Mechanistic aspects of the DNA strand exchange activity of *E. coli* recA protein

Stephen C. Kowalczykowski

The recA protein Escherichia coli *catalyses a reaction that is unique in nucleic acid enzymology: it can promote the exchange of single strands of DNA between different duplex DNA molecules with sequence complementarity. The DNA strand exchange reaction comprises several distinct biochemical steps, requires ATP hydrolysis, and is stimulated by the* E. coli *single-stranded DNA binding (SSB) protein. The molecular mechanism of each of these steps is discussed.*

Genetic recombination involves reciprocal exchange of homologous regions of DNA between two different double-stranded DNA molecules; the resulting recombinant DNA molecules contain genetic information originally present in each of parental molecules. Genetic studies have demonstrated that, in *E. coli*, many protein products (the products of genes *recA* through *recJ*) are involved in this complex biological process[1]. One of these, the recA protein, has been shown to be essential in all pathways of homologous recombination, and some of its biochemical properties will be discussed here.

The recA protein is a relatively small protein with a molecular mass of 37 842 Da, yet it has a remarkable number of enzymatic activities (see Refs 2–8 for detailed reviews and citations to early references). It can catalyse the following related reactions: the DNA-dependent hydrolysis of ATP; the ATP-stimulated renaturation of complementary single-stranded (ss) DNA molecules; the ATP-dependent reciprocal exchange of single strands of DNA between homologous double-stranded (ds) DNA molecules; and the cleavage of the lexA and λ repressor proteins in an ATP- and DNA-dependent reaction. This latter activity is not directly involved in the recombination process although it is critical for the activation of a variety of DNA repair processes known collectively as the SOS response[9].

The strand exchange activity of recA protein is unique (in *E. coli*) and is presumed to play an important role in the recombination process. Proteins with strand exchange activity have now been identified in organisms as diverse as bacteriophage T4[10], Gram-positive bacteria[11], lower eukaryotes[12] and human cells[13]. This implies that DNA strand exchange activity is a property of a class of ubiquitous proteins that can be referred to broadly as 'recombinases'.

S. C. Kowalczykowski is at the Department of Molecular Biology, Northwestern University Medical School, Chicago, IL 60611, USA.

The DNA strand exchange reaction

The enzymological requirements for the *in vitro* strand exchange activity of recA protein have been investigated in great detail[2,3,8]. Reciprocal strand exchange events can be detected between two different duplex DNA molecules, provided that the following criteria are met: (1) one of the dsDNA molecules contains a region of ssDNA; (2) the ssDNA region occurs at a site homologous to the other dsDNA molecule; and (3) for topological reasons, one of the substrate molecules has an end.

A reaction that has become a model for mechanistic studies involves the exchange of a circular ssDNA molecule (φX174 or M13 phage) for its homolog within a linear dsDNA molecule (Fig. 1, top portion). The products are a nicked circular dsDNA molecule and a linear ssDNA molecule[4,5], demonstrating that recA protein can completely exchange as much as 6000–7000 bp of DNA in 20–30 minutes[4]. Continuous ATP hydrolysis is required throughout this reaction and, despite the effectiveness of the protein-catalysed DNA strand exchange process, the apparent efficiency of the reaction with regard to ATP hydrolysis is quite poor: anywhere from 16 to 100 molecules of ATP are hydrolysed per base pair exchanged[4].

Minimal mechanistic considerations of the DNA strand exchange process require that recA protein binds to DNA substrates, pairs the DNA substrates, locates regions of sequence complementarity, locally disrupts dsDNA structure, and exchanges DNA strands. Thus, it is hardly a surprise that the strand exchange reaction catalysed by recA protein occurs by a series of kinetically distinct phases[5,6] which can be subdivided even further. At least five experimentally distinguishable steps are involved in strand exchange (Fig. 1).

(1) *Presynapsis*

At its most elementary level, presynapsis is simply the binding of recA protein to ssDNA, resulting in a complex that is capable of participating in strand exchange. Studies of the strand exchange reaction have demonstrated that stoichiometric amounts of recA protein, relative to the ssDNA concentration, are required[2,4,5,8]. Maximum exchange rates are observed at ratios of 1 recA protein monomer per 3–6 nucleotides of ssDNA, implicating the importance of the recA protein–ssDNA complex in the strand exchange process.

Direct ssDNA binding studies have confirmed the binding stoichiometries inferred from the strand exchange studies. By use of native ssDNA, it was demonstrated that conditions which favor the formation of stable secondary structure in the DNA (e.g. high $MgCl_2$ concentrations) prevent saturation of the ssDNA by recA protein[14,15]. As a consequence, the resulting subsaturated presynaptic recA protein–ssDNA complexes are five- to tenfold less proficient in the strand exchange reaction[15]. The apparent binding stoichiometry of these complexes is only approximately 1 recA protein monomer per 10–12 nucleotide residues, changing to ≃4 nucleotides per monomer if the complexes are formed at low $MgCl_2$ concentrations or if the *E. coli* SSB (single-stranded DNA binding) protein is present[15]. Similar optimal molar ratios are observed for the ssDNA-dependent ATPase activity of recA protein under corresponding conditions, demonstrating that DNA secondary structure also impedes ATPase activity[16]. Since SSB protein can alleviate this block and stimulates both

