation uniquely provides an explanation for the requirement for ATP hydrolysis in four-strand-exchange reactions as well as the unusual properties of that reaction outlined in BOX 3. Satisfactorily, this model also provides a function for essentially all of the ATP hydrolysis that occurs in a RecA filament. The RecA-dissociation model is difficult to reconcile with the indirect helicase activity and the bypass of heterologous insertions. The RecA-redistribution model is difficult to reconcile with the absolute dependence of four-strand exchanges on ATP hydrolysis (a contiguous filament is insufficient to allow a continuation of exchange into the four-strand region when ATP is not hydrolysed). Both the dissociation and redistribution proposals are incompatible with four-strand exchanges unless the filament is ultimately shown to have internal binding sites for two duplex DNAs in its interior.

The facilitated-DNA-rotation model has unresolved issues of its own, and most of these are structural. DNA-binding sites on the exterior of the filament have not been identified. The DNA arrangement shown in FIG. 5 also requires that the external DNA be extended at least as much as the DNA bound in the filament, and the structural requirements of the proposed DNA loops have not been explored. A careful analysis of pathways for the formation of an intermittent DNA-pairing intermediate — such as that shown in FIG. 5, which incorporates key structural parameters of both duplex DNA and active RecA filaments — has not been carried out.

What remains abundantly clear is that RecA has a substantial capacity to manipulate the DNA in its vicinity so as to move DNA branches in ways that can facilitate DNA repair. Enough ATP hydrolysis is available in these reactions to overcome substantial barriers. Although many of these reactions seem not to be promoted by the eukaryotic and archaeal homologues of RecA, some or all of these activities might eventually be attributed to auxiliary proteins in these organisms.

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Competing interests statement
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