Why does RecA protein hydrolyse ATP?

Michael M. Cox

RecA is a DNA-dependent ATPase involved in DNA-strand repair. Most of the ATP hydrolysis that occurs in a RecA nucleoprotein filament is implicitly considered to be irrelevant in many current models for RecA-mediated DNA-strand exchange. However, preventing RecA from hydrolysing ATP alters its behavior, suggesting that ATP hydrolysis by RecA is more than incidental. This review explores recent results detailing the effects and rates of ATP hydrolysis by RecA, and models are proposed that permit us to account quantitatively for ATP consumption by this protein.

THE MAJOR CONSUMPTION of nucleoside triphosphates in a typical cell occurs not in intermediary metabolism, but in the biosynthesis of information-containing macromolecules, principally the proteins and nucleic acids. In a bacterial cell, protein biosynthesis alone can account for 90% of the chemical energy used in all biosynthetic processes. If we set up a thermodynamic balance sheet, with nucleoside triphosphate consumption in the debit column and information in the credit column, we find that information is invariably expensive.

Living systems have little tolerance for errors in reactions involving information transfer. The processes of DNA replication, protein biosynthesis and even messenger RNA splicing incorporate complex proofreading mechanisms to enhance fidelity. Proofreading consumes nucleoside triphosphates in addition to those used in the polymerization reactions themselves[1]. In the case of DNA, maintaining the integrity of the information exacts an additional energetic cost in the form of repair processes. A cell's proteins and RNAs can be replaced, but the genomic DNA cannot. Every cell has multiple and redundant systems to repair the thousands of DNA lesions of all types that burden the genome in a given day[2].

DNA repair processes are notoriously profligate consumers of chemical energy. To repair one mismatched base pair, a bacterial cell may remove and replace 1000 nucleotides in one DNA strand between the mismatch and a GATC signal sequence[3]. To remove a methyl group from O^6-methylguanine, an alkyltransferase is used that is irreversibly inactivated in the reaction; thus, an entire 19 kDa protein is consumed for each O^6-methylguanine that is repaired[4].

In 1978, Ogawa and colleagues[5] reported that the *Escherichia coli* RecA protein was a DNA-dependent ATPase. In the intervening years, this enzymatic activity has been the subject of much debate, speculation and experimentation. The role of ATP hydrolysis in RecA function remains a controversial topic, with many workers favoring the view that ATP hydrolysis serves primarily to recycle RecA filaments. We have argued that RecA-mediated ATP hydrolysis is directly coupled to a synchronized rotation of two DNA molecules undergoing DNA-strand exchange[6,7], and that this coupling is critical to the function of RecA in recombinational DNA repair[8]. This review examines the data that address the function of ATP hydrolysis, and current models that attempt to explain how coupling between ATP hydrolysis and DNA-strand exchange might occur. The first step is to establish a link between ATP hydrolysis and DNA-strand exchange.

RecA-mediated ATP hydrolysis is coupled to DNA-strand exchange

The RecA protein promotes a DNA-strand-exchange reaction *in vitro* that reflects its function in recombinational DNA repair and homologous genetic recombination *in vivo*. As illustrated in Fig. 1, this reaction can involve either three or four DNA strands. The active species of RecA is a nucleoprotein filament, formed on the single-stranded or gapped DNA and containing one RecA monomer for every three nucleotides or base pairs (bp). The DNA within the filament is extended and, if it is in duplex form, underwound relative to B-form DNA. There are six RecA monomers and 18 bp or nucleotides of DNA per turn of the RecA nucleoprotein filament. The single-stranded (ss)-DNA-binding protein (SSB) facilitates filament formation on ssDNA. A second homologous DNA molecule is paired in a single-stranded region of the DNA within the nucleoprotein filament, and strand exchange ensues. The initial pairing of the two DNAs may involve the formation of a novel DNA triplex structure[9–11].

