Relating Biochemistry to Biology: How the Recombinational Repair Function of RecA Protein is Manifested in its Molecular Properties

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Summary

The multiple activities of the RecA protein in DNA metabolism have inspired over a decade of research in dozens of laboratories around the world. This effort has nevertheless failed to yield an understanding of the mechanism of several RecA protein-mediated processes, the DNA strand exchange reactions prominent among them. The major factors impeding progress are the invalid constraints placed upon the problem by attempting to understand RecA protein-mediated DNA strand exchange within the context of an inappropriate biological paradigm – namely, homologous genetic recombination as a mechanism for generating genetic diversity. In this essay I summarize genetic and biochemical data demonstrating that RecA protein evolved as the central component of a recombinational DNA repair system, with the generation of genetic diversity being a sometimes useful byproduct, and review the major in vitro activities of RecA protein from a repair perspective. While models proposed for both recombination and recombinational repair often make use of DNA strand cleavage and transfer steps that appear to be quite similar, the molecular and thermodynamic requirements of the two processes are very different. The recombinational repair function provides a much more logical and informative framework for thinking about the biochemical properties of RecA and the strand exchange reactions it facilitates.

Introduction

The RecA protein found in *E. coli* and most other bacteria has a central role in homologous genetic recombination and recombinational DNA repair[1-6]. The most important experimental model for these processes is the set of DNA strand exchange reactions promoted by the RecA protein in vitro (Fig. 1). In addition, the coprotease activity of RecA (which facilitates the autocatalytic cleavage of the LexA and related repressors) is essential for induction of the S.O.S. response to DNA damage[7] and the protein has an as yet unidentified role in S.O.S. mutagenesis[8]. This essay focuses on recombination and recombinational repair, with special emphasis on the relationship of these two functions to each other and to the biochemical properties and structure of the RecA protein.

As pointed out by Campbell[9], the real biological function of recombination systems is enigmatic and often ignored. As in other human pursuits, the solutions to scientific problems that we arrive at are influenced by the manner in which the questions are framed. It is therefore useful to consider the framework of ideas – the biological paradigms – that influence thinking about RecA protein. The first sentence of the previous paragraph can be found in some form in the Introduction to almost any paper describing RecA activity in vitro (the major variation being a statement that neglects to mention recombinational repair). The implication is that recombination and recombinational repair are in some manner distinct. An examination of the RecA literature reveals two major and competing perspectives underlying much of the current thinking about the DNA pairing and strand exchange activities of RecA protein:

(1) The *recombination paradigm*. In this view, the primary function of recombination is the creation of genetic diversity through genetic exchanges occurring (to a first approximation) at random locations along the length of homologous chromosomes. This paradigm had its genesis in the first part of this century, with studies of genetic exchanges and their biological consequences in eukaryotes, and it found an early cogent expression in the recombination model of Robin Holliday[10]. A number of additional models have been developed within this paradigm, varying as to whether strand breaks come before or after the pairing of two DNA molecules[11-15].

(2) The *repair paradigm*. In this view, the primary function of recombination is DNA repair, with recombination events initiated at single-strand gaps or double-strand breaks or crosslinks that arise as a consequence of DNA damage. The association between recombination and repair in prokaryotes is derived from studies of bacterial strains defective in recombination functions[3,5,16], and the perspective has been extended to eukaryotes[17]. This paradigm is expressed in a series of interrelated models for post-replication repair and the repair of DNA strand breaks and crosslinks[18-23].

This division can seem artificial, since few researchers in the field would place themselves entirely in one camp. The paradigms obviously overlap in the case of some models that envisage initiation of recombination at double-strand breaks[13-15,18]. These are included within the recombination paradigm above because the breaks are not necessarily seen as resulting from DNA damage. However, there is pedagogical value in treating repair and recombination as distinct paradigms, arising from a variety of ideas that can be uniquely associated with one or the other. For example, the idea of a 4-stranded DNA pairing intermediate, discussed below, has its origins in models that envisage the pairing of two homologous duplex DNAs as an initiating event in recombination.