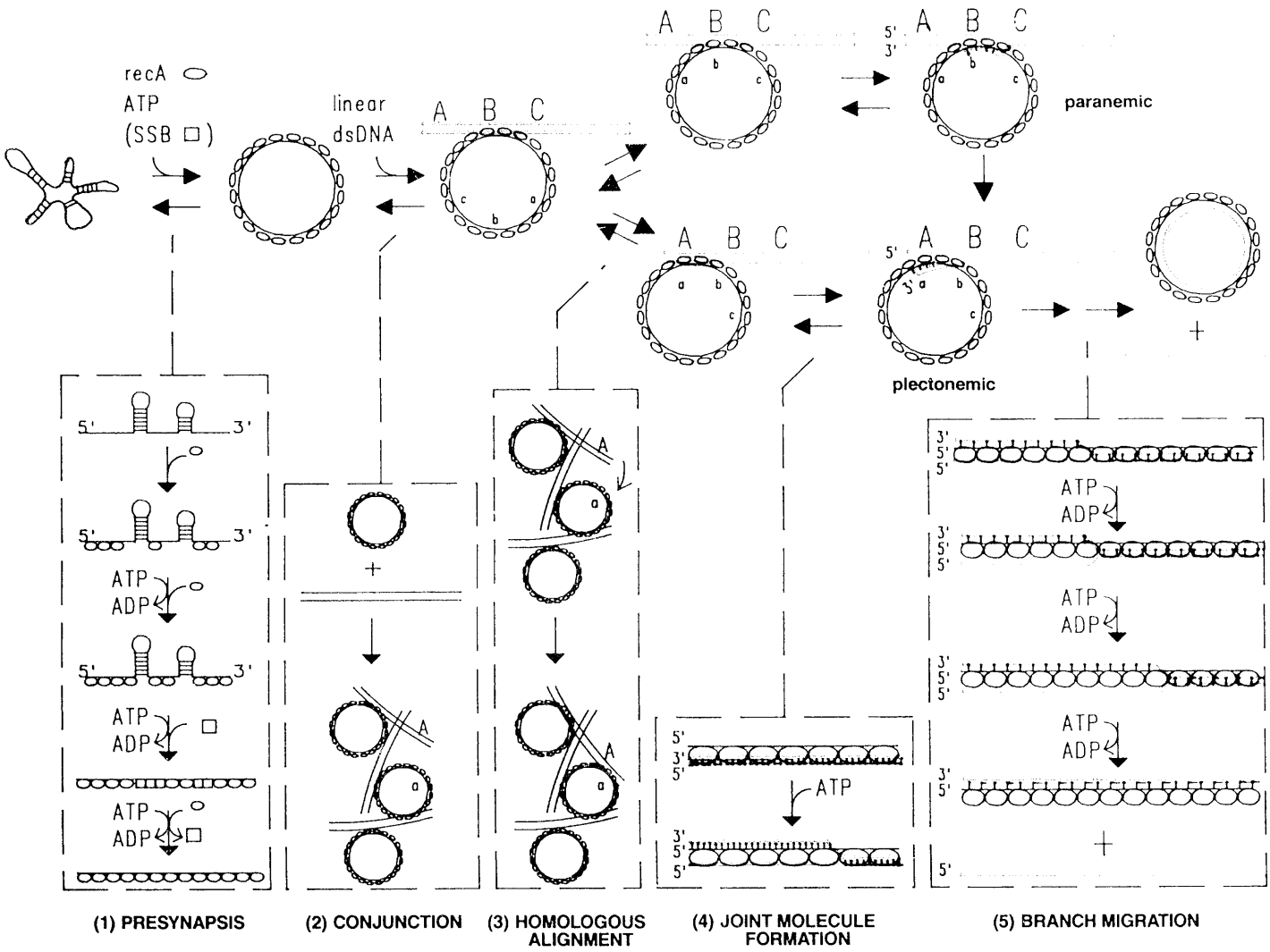

Fig. 1. Schematic representation of the DNA strand exchange reaction catalysed by E. coli *recA protein. The top portion of the figure represents a simplified version of the individual kinetic steps involved in the protein-catalysed exchange of DNA strands between circular single-stranded and linear double-stranded DNA molecules. Each step is described in further molecular detail in the boxed panels beneath it. The illustrations are based on work described in the references cited in the text; the drawings are not to scale and the scale of each panel is different. A, B, C and a, b, c represent sites of homology on different DNA molecules. The individual panels depict (1)* Presynapsis. *A magnified view of recA protein (◯) binding, with a 5'→3' polarity, to regions of ssDNA devoid of secondary structure. SSB protein (□) is required for destabilization of the DNA stem–loop structures and is subsequently displaced by recA protein. All of the DNA-bound recA protein molecules hydrolyse ATP. (2)* Conjunction. *ssDNA and dsDNA molecules are associated non-homologously by recA protein to form extensive networks of ternary complexes of recA proteins, ssDNA, and dsDNA referred to as coaggregates. (3)* Homologous alignment. *The search for DNA sequence homology is depicted as occurring within the coaggregate structure and is facilitated by the concentration effect of coaggregation. (4)* Joint molecule formation. *An enlarged view of the formation of a nascent heteroduplex structure (either plectonemic or paranemic) with the dsDNA in an 'open' conformation induced by the ATP-recA protein complex; ATP hydrolysis is not essential. (5)* Branch migration. *A nascent heteroduplex joint is shown being extended in a 5'→3' direction by the dsDNA-dependent ATPase activity of recA protein; all of the DNA-bound recA protein molecules are hydrolysing ATP. The recA protein is shown bound to ssDNA strands of identical polarity, on the basis of the work of Chow* et al.[38]