Strand exchange is unidirectional (5' to 3' with respect to the single strand within the filament) and relatively slow

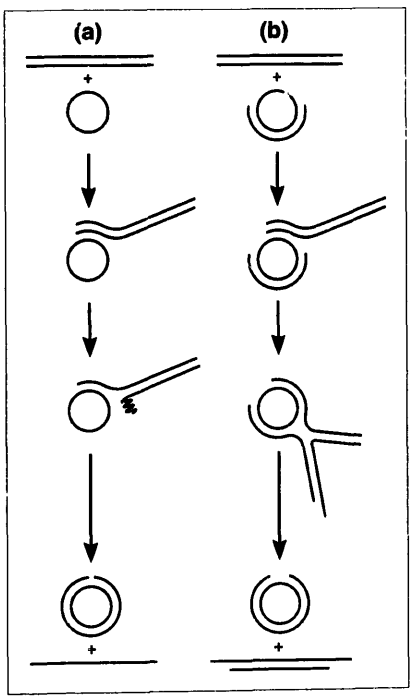

Figure 1
RecA-mediated DNA-strand-exchange reactions. Reactions are shown involving **(a)** three and **(b)** four DNA strands. In both cases, the initial pairing interaction involves only three strands. The substrates are generally derived from bacteriophage DNAs and are typically 5000–8000 nucleotides or base pairs in length.

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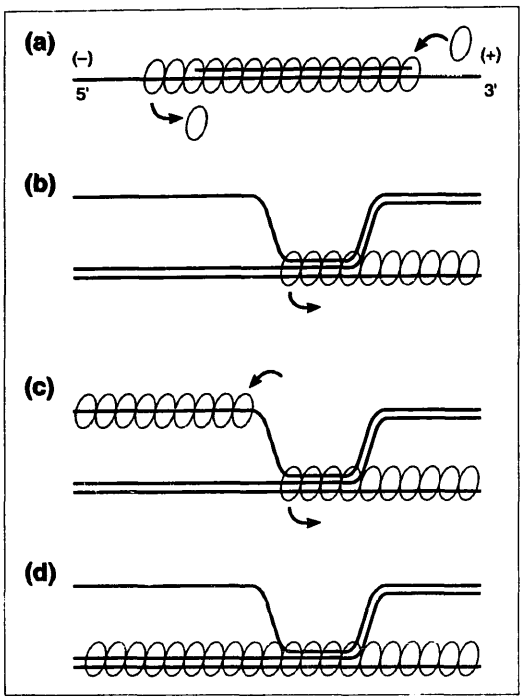

Figure 2
Models that couple DNA-strand exchange with assembly and/or disassembly of RecA filaments. **(a)** Filament assembly occurs in a 5' to 3' direction on single-stranded DNA, and can readily be extended unidirectionally into adjacent regions of duplex DNA. If the end to which monomers are added is defined as (+), dissociation is found to occur predominantly on the opposite (−) end. **(b)** A model in which disassembly of RecA filaments at the branch point is coordinated with unidirectional DNA-strand exchange. Many variations on this theme can be found in the literature (see Refs 7, 18, 19 and 25). **(c)** A variation of the disassembly model in which an assembly process is also visualized on the displaced strand[20]. Either process could be linked to DNA-strand exchange. **(d)** DNA-strand exchange without associated assembly or disassembly.

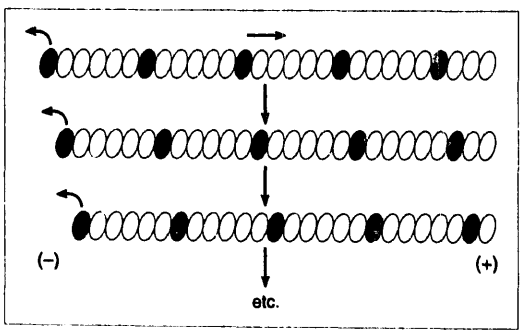

Figure 3
Proposed coordinated movement of waves of ATP hydrolysis through a RecA filament. Filled ovals represent monomers in which ATP hydrolysis is occurring. The other monomers need not be dormant, and would presumably be in other stages of a coordinated ATP hydrolytic cycle. Dissociation of monomers at the (−) end is represented by small curved arrows.

(3–10 bp s^{-1}). A key property of the RecA-mediated three-strand exchange reaction (Fig. 1a) is its capacity to bypass structural barriers in one or both DNA substrates, including heterologous inserts of 50–100 bp.

A four-strand exchange (Fig. 1b) is invariably initiated as a three-strand reaction in the single-stranded gap of the first DNA. As strand exchange proceeds beyond the gap, a Holliday intermediate is formed.