Accurate DNA repair is made possible to a large extent because DNA is double-stranded. A lesion in one strand can be repaired by removing a segment containing the lesion, and then using the undamaged strand as a template to guide synthesis of a replacement segment. When both strands are damaged (eg. a double-strand break or crosslink) or when the lesion is in a single-stranded region of DNA, the information

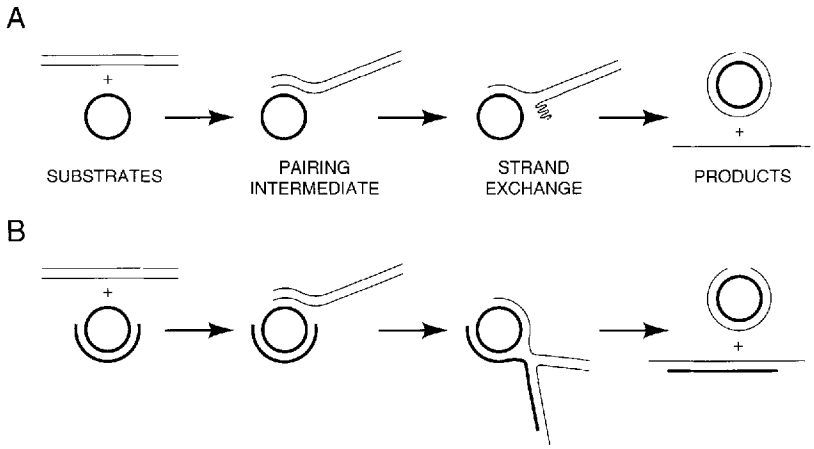

Fig. 1. RecA protein-mediated DNA strand exchange reactions with three (A) or four (B) strands. RecA nucleoprotein filaments form on the circular single-stranded or gapped DNA substrates. The homologous linear duplex is paired with the DNA within the filament, forming a putative triplex DNA pairing intermediate[61]. In the 4-strand reaction, this initial pairing interaction is restricted to the single strand gap. After the strand switch, a RecA-mediated unidirectional branch migration completes the strand exchange.

for accurate repair must come from another homologous DNA molecule via recombination.

The apparent mechanistic similarities between the two paradigms can be seen in Fig. 2. A generic model for homologous recombination as it might be presented within the recombination paradigm is shown in Fig. 2A. A model for one type of recombinational repair (post-replication repair) is presented in Fig. 2B. A DNA strand exchange reaction, mediated by RecA protein, is the centerpiece of both pathways. However, comparisons such as the one in Fig. 2 can be misleading.

To paraphrase Campbell[9]: it is widely appreciated (if rarely stated) that the immediate selective value of recombination genes and their products does not depend on their role in reshuffling genes in natural populations. Nevertheless, genetic exchange between homologous duplex chromosomes during meiosis in eukaryotes and during conjugation and transduction in bacteria provides the intellectual framework for most discussions about recombination mechanisms. A bias favoring the recombination paradigm is evident in much of the literature describing RecA activities in vitro, as well as in the fact that the enzymology of RecA and related enzymes is generally discussed at international meetings dealing with recombination as opposed to meetings focused on DNA repair. One result of this is that the RecA problem has long been framed by expectations, hypotheses and experimental designs arising from the recombination paradigm. We now consider two questions: (1) Which of these processes, generating genetic diversity or DNA repair, is more important to the bacterial cell and thus would have guided the evolution of the RecA system? (2) Which of these two paradigms provides the most appropriate framework within which to seek an understanding of the mechanism of RecA-mediated DNA strand exchange?

RecA is a Recombinational Repair System

Although occasional genetic exchanges between two cells are very useful to geneticists, the biological advantage conferred on the cell by increased genetic diversity is difficult to quantify and is probably not too important to *E. coli* growing in L broth. Recombinational DNA repair, on the other hand, is critical to cell survival every minute.

While the precise levels of DNA damage encountered by a bacterial cell growing under aerobic conditions are unknown, the measurements that have been done indicate that every cell must deal with thousands of lesions in each each generation, most of them oxidative in origin[24]. These include multiple single- and double-strand breaks[25,26]. The strand breaks, and an unknown fraction of the other lesions, are repaired by RecA-dependent pathways[20,27-29].