strand exchange and ATPase activity, its role in recA protein function is critical and will be discussed separately below.

Binding studies utilizing a fluorescent ssDNA substrate devoid of secondary structure avoid the complications introduced by DNA structure and yield a DNA binding site size of 6–8 nucleotides per recA protein monomer[17], which agrees well with ATPase activity studies[16]. The equilibrium binding affinity is highly dependent on the salt concentration, decreasing by $\simeq 10^{10}$ for a tenfold increase in NaCl concentration. The binding is also cooperative in protein concentration; the apparent cooperativity parameter, ω, is 50 (based on monomer binding) and is independent of salt concentration[17].

Since ATP is not required for ssDNA binding, it is possible to determine the roles of ATP binding and hydrolysis with regard to ssDNA binding. Neither ATP nor ADP have any effect on the binding stoichiometry of recA protein to ssDNA[17,18], but they do have striking effects on the equilibrium binding affinity of recA protein to the DNA. Both ATP and the non-hydrolysable ATP analogue, ATPγS, significantly increase the affinity of recA protein for ssDNA[17]. Surprisingly, the effect of ADP is to decrease the affinity of the recA protein–ssDNA complex by at least tenfold (depending on salt concentration) compared to the affinity of the nucleotide cofactor-free complex. Thus, the *binding* of ATP results in a high ssDNA binding affinity form of recA protein whereas ADP, the product of ATP *hydrolysis*, results in a form with low ssDNA binding affinity. This implies that the ATP hydrolytic cycle serves to modulate the affinity of recA protein between two different ssDNA affinity states and thereby facilitate the cyclic binding and dissociation of recA protein from ssDNA[17]. In this mechanism, the energy of the actual ATP hydrolysis event is not 'used' directly. Rather it is the free energy change associated with ATP binding that