RecA protein contains a highly conserved ATP-binding site[7,12]. The controversy surrounding the role of ATP hydrolysis in DNA-strand exchange begins with the inherent inefficiency of the process. ATP is hydrolysed uniformly by monomers throughout a filament formed on ssDNA, with a monomer k_{cat} of 30 min^{-1}. When a homologous duplex DNA is added, the k_{cat} decreases abruptly to 20–22 min^{-1}, then remains at that rate throughout the ensuing reaction. Typically, about 100 ATP molecules are hydrolysed per base pair of heteroduplex DNA generated.

Several experimental findings have tended to minimize the function of ATP hydrolysis in DNA-strand exchange. Reports from the Kowalczykowski[13] and Stasiak[14] groups demonstrated that substantial DNA-strand exchange can occur in the presence of ATPγS, an ATP analog that is not readily hydrolysed by RecA. Under a narrow set of reaction conditions, as much as 3000 bp of heteroduplex DNA are formed in each set of paired DNA substrates in 2 min (Ref. 13). Furthermore, a RecA mutant that cannot hydrolyse ATP (RecA K72R) can also promote a limited degree of DNA-strand exchange *in vitro*[15]. Eukaryotic DNA-pairing proteins have been found that do not hydrolyse ATP[16] and, in bacteria, the RuvA and RuvB proteins promote a branch-migration reaction that might augment or even supplant the directed branch migration seen in RecA-mediated DNA-strand exchange[17]. Together, these results have led to the widespread view that RecA is primarily a DNA-pairing activity, and that ATP hydrolysis has no function, or at most a limited function, in the disassembly of the filament[18,19]. This interpretation is compelling in its simplicity, but it leaves most of the RecA-mediated ATP hydrolysis and a number of additional observations unexplained.

Closer examination yields an important counterpoint: the properties of the DNA-strand-exchange reaction change when ATP is hydrolysed. Recent studies have revealed limitations of the ATPγS reaction that begin to define an important and direct contribution of ATP hydrolysis to DNA-strand exchange. In the presence of ATPγS, DNA-strand exchange is limited in extent, bidirectional[20], does not bypass even minor structural barriers in the DNA[21,22], and will not accommodate four DNA strands[23]. The corresponding properties of the DNA-strand-exchange activity of RecA K72R or the eukaryotic DNA-pairing proteins have not yet been adequately investigated.

The RecA nucleoprotein filament has an intrinsic capacity to bind at least three strands of DNA and promote a strand exchange between them, as originally proposed by Howard-Flanders and colleagues[24]. ATP hydrolysis is not required for this activity, but without it the DNA-strand exchange is undirected and ill-suited to its function in recombinational DNA repair. ATP hydrolysis alters the properties of the strand-exchange reaction fundamentally: (1) it is slower, taking 5–10 min to proceed by 3000 bp; (2) it is unidirectional; (3) it readily bypasses substantial structural barriers[21,22]; and (4) it now accommodates four DNA strands[23]. These observations provide ample evidence that ATP hydrolysis is coupled in some manner to DNA-strand exchange, conferring on it properties that are critical to recombinational DNA repair[8].

The results do not establish a molecular basis for this coupling. Models can be divided loosely into two classes: those that focus on molecular events

limited to filament ends (assembly and/or disassembly), and those that envision a function for the entire filament (the DNA rotation models). As outlined below, a role for ATP hydrolysis in the disassembly of filaments has been demonstrated, but filament disassembly is not a mechanistic requirement for DNA-strand exchange.

ATP hydrolysis in RecA filament disassembly

Early recognition that the active species in RecA reactions was a filament led to inevitable comparisons with other filamentous NTPases such as actin and tubulin, yielding both insights and dead ends.

The assembly and disassembly of RecA nucleoprotein filaments has been examined in some detail. Both processes are almost entirely end dependent. Within limits reviewed elsewhere[7], RecA monomers in a filament interior do not dissociate. Assembly occurs unidirectionally, 5' to 3' on ssDNA. Disassembly is also unidirectional. Monomer addition occurs predominantly on one end (+); monomer dissociation occurs predominantly on the other (–); and both processes track along the DNA in the same direction (Fig. 2). This direction is the same as that taken by RecA-mediated DNA-strand exchange, giving rise to models[18–20,25] coupling strand exchange to filament assembly and/or disassembly (Fig. 2).