The importance of RecA-dependent recombinational repair to cell survival and growth is well documented. The prototypical phenotype of many RecA mutants is an extreme sensitivity to DNA damaging agents. Bacteria lacking RecA activity grow very poorly. Many of the characteristics of RecA mutants reflect a sensitivity to oxidative damage. Even in the absence of other DNA damaging agents, about half of the cells in an aerobically grown culture are dead and 10% contain no DNA[30]. Recombinational DNA repair represents a critical second line of defense to repair the DNA damage caused by reactive oxygen species not removed by catalase or superoxide dismutase[31,32]. It is the recombination activity of RecA, rather than the induction of S.O.S. functions by this same protein, that is required in recombinational repair[25,33].

A strong correlation between DNA damage and recombination frequencies is evident in the repair and recombination literature. Many separate lines of evidence point to DNA damage as the precipitating event in recombination, including:

(1) Mutations in genes such as *xth*, *ung*, *lig*, *polA* and *dam*, that cause an increase in the number of DNA strand breaks, generally exhibit a hyper-rec phenotype[34]. Cells that lack one of these activities and RecA (or other key rec functions such as recB or recC) are inviable[28,35-38], suggesting that the recombinational repair of strand breaks is critical to the survival of these strains. Notably, *xth, RecA* and *polA1, recB* cells exhibit restored viability when they are grown anaerobically[36,39].

(2) There is *no* corresponding correlation between levels of RecA protein in the cell and recombination frequencies[40].

(3) Undamaged and unreplicated bacteriophage λ DNA is effectively inert as a substrate for homologous genetic

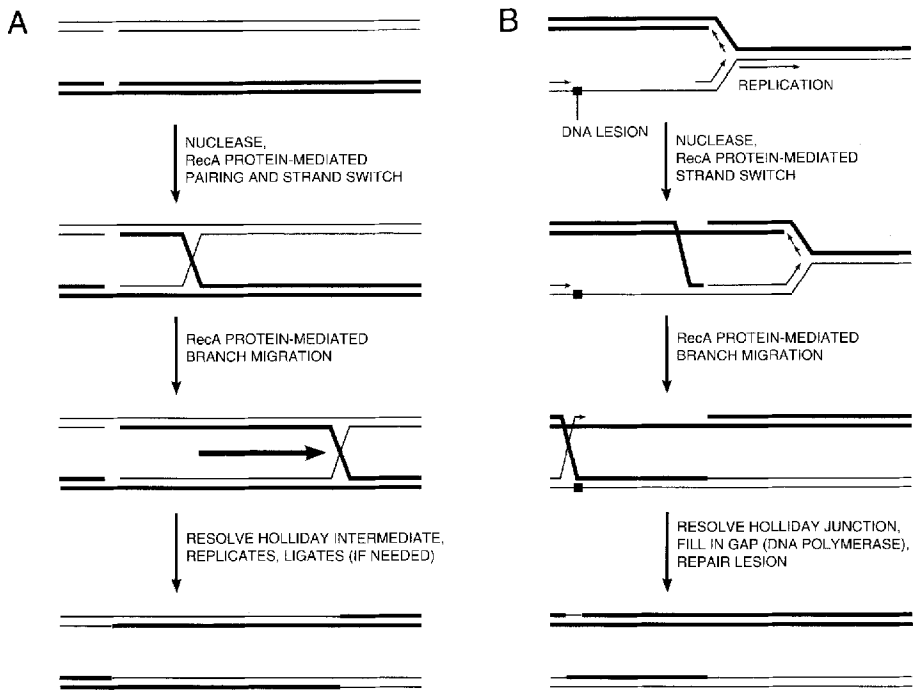

Fig. 2. Recombination versus recombinational DNA repair. (A) A generic model for a genetic exchange between two homologous chromosomes. (B) Post-replication repair of a lesion left in a single-stranded region by replication bypass. Comparable steps in the two pathways are arranged in parallel.

recombination in vivo unless it is irradiated with UV light or otherwise damaged prior to infection[40].