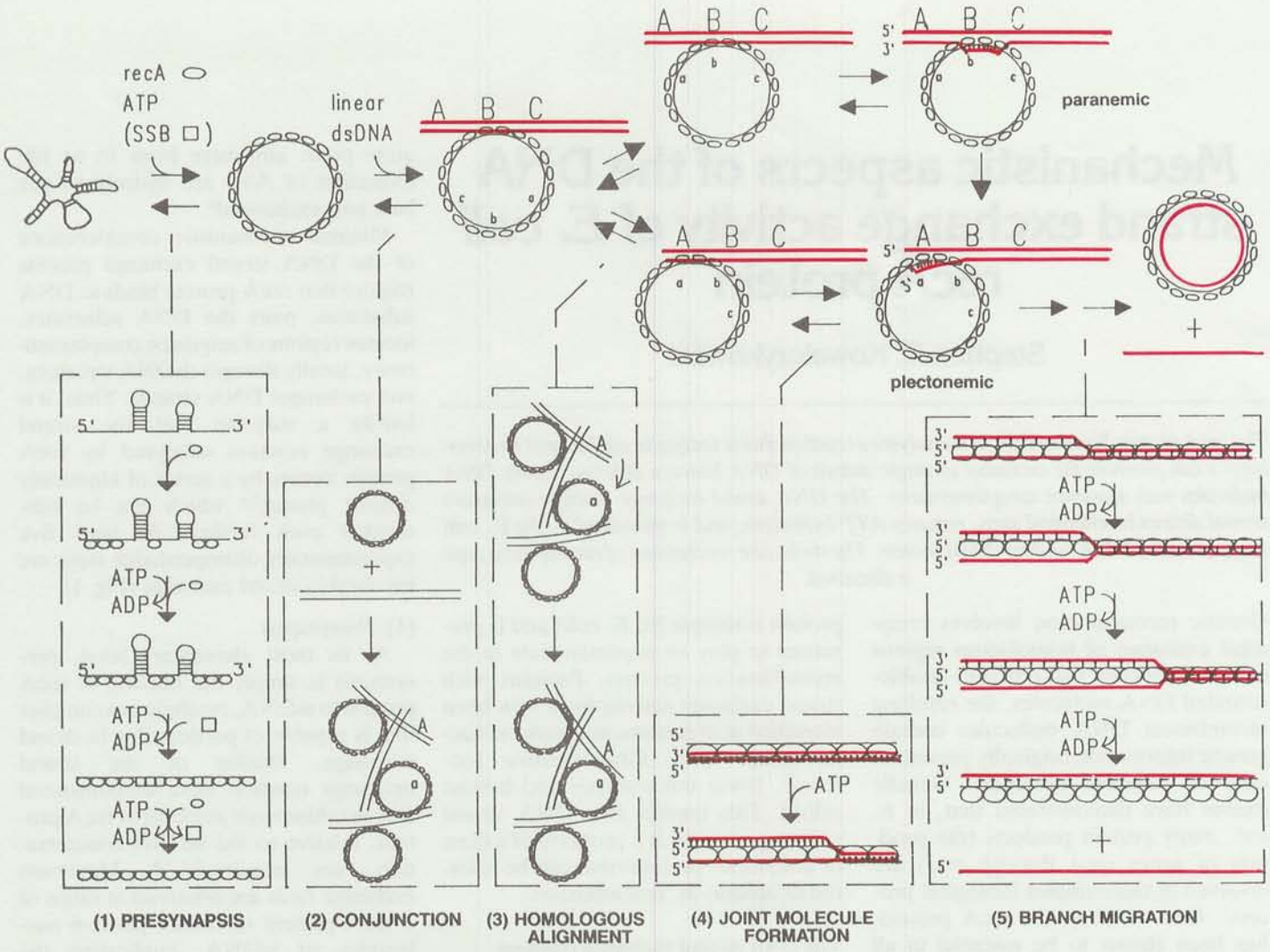

Fig. 1. Schematic representation of the DNA strand exchange reaction catalysed by E. coli *recA protein. The top portion of the figure represents a simplified version of the individual kinetic steps involved in the protein-catalysed exchange of DNA strands between circular single-stranded and linear double-stranded DNA molecules. Each step is described in further molecular detail in the boxed panels beneath it. The illustrations are based on work described in the references cited in the text; the drawings are not to scale and the scale of each panel is different. A, B, C and a, b, c represent sites of homology on different DNA molecules. The individual panels depict (1)* Presynapsis. *A magnified view of recA protein (◯) binding, with a 5′→3′ polarity, to regions of ssDNA devoid of secondary structure. SSB protein (□) is required for destabilization of the DNA stem–loop structures and is subsequently displaced by recA protein. All of the DNA-bound recA protein molecules hydrolyse ATP. (2)* Conjunction. *ssDNA and dsDNA molecules are associated non-homologously by recA protein to form extensive networks of ternary complexes of recA proteins, ssDNA, and dsDNA referred to as coaggregates. (3)* Homologous alignment. *The search for DNA sequence homology is depicted as occurring within the coaggregate structure and is facilitated by the concentration effect of coaggregation. (4)* Joint molecule formation. *An enlarged view of the formation of a nascent heteroduplex structure (either plectonemic or paranemic) with the dsDNA in an 'open' conformation induced by the ATP-recA protein complex; ATP hydrolysis is not essential. (5)* Branch migration. *A nascent heteroduplex joint is shown being extended in a 5′→3′ direction by the dsDNA-dependent ATPase activity of recA protein; all of the DNA-bound recA protein molecules are hydrolysing ATP. The recA protein is shown bound to ssDNA strands of identical polarity, on the basis of the work of Chow* et al.[38]

strand exchange and ATPase activity, its role in recA protein function is critical and will be discussed separately below.

Binding studies utilizing a fluorescent ssDNA substrate devoid of secondary structure avoid the complications introduced by DNA structure and yield a DNA binding site size of 6–8 nucleotides per recA protein monomer[17], which agrees well with ATPase activity studies[16]. The equilibrium binding affinity is highly dependent on the salt concentration, decreasing by $\simeq 10^{10}$ for a tenfold increase in NaCl concentration. The binding is also cooperative in protein concentration; the apparent cooperativity parameter, ω, is 50 (based on monomer binding) and is independent of salt concentration[17].

Since ATP is not required for ssDNA binding, it is possible to determine the roles of ATP binding and hydrolysis with regard to ssDNA binding. Neither ATP nor ADP have any effect on the binding stoichiometry of recA protein to ssDNA[17,18], but they do have striking effects on the equilibrium binding affinity of recA protein to the DNA. Both ATP and the non-hydrolysable ATP analogue, ATPγS, significantly increase the affinity of recA protein for ssDNA[17]. Surprisingly, the effect of ADP is to decrease the affinity of the recA protein–ssDNA complex by at least tenfold (depending on salt concentration) compared to the affinity of the nucleotide cofactor-free complex. Thus, the *binding* of ATP results in a high ssDNA binding affinity form of recA protein whereas ADP, the product of ATP *hydrolysis*, results in a form with low ssDNA binding affinity. This implies that the ATP hydrolytic cycle serves to modulate the affinity of recA protein between two different ssDNA affinity states and thereby facilitate the cyclic binding and dissociation of recA protein from ssDNA[17]. In this mechanism, the energy of the actual ATP hydrolysis event is not 'used' directly. Rather it is the free energy change associated with ATP binding that

is responsible for the structural transition into the high ssDNA binding form of the protein. The destruction of the ATP by hydrolysis returns the system to its starting point. This mechanism may be of general importance for energy transduction in nucleic acid enzymology since it solves the 'tight binding' dilemma – a nucleic acid enzyme faces two contradictory functional requirements: it must have a sufficient DNA binding affinity to bind tightly to its substrate, yet it must also dissociate rapidly to act catalytically. The ATP hydrolytic cycle resolves this problem.