A link between ATP hydrolysis and filament disassembly has been clearly established. ATP hydrolysis weakens the interaction of RecA with DNA[19]. ATPγS permits filament assembly but blocks disassembly.

However, there is no demonstrable mechanistic link between filament assembly or disassembly and DNA-strand exchange. Filament assembly is too fast. Optimal assembly rates are at least 30–40 monomers s^{-1}, exceeding the fastest observed rates for unidirectional strand exchange by more than an order of magnitude. Rates of filament disassembly [which are readily measured only for filaments bound to linear double-stranded (ds)DNA] peak at about three monomers s^{-1} at pH values above 8.0 (Ref. 26). This correlates well with observed rates of strand exchange if it is assumed that each monomer is bound to three base pairs of DNA, but the correlation ends there.

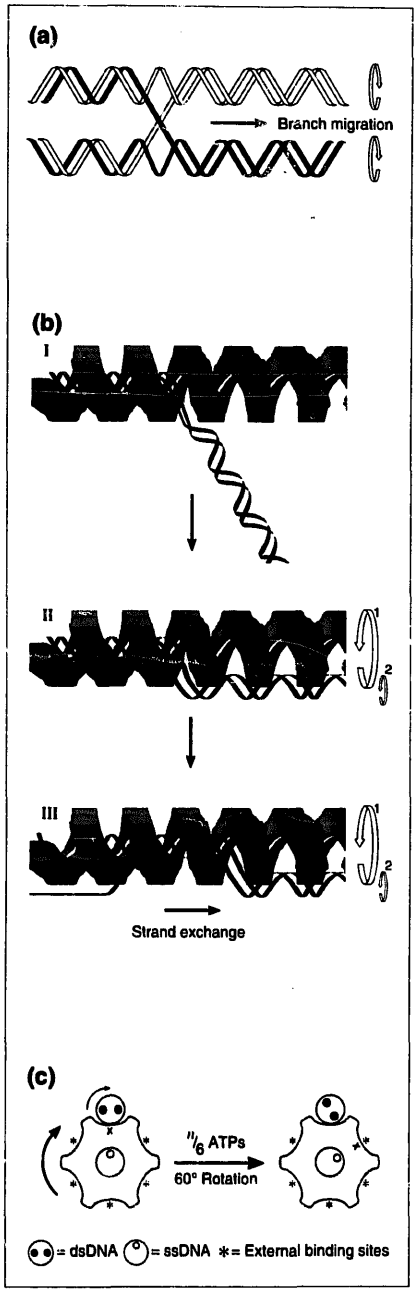

Figure 4
A model for ATP-facilitated DNA rotation to produce unidirectional DNA-strand exchange. **(a)** Two DNA molecules undergoing DNA-strand exchange must be rotated to move the branch point. **(b)** Coupling rotation to RecA-mediated ATP hydrolysis. The initial pairing process is very similar to that proposed by Howard-Flanders and colleagues[21], with three DNA strands homologously aligned within the filament. In the absence of ATP hydrolysis, binding energy within the helical groove of the filament will tend to draw additional dsDNA in and extend the heteroduplex DNA in either direction by means of rotary diffusion (panel I). This mechanism operates whether or not ATP is hydrolysed. When ATP is hydrolysed, that part of the second DNA molecule outside the filament becomes bound to one of a series of external DNA-binding sites, and rotary diffusion is constrained (panel II). ATP hydrolysis is coupled to a coordinated rotation of the two DNA substrates that produces a facilitated and unidirectional strand exchange. One 360° rotation of both DNAs is shown in moving from panel II to panel III. Arrow 1 in panels II and III indicates the rotation of the RecA filament and the DNA bound within it; arrow 2 indicates the rotation of the external DNA molecule. **(c)** A view looking down the filament from one end. Since there are six RecA monomers per turn in the nucleoprotein filament, it is assumed that there are six external DNA-binding sites. ATP hydrolysis is coupled to the transfer of the external segment of the DNA from one external DNA-binding site to the next in a kind of ratcheting motion. Six of these steps would be required to produce the 360° rotation shown in **(b)**. X marks a single monomer near the end of the filament to provide a point of reference. The facilitated DNA rotation forces the strand-exchange process in one direction at a rate dictated by the rate of ATP hydrolysis, drawing additional DNA into the groove and extruding a displaced ssDNA product as the reaction proceeds. The external DNA-binding sites extend the length of the filament, with each site formed by 1/6 of the monomers. Coordinated ATP hydrolysis by the monomers making up each of these sites in succession would produce the coordinated waves of ATP hydrolysis described in Fig. 3.