(4) RecA is required to repair DNA crosslinks[29]. A wild-type *E. coli* strain can survive conditions that generate 65 psoralen crosslinks per cell; a *uvrA6 RecA13* mutant cell cannot survive one crosslink[5].

(5) About half of the spontaneous mutagenesis that occurs in a bacterial cell can be traced to RecA-dependent DNA repair[41]. Interestingly, this links recombinational repair to one of the key enabling processes in evolution.

The levels of DNA damage-precipitated strand breaks and the clear correlation between DNA damage and rates of genetic exchange[3,42] strongly suggest that genetic exchanges in bacteria are *always* associated with DNA damage. The only exceptions might be recombination during conjugation or transduction, which takes advantage of the cellular DNA strand break repair system by presenting the cell with a free DNA end[15].

RecA is present in almost all bacteria. *RecA* genes have been cloned and sequenced from at least 40 different bacterial species, including mycoplasma[2]. All of these *RecA* genes are closely related in sequence. At least 71 of the 352 amino acid residues of the protein are completely conserved in bacteria that diverged over 1.5 billion years ago, and ongoing research promises to trace the origins of RecA even further back in evolutionary time. The occurrence of RecA depends to a degree on environment. Among spirochetes, free-living species have inducible RecA proteins while some virulent parasitic species (which inhabit environments where they encounter less oxygen and UV radiation) are among the very few examples of bacteria that lack detectable RecA[43]. The strong selective pressure needed to develop and maintain a molecule as complex as RecA is most readily rationalized in terms of the challenge to cell survival posed by DNA damage.

The Mechanistic Constraints Imposed by the Recombination and Repair Paradigms are Distinct

Operationally, the two processes depicted in Fig. 2 exhibit many similarities. In both cases, two DNA molecules are aligned, DNA strands are exchanged, and the resulting crossover undergoes branch migration and resolution. However, the molecular and thermodynamic requirements of these processes are very different.

First, consider the recombination pathway (of Fig. 2A). If recombination is viewed as a genetic exchange between two homologous DNA molecules which is not necessarily triggered by DNA damage, then the requirements are quite simple. Since the number of base pairs in products and substrates is the same, the process is isoenergetic, and no input of chemical energy should be required. Enzymes that catalyze the formation of Holliday intermediates without the need for high energy cofactors are known (eg. the integrase class of site-specific recombinases). If needed, double-strand breaks could be generated by a dedicated enzyme, with the resulting ends remaining bound to the complex to ensure efficient processing. Enzymes that resolve Holliday intermediates are also known[4], and branch migration itself is a spontaneous reaction in vitro[44]. The entire process might be effected by a complex of 2–4 enzymes, using no high energy cofactors except for DNA ligation. The enzyme complex might be expected to bind almost anywhere along the length of a duplex DNA molecule. The only mechanistic problem arises in the step where the two DNA molecules are aligned. This could involve the formation of a novel 4-stranded DNA pair-

ing intermediate[11,45]. Since the creation of genetic diversity implies an element of chance, the overall reaction need not be efficient and the enzymes involved could be present at very low levels.

The repair pathway of Fig. 2B is significantly more demanding in its requirements. Since failure to repair such damage could result in cell death, efficiency is critical. The system must be targeted to those locations in the DNA where repair is required. Random binding to duplex DNA would be counterproductive. To make the depicted strand exchange efficient, the branch migration must be unidirectional, other DNA binding proteins present in the cell must be removed from the path of the migrating branch, and enough energy must be applied to ensure that the branch bypasses whatever type of DNA lesion might be present. The initial DNA pairing interaction that aligns the two DNA molecules need not involve more than three DNA strands. The enzymes involved must be present at levels commensurate with the DNA damage load faced by the cell.

The in vitro properties of RecA protein can now be examined with these requirements in mind.

The Molecular Properties of RecA Protein Reflect its DNA Repair Function

A more detailed description of some of the experimental facts and arguments in the following discussion, and the literature on which they are based, can be found in a recent review[2]. There are also several detailed and current reviews featuring different perspectives[1,4]. The short summary by Radding is also recommended[6]. The sources for information presented below but not supported by citations may be found in one or more of these reviews. Studies published during the last two years are cited individually.