The interpretation of the equilibrium experiments is also consistent with kinetic lifetime data[18,19]. The lifetime of recA protein–ssDNA complexes is decreased by ADP but is increased by ATPγS, relative to the cofactor free complex. At low ATP concentrations, the lifetime is similar to the ADP-induced form; however at high ATP concentrations, the apparent kinetic lifetime increases[18,20]. This is because the ATPase activity becomes processive at high ATP concentrations, with as many as 50 ATP molecules hydrolysed per protein transfer event[20]. These results imply that following ATP hydrolysis, the resultant low affinity ADP–recA protein–DNA complex can follow at least two kinetic pathways: either the complex can dissociate from the DNA, or ADP can dissociate from the recA protein–DNA complex which then binds another ATP molecule. At low ATP concentrations, the former dispersive ATP hydrolysis pathway predominates, whereas at high ATP concentrations, the latter processive pathway predominates[20]. Although under most conditions recA protein is a ssDNA-dependent ATPase, it has been shown that both the binding of ATP and ssDNA to recA protein are necessary, but not sufficient, to activate its ATPase activity. The specific molecular nature of this additional requirement is unknown, but it has been suggested that ATP hydrolysis requires the formation of contiguous clusters of 15 or more DNA-bound recA protein molecules[21].

Finally, the binding of recA protein to ssDNA is polar, proceeding in a 5′→3′ direction[22]. This is the same direction as the branch migration reaction promoted by recA. Thus, although at the simplest level presynapsis involves the binding of recA protein to ssDNA, the mechanism of binding bears considerable similarity to a polymerization process such as that observed for actin or tubulin. Electron microscopic observation of recA protein–DNA complexes can clearly visualize filamentous protein–nucleic acid complexes that are actively involved in the strand exchange process[23].

(2) *Conjunction*

Once an active presynaptic complex of recA protein and ssDNA has been formed, that complex must then pair with dsDNA. On the basis of probability, this initial contact must be at regions of non-homology since even with DNA substrates as small as ϕX174 or M13 phage DNA molecules, only one orientation out of ≈6000 represents perfect alignment of homologous sequences.

RecA protein is capable of forming a complex between ssDNA and dsDNA molecules[24–26] or two different ssDNA molecules[18,19] that have little or no sequence complementarity, implying that the functional recA protein–DNA complex possesses two DNA binding sites. The non-homologously paired complexes that form between ssDNA and dsDNA molecules do not simply involve two individual molecules. Instead, recA protein can join many DNA molecules together in complex three-dimensional networks that can be sedimented in a low speed centrifuge[24]. These complexes are referred to as coaggregates (Fig. 1, panels 2 and 3) because they contain both ssDNA and dsDNA. Their formation requires ATP, elevated magnesium ion concentration, and saturation of the ssDNA by recA protein – all of which are required for the strand exchange reaction. These coaggregates form rapidly (in minutes) and kinetically precede joint molecule formation (see below). Thus, it has been inferred that coaggregates of ssDNA and dsDNA molecules at regions of non-homology are intermediates on the pathway of the strand exchange reaction[24].

(3) *Homologous alignment*

Perhaps one of the most intriguing aspects of recA protein-catalysed strand exchange is the kinetic mechanism by which DNA sequence homology is found. Although recA protein shows no sequence-specific binding, the homology search problem is analogous to the problem that sequence-specific DNA binding proteins face in locating their DNA target site[27]. In the case of recA protein, however, the 'binding protein' is the entire recA protein–ssDNA complex and the 'target' is the unique complementary sequence within the dsDNA. Thus, mechanisms invoking simple diffusion, one-dimensional sliding, intersegmental transfer and, for an ATPase, active translocation driven by ATP hydrolysis could be formally considered.

Insight into this process was provided by experiments which demonstrated that the addition of increasing lengths of non-homologous dsDNA onto homologous dsDNA increased the rate at which homologous pairing occurred[6,26]. This result implied that non-specific DNA increased the rate by which complementary sequences are located, a result at least superficially analogous to that observed for sequence-specific DNA binding proteins. Similar experiments carried out under somewhat different experimental conditions demonstrated that heterologous dsDNA had no effect on the rate of pairing, implying that the rate-limiting step in the homologous pairing reaction may be sensitive to changes in experimental conditions[25]. From the experiments where heterologous DNA increased the rate of the homology search, it was concluded that the coaggregates were instrumental in the pairing process and that the search occurred by three-dimensional diffusion rather than one-dimensional sliding[6,26]. The three-dimensional diffusion process is so efficient in the recA protein-catalysed search because coaggregation acts to increase the local concentration of the DNA within domains, thereby facilitating the diffusion process (Fig. 1, panel 3). Therefore, the homology search mechanism *in vitro* may be as 'simple' as a passive diffusion process that is accelerated by the concentrating effect of the coaggregation reaction, thus yielding apparent first-order kinetics[26].