The rate of disassembly falls off sharply as pH values decrease to 6.5, while the rate of RecA-mediated DNA-strand exchange and the k_{cat} for ATP hydrolysis are constant over this pH range[26]. In addition, disassembly can be blocked with low ATPγS concentrations (2–3 μM) that have only modest effects on ATP hydrolysis or unidirectional DNA-strand exchange[26]. Finally, when SSB and ATP regeneration are included in a reaction, the RecA protein and SSB are found on the heteroduplex DNA and the displaced ssDNA, respectively, after the reaction has been completed[27,28]. Under these conditions, measurable RecA filament disassembly does not occur, even without ATPγS.

With ATP hydrolysis occurring uniformly throughout the filament, the disassembly process rarely accounts for more than a minute fraction of ATP hydrolytic events. A DNA-strand-exchange mechanism that relies on disassembly must discount the significance of most of the observed ATP hydrolysis. Disassembly models also do not explain the bypass of heterologous inserts or the ATP requirement for four-strand exchanges.

If disassembly is not coupled to DNA-strand exchange, then the focus must

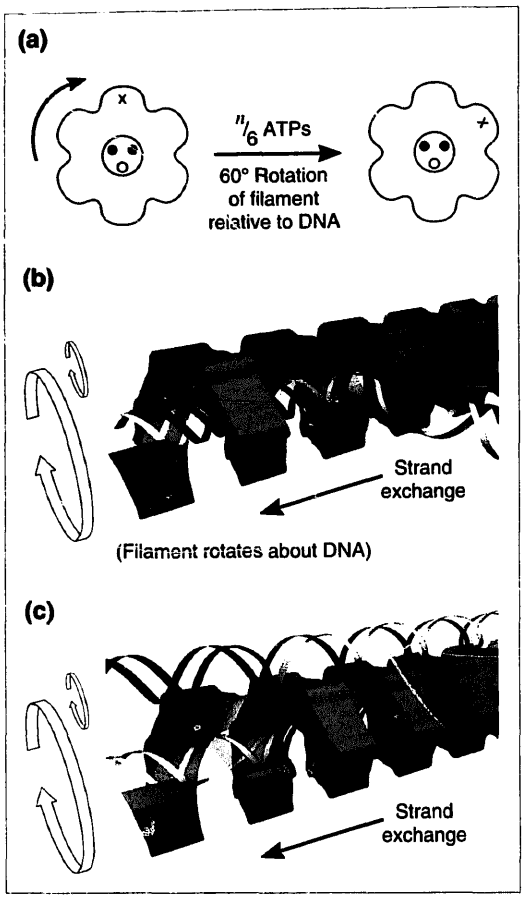

Figure 5

(a) The Radding proposal for coaxial rotation of DNA molecules to bring about unidirectional DNA-strand exchange. Either three or four strands of DNA would be rotated within the filament. **(b), (c)** Comparison of two models for DNA-strand exchange in which ATP hydrolysis is coupled to DNA rotation [coaxial **(b)** versus side-by-side **(c)**]. Side views are shown depicting a four-strand exchange. In the coaxial rotation model, heteroduplex DNA is extruded progressively as one result of rotation. In **(b)** and **(c)**, part of the filament has been rendered transparent to better display the strand-exchange process occurring in the interior.

shift to molecular events in the filament interior. One possibility is that ATP hydrolysis results in programmed conformation changes in every monomer, coupled to DNA-strand exchange and resulting in dissociation only if the monomer is at the (–) end. In this scenario, the study of disassembly might provide insights into the dynamics of the filament as a whole.