The RecA filament

The active species in RecA-mediated DNA strand exchange reactions is not a dimer, tetramer or some other familiar enzymatic form. Instead, it is a nucleoprotein filament which can contain thousands of monomers of RecA protein. The synthesis of the required protein components represents a substantial investment of cellular biosynthetic energy. RecA is also a DNA-dependent ATPase, and the nucleoprotein filament hydrolyzes ATP. The DNA in a RecA nucleoprotein filament formed in the presence of DNA is extended and underwound. The rise per base pair is increased from 0.34 to 0.51 nm, disrupting base stacking interactions, and 40% of the helical turns are removed to give a helical repeat of 18 bases or base pairs per turn. The protein component also exhibits an ordered helical structure, with 6 RecA monomers per turn or 3 bases per RecA monomer. The helical structure is always right-handed.

This complex structure may be viewed as a scaffold to facilitate DNA pairing and strand exchange, and it clearly serves this function. Within the repair paradigm, the filamentous form would have the additional function of excluding obstacles such as other DNA binding proteins from the path of the migrating branch. This alone could have a substantial effect on the efficiency of the overall strand exchange reaction.

DNA binding

RecA protein binds to single-stranded DNA rapidly. Nucleating anywhere, the filament is assembled in the 5′ to 3′ direction along the DNA. Since nucleoprotein filaments containing 2000 or more RecA monomers can be formed in less than two minutes, the optimal rate of filament extension may approach 1000 monomers per minute. Binding to single-stranded DNA does not require ATP, although the structure of the filament formed in the absence of ATP is quite distinct and inactive.

In contrast, RecA protein binds to duplex DNA very slowly. The rate-limiting step is nucleation, and the slow binding of the initial RecA monomer or oligomer can lead to a lag in binding of several hours under physiological conditions. Following nucleation, extension of a RecA filament on the duplex DNA is rapid and the resulting nucleoprotein filament is quite stable. Binding to duplex DNA exhibits an absolute requirement for ATP or its non-hydrolyzable analog ATPγS. A major factor in the slow rate of nucleation is the requirement for underwinding of the DNA. Any structural perturbation or sequence that facilitates DNA underwinding has a stimulatory effect on nucleation. A single-strand gap provides an optimal nucleation site, and RecA filaments extend rapidly from the gap to incorporate adjacent duplex DNA. The filament lengthening occurs in the 5′ to 3′ direction relative to the single DNA strand in the gap. The major consequence of this molecular design is that RecA binding is largely limited to regions in the DNA containing suitable nucleation sites, especially single-strand gaps, effectively targeting the protein to regions where repair is likely to be required.

A variety of DNA lesions also facilitate nucleation of RecA protein binding to duplex DNA, and this is the molecular basis for the observed preferential binding of RecA to DNA molecules containing such lesions[46]. RecA protein does not specifically recognize such lesions as binding sites, but the structural perturbations they cause provide favorable nucleation sites.

The overall binding pathway can be readily rationalized within the repair paradigm as a mechanism for restricting binding to chromosomal regions requiring repair. The same binding pathway provides a strong argument against those models within the recombination paradigm that envisage the pairing of two duplex DNAs prior to strand breakage, at least in prokaryotes.

DNA pairing: 3 strands versus 4 strands

One consequence of framing the DNA strand exchange reaction within the recombination paradigm is the widespread expectation that RecA protein will promote the formation of a 4-strand DNA pairing intermediate. Models for 4-stranded recombination intermediates have been developed and widely discussed for over 20 years[4,11,45,47,48], and the 4-strand exchange reaction illustrated in Fig. 1 is often cited as the one that is more relevant to recombination. Some evidence for a 4-strand pairing intermediate has appeared in the literature[2,49].