(4) *Joint molecule formation*

After the two DNA molecules are homologously aligned, recA protein must catalyse the nascent exchange of DNA strands. For the reaction involving a dsDNA and ssDNA molecule, this requires local denaturation of the dsDNA molecule and subsequent exchange of the identical single strands of DNA. These steps may be simultaneous or separated in time. Such intermediates are referred to as joint molecules or D-loop structures.

Two types of joint molecule can form depending on the topological constraints imposed on them: either plectonemic or paranemic. A plectonemic joint is one in which the incoming single strand of DNA is intertwined around its complement as in native dsDNA. A paranemic joint is one in which the individual complementary strands do not intertwine, resulting in a molecule that is base paired

though not topologically linked. For the two DNA substrates typically employed in the recA protein-catalysed strand exchange (i.e. linear dsDNA and circular ssDNA), a plectonemic joint can form only at the ends of the linear dsDNA whereas a paranemic joint will form in the interior of the dsDNA (Fig. 1). RecA protein can form both types of joint molecule structures[6,28–30]. The plectonemic joints that form are estimated to contain ≈300 base pairs of heteroduplex DNA[30] and, once formed, do not require recA protein for stability[29,30]. Paranemic joints, however, are unstable in the absence of recA protein[28]. Since paranemic joints form somewhat more rapidly than plectonemic joints, it appears that paranemic joint molecules are intermediates on the pathway to the formation of the more stable plectonemic molecules[29].

What is the role of ATP binding and hydrolysis in joint molecule formation? Since the local denaturation of dsDNA requires the input of some form of energy, it is reasonable to suspect the involvement of ATP in this process. ATP hydrolysis may not be an absolute requirement for paranemic joint formation: the non-hydrolysable analogue, ATPγS, will support paranemic joint formation, although the yield is variable[29]. Thus, it appears that the ATP-bound high ssDNA affinity form of recA protein can act locally to 'open' the dsDNA (Fig. 1, panel 4). This view of joint molecule formation is also consistent with the properties of its dsDNA-dependent ATPase activity[31]. The hydrolysis of ATP shows a distinct time lag with dsDNA that is not observed when ssDNA is employed. This lag is due to a rate-limiting step involving a transition of the protein–dsDNA complex from a 'closed' double-helical form to an 'open' locally denatured form[31]. ATP hydrolysis appears to be a consequence of transient ssDNA formation rather than being necessary for the denaturation event itself.

(5) *Branch migration*

After formation of the plectonemic heteroduplex joint, the branch migration phase commences. During branch migration, the nascent heteroduplex joint is extended until complete exchange of DNA single strands occurs. The branch migration phase actually may not be a mechanistically separate step but, instead, represent continuation of plectonemic joint formation[29]. However, these steps can be distinguished experimentally since joint molecule formation requires the exchange of only ≈300 bp whereas complete branch migration requires the exchange of ≈6000 bp.

The branch migration reaction proceeds in a polar manner, 5′→3′ relative to the incoming ssDNA, at a rate of ≈20 nucleotides per second[4,7]. The recA protein-catalysed branch migration is relatively tolerant of nucleotide sequence mismatches and can readily traverse short mismatches[6]; the reaction is inhibited (>90%) by mismatches of 500 base pairs or more within the dsDNA molecules. Continued ATP hydrolysis is required throughout the branch migration process. When the total amount of ATP hydrolysed is compared to the number of base pairs exchanged, the apparent efficiency of ATP utilization ranges from 16 to 100 ATP molecules hydrolysed per base pair branch migrated[4,7]. Since recA protein readily hydrolyses ATP in the presence of ssDNA alone (i.e. when there is no branch migration), much of the observed ATP hydrolysis must be non-productive with regard to the branch migration process. Consistent with this view is the absence of a correlation between the total amount of ATP hydrolysed and the extent of strand exchange[32]. Also, on the basis of differences in sensitivity to inhibition by ADP, it was concluded that continued association of recA protein with ssDNA was unnecessary for strand exchange after joint molecule formation had been complete[30].

However, recA protein also possesses a dsDNA-dependent ATPase activity[31] (described above), although it actually occurs via a transient ssDNA intermediate resulting from local denaturation of the dsDNA. When the amount of ATP hydrolysed in this dsDNA-dependent reaction is compared to the extent of branch migration, a linear correlation is found. Early in the reaction, the ATP utilization is approximately 1 ATP molecule per base pair branch migrated[32]. Thus, the strand exchange reaction is much more efficient than previously recognized when the non-productive ssDNA-dependent ATP hydrolysis is discounted.