For example, some results suggest an important cooperative interaction between adjacent RecA monomers: ATP hydrolytic events might progress unidirectionally down the RecA filament in coordinated waves. This idea is derived from the measured rates of ATP hydrolysis and end-dependent filament disassembly from linear duplex DNA at high pH[7]. The k_{cat} for ATP hydrolysis by RecA monomers in a filament bound to duplex DNA is about 24 min^{-1} (somewhat less than the rate when bound to ssDNA), so that each monomer in the filament is hydrolysing one ATP about every 2.5 seconds. If adjacent monomers hydrolysed ATP independently, the monomer at the (–) end would hydrolyse an ATP anywhere from 0 to 2.5 seconds (1.25 seconds on average) after the previous monomer dissociated. If ATP hydrolysis and dissociation were coupled, this would yield a maximum rate of dissociation equivalent to one monomer every 1.25 seconds. However, the maximum rate of disassembly is four times greater than this, or three monomers s^{-1}; therefore disassembly can be coupled to ATP hydrolysis only if hydrolytic events in adjacent monomers are coordinated and staggered by 0.33 seconds. This is compatible with the observed k_{cat} for ATP hydrolysis if the monomers promoting hydrolytic events at any moment are separated by five or six monomers that are not promoting hydrolysis.

An intriguing (albeit speculative) picture emerges in which ATP hydrolysis advances cooperatively in waves separated by five or six RecA monomers, each wave moving unidirectionally through the filament at three monomers s^{-1} (Fig. 3). A new wave would reach a given monomer every 2.5 seconds, accounting for the observed k_{cat} for ATP hydrolysis. At the (–) end of the filament, the hydrolytic event may result in monomer dissociation, with the probability of dissociation being a function of pH and other factors. This dissociation is no doubt critical to the recycling of the RecA protein *in vivo*. It may also be one manifestation of a potentially fascinating molecular process under way in the filament interior.

ATP hydrolysis can produce unidirectional DNA-strand exchange if it is linked to DNA rotation

DNA is a helical molecule. Strand exchange between two DNA molecules requires DNA rotation (Fig. 4a). The direction of rotation determines the direction of strand exchange. We have proposed a model for DNA-strand exchange[6,7] that couples ATP hydrolysis to DNA rotation, shown in Fig. 4 (b, c). The coupling mechanism relies on external DNA-binding sites, lining the filament surface longitudinally, to effect the rotation. The DNA molecules are rotated about separate, parallel axes. RecA monomers throughout the filament hydrolyse ATP to bring about movement of a single strand-crossover point. This couples more chemical energy to rotation than is needed for a simple exchange of homologous DNA strands. The surplus permits the strand exchange to proceed efficiently and unidirectionally through structural barriers in the DNA.

Radding and colleagues[29] have recently proposed an alternative coupling scheme. In their model, ATP hydrolysis is coupled to a coaxial rotation of the two interwound DNA substrates relative to the filament, as shown in Fig. 5a. This model has the advantage that it does not rely on external DNA-binding sites, the existence of which is currently hypothetical. In effect, the motile force is generated by a series of internal DNA-binding sites. The two models are compared in Fig. 5 (b, c), as they might be applied to a four-strand exchange reaction.

Either model explains the properties conferred on DNA-strand-exchange reactions by ATP hydrolysis, and provides a quantitative rationalization for ATP consumption in DNA-strand exchange. Every RecA monomer in the filament is associated with one of the external (or internal) DNA-binding sites, so that each monomer would have to hydrolyse one molecule of ATP to bring about one 360° rotation. A typical filament containing about 2000 monomers will therefore consume 2000 ATP molecules to move the crossover by one helical turn (which is equivalent to 18 bp in a RecA filament). The predicted efficiency of 2000/18 (just over 100) ATP molecules per base pair of heteroduplex is very close to the observed efficiency of the reaction.