However, critical examination of the RecA literature compels one to conclude that RecA is fundamentally a 3-strand

system and that only three strands can be accommodated within the filament. The single-strand gap in the duplex substrate in Fig. 1B is not just the nucleation site for RecA binding; it is also the place where the strand exchange reaction is initiated. The linear duplex substrate must overlap the gap by 50-100 base pairs for an optimal reaction[5], ie. 4-strand exchanges begin as 3-strand exchanges. Direct analyses of RecA binding to DNA by a variety of physical methods indicate that three, but not four DNA strands can be accommodated in the major filament groove[50]. As noted below, 3-strand exchanges involving homologous DNA substrates do not require ATP hydrolysis. This ATP-independent mode of strand exchange, however, does not accommodate four strands[51]. Finally, the evidence for a 4-strand interaction involves a very weak signal that appears only when the second duplex substrate is supercoiled[49]. This suggests the possibility that this apparent 4-strand interaction actually reflects a 3-strand interaction in which only one strand of the supercoiled duplex (generated by transient strand separation) is incorporated into the filament.

There is, at present, no unambiguous evidence indicating the existence of a 4-stranded DNA pairing intermediate that has a significant role in the alignment of two DNA molecules during recombination in bacteria. When considered from the vantage point of the repair paradigm, there is no reason to expect such an intermediate. Once pairing and strand exchange is initiated within a 3-stranded region, branch migration can be propagated through adjacent regions in a 3- or 4-stranded mode without the need for unusual pairing interactions, as long as the two DNA molecules involved are homologous over those adjacent regions. Progress in characterizing 3-strand pairing intermediates has recently been reviewed[52]. Some indirect evidence exists for a limited 4-strand interaction around a Holliday structure that may involve less than one helical turn of a RecA-bound DNA[4].

ATP hydrolysis

DNA-dependent ATP hydrolysis was the first activity reported for purified RecA protein. The function of this activity remains one of the most controversial mechanistic issues in this system, simply because the function is not obvious in the context of a genetic exchange between homologous chromosomes. During a typical DNA strand exchange reaction in vitro, about 100 ATPs are hydrolyzed for every base pair of heteroduplex DNA generated, an expenditure that one is tempted to dismiss as artifactual.

Other work has shown that ATP hydrolysis is not required for the reaction illustrated in Fig. 1A. A substantial 3-strand exchange involving homologous substrates can be observed in the presence of ATPγS, which is not appreciably hydrolyzed by RecA protein[53]. In addition, one recA mutant and several eukaryotic proteins have been found that have greatly reduced or no ATP hydrolytic activity but promote DNA strand exchange[48,54]. One prevailing view arising from this work is that much of the observed ATP hydrolysis by RecA is wasted.

Nevertheless, the ATPase activity is not an in vitro artifact. RecA protein has one ATP-binding site per monomer. When RecA protein is bound to single-stranded DNA, ATP is hydrolyzed uniformly throughout the filament with a monomer K_{cat} of 30 min^{-1}. The monomer K_{cat} is reduced to about 22 min^{-1} when RecA is bound to duplex DNA. Hydrolysis of ATP occurs in RecA complexes on single-stranded DNA prior to strand exchange. When the homologous duplex substrate is added, the K_{cat} shifts rather abruptly to the lower rate characteristic of the complex with duplex DNA, and it remains at that rate throughout the strand exchange reaction and following the completion of strand exchange as long as ATP is regenerated.

Several possible functions for this ATP hydrolysis remain. One is the dissociation of RecA protein from one or both DNA substrates, and this has been described as perhaps its only function in some mechanistic schemes[48,55]. RecA protein dissociates in a polar fashion from the filament end opposite to that where assembly takes place, and this must be coupled in some way to ATP hydrolysis[2]. Furthermore, RecA must logically dissociate from the DNA at some point during or following strand exchange.

However, dissociation provides an incomplete rationale for the ATP hydrolytic activity. ATP hydrolysis occurs uniformly throughout the filament, and that coupled to RecA dissociation from one filament end rarely accounts for more than a minute fraction of ATP hydrolytic events. Under some conditions ATP hydrolysis proceeds without any detectable dissociation. Furthermore, no correlation exists between measurable RecA dissociation processes and rates of strand exchange. The observed disposition of RecA protein following strand exchange indicates that dissociation from the heteroduplex DNA is not a mechanistic requirement for the reaction. When the *E. coli* single-strand binding protein (SSB) is included in a 3-strand reaction (as it often is, due to the stimulatory effect it affords), essentially all of the RecA protein initially bound is found on the heteroduplex DNA product[2], and SSB is bound to the displaced single strand[56].