Three molecular mechanisms have been proposed to explain the branch migration process: treadmilling[7], rotation mechanisms[7] and polar polymerization[31]. Each of these mechanisms share the realization that filaments of recA protein are important to the strand exchange process[33]. The treadmilling mechanism is based on the classical mechanism used to describe the polymerization of actin and tubulin: ATP–recA protein complexes bind to the 'front' of a recA protein–DNA filament, ATP is hydrolysed, and dissociation of the ADP–recA protein occurs at the filament 'end'[7]. However, one prediction of this classical view is that ATP hydrolysis rate might parallel the number of filament ends. The experimental observation is that essentially all recA protein molecules bound to ssDNA can hydrolyse ATP and hydrolysis is not limited to ends[8,16]. The rotation mechanisms propose that multiple contacts between two recA protein–DNA filaments are responsible for branch migration. The apparent inefficiency of ATP utilization is inherent to the process and results from the high number of contacts that must be broken between filaments for the branch migration of just one base pair[7]. The polar polymerization model can be viewed as a variant of the classical treadmilling: the binding of an ATP–recA protein complex to a ssDNA region within dsDNA occurs in a polar manner at the growing end of a filament; this event is responsible for both the opening of the dsDNA and strand exchange (either actively or passively)[31]. However, in contrast to the classical model, the recA protein–DNA complex is capable of several rounds of processive ATP hydrolysis prior to dissociation and these additional hydrolytic events are not productive in strand exchange; only the newly added ATP–recA protein complex is responsible for driving branch migration in a polar direction[31]. In one limiting view of this mechanism, ATP hydrolysis is merely a consequence of the dsDNA opening event and is not used to actively 'drive' the branch migration process but rather to dissociate the transient recA-ssDNA complex. Consistent with the polar polymerization model for branch migration is an activity of recA protein referred to as 'processive unwinding'[34]. In the presence of ATP or ATPγS, recA protein can processively polymerize onto dsDNA and induce a topological unwinding of the dsDNA[34] in a reaction that has several parallels with the dsDNA-dependent ATPase activity. Clearly, a more complete understanding of the roles of ATP binding and hydrolysis is critical for the elucidation of the mechanism of this recA protein function.

The role of SSB protein

The role of SSB protein in the strand exchange reaction is of considerable mechanistic importance. It affects the binding of recA protein to ssDNA[4,6,7], greatly stimulating the strand exchange process. Consideration of how this is achieved led to two limiting proposals.

On the basis of kinetic competition studies, it was proposed that SSB protein increases the lifetime of the recA protein-ssDNA complex through the formation of an SSB protein–recA protein interaction[4,7]. However, direct equilibrium and kinetic studies failed to demonstrate any direct effect of SSB protein on the affinity of recA protein for ssDNA; in fact, the binding of the two proteins is competitive for ssDNA[35]. In addition, despite attempts, direct evidence for a specific interaction between these two proteins is absent.

The alternative proposal for the role of SSB protein is that it removes secondary structure from ssDNA which is inhibitory to the formation of a saturated presynaptic complex[30,36] (Fig. 1, panel 1). This is consistent with the role of SSB protein as a helix-destabilizing protein and the observation that other helix destabilizing proteins can substitute in the strand exchange reaction *in vitro*[36]. The stimulatory effect of SSB protein on joint molecule formation can be mimicked by conditions that decrease the stability of DNA secondary structure (e.g. low magnesium ion concentration or elevated temperature[36]). Similar trends are observed in the ssDNA-dependent ATPase activity of recA protein: SSB protein, bacteriophage T4 gene 32 protein, or low magnesium ion concentrations eliminate the inhibitory effect of secondary structure on recA protein binding and subsequent ATP hydrolysis[16]. In fact, SSB protein is not required to achieve the optimal DNA binding stoichiometry for ATPase activity when DNA substrates devoid of secondary structure are used[16]. An added complexity of SSB protein participation is that it is required continually during recA protein function and not just to initiate presynaptic complex formation[16,37]. Thus it appears that upon recA protein dissociation from the ssDNA (after anywhere from 1 to 50 rounds of ATP hydrolysis), DNA secondary structure can reform, requiring the repeated action of SSB protein.

Concluding remarks

The biochemical complexity of both recA protein and the strand exchange reaction is remarkable. Thus, it is a fair question to ask which of these activities are important to biological function. Fortunately, many different mutations in the recA gene have been genetically characterized and biochemical study of such mutant recA proteins should provide insight into biologically important activities. For example, the mutant recA142 protein is defective in genetic recombination *in vivo* and cannot carry out strand exchange *in vitro*. The *in vitro* basis for this defect is that recA142 is unable to compete effectively with SSB protein for ssDNA binding, is unable to coaggregate, and is deficient in its dsDNA-dependent ATPase activity. The first and third defects result from the inability of recA142 protein to form the high ssDNA-binding affinity state induced by ATP (Kowalczykowski *et al.*, submitted). Thus, any one or all of these activities must be important to biological function *in vivo*.

Much has been learned about the properties of recA protein, but even more remains to be determined. The details of the homology search, the mechanism of energy transduction in this system, the detailed mechanism and role of the DNA renaturation reaction, properties of the reaction between duplex DNA molecules, and biological importance of various biochemical events are examples. In addition, since recA protein does not act in the cell in isolation, its interaction with other recombinationally important proteins, such as the recBCD enzyme remains to be solved.

Acknowledgements

The work in this laboratory and the preparation of this article was supported by grants from the National Institutes of Health (AI-18987) and by the American Cancer Society (JFRA-70). The author is grateful to Lester Lau, Scott Lauder, Joseph Menetski, and Richard Scarpulla for their comments on this manuscript.