More importantly, the rate of ATP hydrolysis can be directly related to the

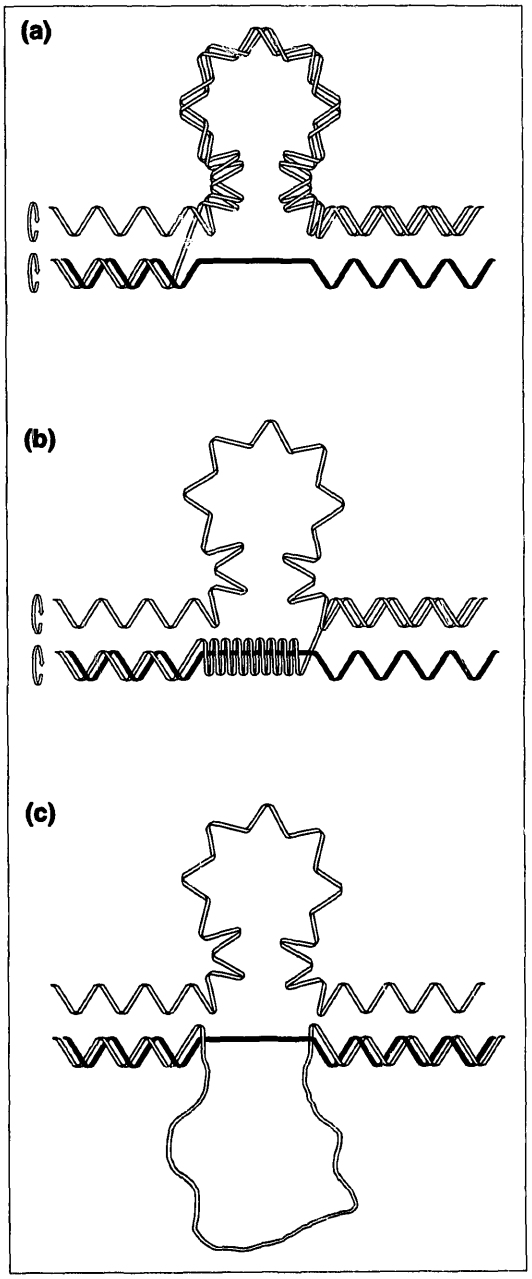

Figure 6

Bypassing a heterologous insertion in the duplex DNA substrate by means of torsional stress (generated by facilitated rotation of the DNA substrates). **(a)** The two DNAs are paired and strand exchange has proceeded normally up to the heterologous insert (represented by a DNA loop). **(b)** Continued rotation of the two DNAs in place will unwind the DNA in the insert and allow strand exchange to continue on the other side of the insert. The energy required for strand separation would be derived from ATP hydrolysis coupled to DNA rotation, which would generate a torsional stress on the insert. **(c)** The structure generated by this process would relax spontaneously upon removal of RecA protein, or could be resolved by topoisomerase action. In a four-strand exchange, the DNA structure generated by insertion bypass would be topologically much more complex and constrained. Notably, the capacity of RecA to bypass heterologous inserts during a four-strand exchange is much reduced.

rate of strand exchange. If a given monomer hydrolyses one molecule of ATP per DNA rotation, a k_{cat} of 20 min^{-1} for ATP hydrolysis translates into 20 rotations min^{-1}. Since each rotation moves the branch by 18 bp, the rate of strand exchange should be about 360 bp min^{-1} or 6 bp s^{-1}, consistent with the observed range of 3–10 bp s^{-1}.

A facilitated rotation model provides a mechanism for bypassing heterologous inserts (Fig. 6) by means of the torsional stress that would be generated on the DNA upon encountering the insert[30]. ATP-facilitated rotation of DNA on either side of the insert would simply unwind the insert DNA. Radding and colleagues[29] have demonstrated that bypass does not occur if a nick is incorporated into the insert, elegantly demonstrating that the bypass mechanism involves the generation of torsional stress. Since barrier bypass also requires ATP hydrolysis, this result provides an experimental link between ATP hydrolysis and DNA rotation.

The accommodation of three-, but not four-stranded exchanges in the presence of ATPγS is one of several pieces of evidence suggesting that the filament can bind only three strands of DNA[8]. The coupling mechanism of Figs 4 and 5c provides a rationalization for how a four-strand exchange could occur with a filament that can bind only three strands within the groove. Rotation will remove one of the strands within the groove before its replacement appears (Figs 4a and 5c). Once the DNA substrates are aligned in a three-stranded region (Fig. 1b), the four-strand exchange could proceed without a continued pairing interaction between the DNA substrates. A distinguishing characteristic of the coaxial rotation of Fig. 5b is that it can work for four-strand exchanges only if all four strands are bound inside the RecA filament.