A more compelling rationale for ATP hydrolysis in this system can be developed by returning to the repair paradigm. During post-replication repair, RecA protein-mediated DNA strand exchange must bypass a variety of DNA lesions, and the reaction is no longer isoenergetic. In vitro, RecA-mediated strand exchange readily bypasses lesions, mismatches and even heterologous sequence insertions ranging up to a few hundred base pairs in one or both DNA substrates[2].

The bypass of heterologous inserts exhibits an absolute requirement for ATP hydrolysis. When ATP is hydrolyzed, an insert of 52 base pairs in the linear duplex substrate is bypassed and products are formed nearly as efficiently as in the reaction between homologous substrates. This same insert is a reflecting barrier to strand exchange when ATPγS is used[57]. Similar results have been obtained with a barrier as small as six base pairs[57]. ATP hydrolysis is also required to render strand exchange unidirectional[55]. ATP hydrolysis is therefore mechanistically linked with two properties of the DNA strand exchange reaction that are especially relevant to DNA repair.

The mechanism by which ATP hydrolysis is utilized to effect the bypass of insertions is not established, but it involves the generation of a torsional stress on the duplex

substrate that is used to unwind it in the region of the insert. Bypass of the heterologous insert requires the presence of homologous sequences downstream of the insert[58]. If a nick is present within the heterologous insert, the bypass is blocked[58]. In this report, Radding and colleagues proposed that the torsional stress is generated by an ATP-dependent dissociation of a DNA triplex formed downstream of the insert. An alternative mechanism in which the torsional stress is generated by a coupling of ATP hydrolysis to a ratchet-like rotation of the two DNAs has also been described[2]. The latter model accounts for the apparent inefficiency of the system and relates the rates of ATP hydrolysis to the observed rate of unidirectional strand exchange.

It should be noted that an apparently extravagant application of chemical energy is a theme of DNA repair processes. In the methyl-directed mismatch repair pathway of *E. coli* and other bacteria, a thousand nucleotides or more may be removed from a DNA strand and replaced in order to repair one mismatch[59]. The repair of alkylated guanine residues features a one-turnover alkyl transferase that is inactivated in the process, resulting in the investment of an entire protein for each repaired lesion[60]. In the case of RecA, the observed expenditure of ATP is not excessive if it is required for the bypass of structural barriers in the DNA during recombinational repair, simply because the alternative is cell death. The expenditure is not a serious problem for the cell. If all of the RecA monomers in a normal bacterial cell were hydrolyzing ATP at K_{cat}, the use of ATP would amount to less than 0.1% of that used by the 15,000 ribosomes in the same cell.

The ideas summarized above, combined with current models for DNA strand exchange[2], suggest some promising avenues for continued research. A better understanding of the evolutionary origins and function of RecA could be enhanced by a search among classes of bacteria not yet examined for the presence of *recA* genes, such as the *Archaebacteria*. The fact that ATP hydrolysis is required largely for functions related to DNA repair suggests that mutants may be found that uncouple the repair and recombination functions, producing bacterial strains that are recombination proficient but repair defective. Many other bacterial genes that have been implicated in recombination have a significant repair phenotype, and a consideration of the corresponding gene products from a repair perspective may facilitate the elucidation of their functions. This perspective may also be useful in the ongoing effort to identify and characterize recombination functions in eukaryotes. On the biochemical front, an improved understanding of the function of ATP hydrolysis by RecA protein, and of the mechanism of DNA strand exchange, is likely to come from a closer examination of how strand exchange bypasses structural barriers in DNA substrates.

Maintaining genomic integrity is a biological imperative for every cell. Genomic DNA is subject to a wide variety of chemical insults, and to respond to this damage every cell has an elaborate set of DNA repair systems. The molecular design of the RecA system revealed in the biochemical studies of the past decade reinforces the notion that the primary function of RecA is DNA repair.

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