References

1 Clark, A. J. (1973) *Annu. Rev. Genet.* 7, 67–86
2 Radding, C. M. (1982) *Annu. Rev. Genet.* 16, 405–437
3 Dressler, D. and Potter, H. (1982) *Annu. Rev. Biochem.* 51, 727–761
4 Cox, M. M., Soltis, D. A., Livneh, Z. and Lehman, I. R. (1983) *Cold Spring Harbor Symp. Quant. Biol.* 47, 803–810
5 Radding, C. M., Flory, J., Wu, A., Kahn, R., DasGupta, C., Gonda, D., Bianchi, M. and Tsang, S. S. (1983) *Cold Spring Harbor Symp. Quant. Biol.* 47, 821–828
6 Flory, S. S., Tsang, J., Muniyappa, K., Bianchi, M., Gonda, D., Kahn, R., Azhderian, E., Egner, C., Shaner, S. and Radding, C. M. (1984) *Cold Spring Harbor Symp. Quant. Biol.* 49, 513–523
7 Cox, M. M., Morrical, S. W. and Neuendorf, S. K. (1984) *Cold Spring Harbor Symp. Quant. Biol.* 49, 525–533
8 Cox, M. M. and Lehman, I. R. *Annu. Rev. Biochem.* (in press)
9 Little, J. W. and Mount, D. W. (1982) *Cell* 29, 11–22
10 Yonesaki, T., Ryo, Y., Minagawa, T. and Takahashi, H. (1985) *Eur. J. Biochem.* 148, 127–134
11 Lovett, C. M. and Roberts, J. W. (1985) *J. Biol. Chem.* 260, 3305–3313
12 Kmeic, E. and Holloman, W. K. (1982) *Cell* 29, 367–374
13 Hsieh, P., Meyn, M. S. and Camerini-Otero, R. D. (1986) *Cell* 44, 885–894
14 Morrical, S. W. and Cox, M. M. (1985) *Biochemistry* 24, 760–767
15 Tsang, S. S., Muniyappa, K., Azhderian, E., Gonda, D. K., Radding, C. M., Flory, J. and Chase, J. W. (1985) *J. Mol. Biol.* 185, 295–309
16 Kowalczykowski, S. C. and Krupp, R. A. (1987) *J. Mol. Biol.* 193, 97–113
17 Menetski, J. P. and Kowalczykowski, S. C. (1985) *J. Mol. Biol.* 181, 281–295
18 Bryant, F. R., Taylor, A. R. and Lehman, I. R. (1985) *J. Biol. Chem.* 260, 1196–1202
19 Menetski, J. P. and Kowalczykowski, S. C. (1987) *J. Biol. Chem.* 262, 2085–2092
20 Menetski, J. P. and Kowalczykowski, S. C. (1987) *J. Biol. Chem.* 262, 2093–2100
21 Kowalczykowski, S. C. (1986) *Biochemistry* 25, 5872–5881
22 Register, J. C. and Griffith, J. (1985) *J. Biol. Chem.* 260, 12308–12312
23 Stasiak, A., Stasiak, A. Z. and Koller, T. (1984) *Cold Spring Harbor Symp. Quant. Biol.* 49, 561–570
24 Tsang, S. S., Chow, S. A. and Radding, C. M. (1985) *Biochemistry* 24, 3226–3232
25 Julin, D. A., Riddles, P. W. and Lehman, I. R. (1986) *J. Biol. Chem.* 261, 1025–1030
26 Gonda, D. K. and Radding, C. M. (1986) *J. Biol. Chem.* 261, 13087–13096
27 Berg, O. G., Winter, R. B. and von Hippel, P. H. (1982) *Trends Biochem. Sci.* 7, 52–55
28 Riddles, P. W. and Lehman, I. R. (1985) *J. Biol. Chem.* 260, 165–169
29 Riddles, P. W. and Lehman, I. R. (1985) *J. Biol. Chem.* 260, 170–173
30 Kahn, R. and Radding, C. M. (1984) *J. Biol. Chem.* 259, 7495–7503
31 Kowalczykowski, S. C., Clow, J. and Krupp, R. A. *Proc. Natl Acad. Sci. USA* (in press)
32 Roman, L. J. and Kowalczykowski, S. C. (1986) *Biochemistry* 25, 7375–7385
33 Howard-Flanders, P., West, S. C., Rusche, R. and Varghese, A. (1987) *J. Mol. Biol.* 193, 81–95
34 Shibata, T., Makino, O., Ikawa, S., Ohtani, T., Iwabuchi, M., Shibata, Y., Maeda, H. and Ando, T. (1984) *Cold Spring Harbor Symp. Quant. Biol.* 49, 541–551
35 Kowalczykowski, S. C., Clow, J. C., Somani, R. and Varghese, A. (1987) *J. Mol. Biol.* ???, 81–95
36 Muniyappa, K., Shaner, S. L., Tsang, S. S. and Radding, C. M. (1984) *Proc. Natl Acad. Sci. USA* 81, 2757–2761
37 Morrical, S. W., Lee, J. and Cox, M. M. (1986) *Biochemistry* 25, 1482–1494
38 Chow, S. A., Honigberg, S. M., Bainton, R. J. and Radding, C. M. (1986) *J. Biol. Chem.* 261, 6961–6971