If we set up a thermodynamic balance sheet for RecA, with ATP consumption entered as a debit, our perception of efficiency will depend on what is entered as a credit. The RecA-mediated ATP hydrolysis reaction is inefficient only when credits are limited to the generation of heteroduplex base pairs. Much more is gained in this reaction. Recombinational DNA repair is often a life-or-death proposition for a cell. Properties conferred on DNA-strand exchange by ATP hydrolysis, such as unidirectionality and the bypass of structural barriers, can be viewed as critical to RecA function in DNA repair[8]. In this context, the existence of the ATPase activity of RecA is not a molecular curiosity, but instead becomes an organizing principle for understanding the function of RecA in bacteria. Further study of the coupling between ATP hydrolysis and DNA-strand exchange promises new insights into an extraordinary molecular machine.

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Owing to *TIBS*' policy of short reference lists, the number of references cited in this article has been limited. Some work has therefore unfortunately been left unacknowledged. Omissions may be traced from Refs 7, 8, 18, 19 and 25.

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Signaling by the cytokine receptor superfamily: JAKs and STATs

James N. Ihle, Bruce A. Witthuhn, Frederick W. Quelle, Koh Yamamoto, William E. Thierfelder, Brent Kreider and Olli Silvennoinen

A variety of cytokines, lymphokines and growth factors function by interacting with receptors that are members of the cytokine receptor superfamily. These receptors share extracellular motifs and have limited similarity in their cytoplasmic domains. Although lacking catalytic domains, this family of receptors couples ligand binding with the induction of tyrosine phosphorylation. Recent studies have shown that this is mediated by members of the Janus kinase (JAK) family of cytoplasmic protein tyrosine kinases. JAKs physically associate with the membrane-proximal region of the ligand-bound receptor, leading to their tyrosine phosphorylation and activation. The activated JAKs phosphorylate the receptors as well as cytoplasmic proteins belonging to a family of transcription factors called the signal transducers and activators of transcription (STATs), providing a novel signaling pathway that is shared by all members of the cytokine receptor superfamily.

THE GROWTH, DIFFERENTIATION and functional activities of cells are regulated through the interaction of growth factors or cytokines with their cognate receptors[1,2]. A number of growth factors bind to receptor protein tyrosine kinases and mediate a biological response by activating their intrinsic protein kinase activity[3]. By contrast, a large number of cytokines bind to receptors of the cytokine receptor superfamily[4]. The type I cytokine receptors are characterized by four positionally conserved cysteines and a WSXWS motif in their extracellular domain, while the type II cytokine receptors contain characteristic cysteine pairs at both the amino and carboxyl termini. Unlike the receptor protein tyrosine kinases, receptors of the cytokine receptor superfamily do not have kinase domains, and only limited similarity is found in their cytoplasmic domains. In all the cases examined, the mitogenic function of these receptors requires a membrane-proximal cytoplasmic domain in one or more of the receptor subunits. This region contains a domain, comprising the box 1 and box 2 motifs[5], which shares limited similarity among most cytokine receptors.

Despite the absence of kinase domains in their receptors, cytokines that utilize receptors of the cytokine receptor superfamily rapidly induce tyrosine phosphorylation of cellular substrate proteins as well as of the receptors. Mutagenesis of several of these receptors has demonstrated that the ability of the receptor to couple ligand binding to protein tyrosine phosphorylation requires the membrane-proximal cytoplasmic domain, which is also required for mitogenesis. The rapid induction of tyrosine phosphorylation, the phosphorylation of the receptors and the detection of protein tyrosine kinase activity in receptor immunoprecipitates have all led to the hypothesis that a protein tyrosine kinase physically associates with the receptor and becomes activated following ligand binding.

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Identification and structure of just another family of kinases

During the past year, a number of studies have shown that cytokine receptors associate with and activate members of the JAK family of protein tyrosine kinases. The JAK family was independently identified by low-stringency hybridization[6] and by studies designed to identify novel protein tyrosine kinases by a polymerase chain reaction (PCR) approach[7,8]. Although JAK was initially an acronym for just