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## How strand exchange protein function benefits from ATP hydrolysis

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### Abstract

Members of the RecA family of strand exchange proteins carry out the central reaction in homologous recombination. These proteins are DNA-dependent ATPases, although their ATPase activity is not required for the key functions of homology search and strand exchange. We review the literature on the role of the intrinsic ATPase activity of strand exchange proteins. We also discuss the role of ATP-hydrolysis-dependent motor proteins that serve as strand exchange accessory factors, with an emphasis on the eukaryotic Rad54 family of double strand DNA-specific translocases. The energy from ATP allows recombination events to progress from the strand exchange stage to subsequent stages. ATP hydrolysis also functions to corrects DNA binding errors, including particularly detrimental binding to double strand DNA.

### Overview of StrEx protein function during recombination

Strand exchange proteins (StrEx proteins) of the RecA family carry out the central reaction of homologous recombination. StrEx proteins first polymerize cooperatively into helical nucleoprotein filaments along tracts of single strand DNA (ssDNA) formed at sites of DNA double strand breaks (DSBs) or at stressed replication forks. Recruitment of StrEx proteins to ssDNA involves both direct binding as well as assembly ‘mediator’ proteins [1]. Once formed, StrEx-ssDNA filaments search for complementary sequences within double strand DNA (dsDNA). Upon encountering a sequence match within dsDNA, strand exchange occurs to form hybrid dsDNA (Figure 1a). Thus, the recombining partners are connected in sequence register within a so-called ‘displacement loop’ (D-loop) recombination intermediate.

The bacterial StrEx protein RecA, and its eukaryotic homologs, Rad51 and Dmc1, exist in both ATP-bound and ADP-bound states [1]. The ATP-bound form has higher DNA binding affinity than the ADP bound form; ADP-bound protomers tend to dissociate from

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Conflict of interest statement

DKB has a financial interest in the use of RS-1, which is licenced to Millipore corporation by the University of Chicago. The other authors declare no conflicts.

filament ends. RecA has a relatively robust DNA-dependent ATPase activity with a  $k_{cat}$  of 25–30/min [2,3]. Rad51 and Dmc1 display a much lower level of DNA-dependent ATPase activity compared to RecA ( $k_{cat} = 0.2\text{--}1\text{ min}^{-1}$  [4,5]). The dsDNA-specific translocase Rad54 is an ATPase-dependent motor protein that displaces Rad51 from dsDNA [6]. Dmc1 can be displaced from dsDNA by another member of the Rad54 translocase family, Rdh54 [7]. Thus, there are a number of ATP-hydrolysis-dependent mechanisms for enhancing the dissociation of DNA-bound StrEx protomers from DNA, including ones involving intrinsic DNA-dependent ATPase and others involving ATP-hydrolysis dependent motor proteins in eukaryotes.

Here we review proposed roles of ATP hydrolysis in supporting StrEx protein function. We emphasize the role of ATP hydrolysis in counteracting ‘off-pathway’ dsDNA binding which has not been reviewed previously. Space limitations prevent us from mentioning all relevant and important studies involving the ‘on-pathway’ functions of ATP hydrolysis; we recommend other reviews of the subject [1,8–11]

## Proposed functions of ATPase during StrEx protein-mediated recombination

A critical finding regarding RecA’s ATPase activity was that it is not required for RecA homology search or strand exchange [12–15]. The mechanism driving homology search and exchange has been deduced from RecA-DNA crystal structures and molecular dynamic simulations (Figure 1b–d reviewed in Ref. [11]). Filament polymerization on ssDNA occurs via binding to a high affinity DNA binding site called site I that lies near the central axis of the filament. StrEx ssDNA filaments bind short tracts of dsDNA via a second, low affinity DNA binding site on each protomer called site II. Site II lies distant from the filament axis relative to site I. Site II binding dramatically distorts the dsDNA backbone. When the site II bound dsDNA matches the initiating strand bound to site I, the exchange reaction is energetically favorable; the distorted complementary strand switches basepairing partners, thereby settling into a less distorted conformation near the filament axis. Thus, homology recognition and strand exchange is driven by product stability and does not require chemical energy from ATP hydrolysis [16●●,17●●]. Analysis of Rad51 and Dmc1 mutants defective for ATPase activity found that, like RecA’s, their intrinsic hydrolytic activity is dispensable for homology search and strand exchange *in vitro* and *in vivo* [18–21].

Kowalczykowski *et al.* first proposed that the dynamic DNA binding cycle made possible by RecA’s ATPase activity promotes dissociation of RecA from nascent D-loop intermediates allowing extension of the broken 3’ end by a DNA polymerase (Figure 2a). *In vitro* reconstitution of polymerase-mediated D-loop extension later provided biochemical evidence supporting this model [22]. RecA-ATPase was also found to drive extension of hybrid DNA tracts past sites of heterology [23].

*In vivo* and *in vitro* studies of Rad51-mediated recombination indicate that the ScRad54 translocase carries out a function similar to that observed for RecA’s ATPase in dissociation from D-loops: Rad54 displaces Rad51 from the D-loop to allow for polymerase-mediated extension of the 3’ end [24]. Moreover, biochemical experiments indicate Rad54 can act as a

'heteroduplex pump,' simultaneously generating a contiguous tract of heteroduplex DNA as it removes Rad51, thereby forming a mature D-loop and priming it for extension by a DNA polymerase [25,26<sup>●●</sup>]. Further support for this model is provided by the observations that ScRad54 strongly stimulates ScRad51-mediated D-loop formation *in vitro*, and that it is required for detection of metastable D-loops *in vivo*, as distinguished from earlier less stable Rad51-dependent intermediates in which the homologous donor site is bound by the StrEx filament, but not yet matured to a stable D-loop [27,28].

There is also biochemical evidence that ATP hydrolysis can improve the efficiency of the homology search for both RecA and the eukaryotic StrEx proteins, in spite of previously discussed data indicating that ATP hydrolysis is not essential for either process (Figure 2a). A single-molecule approach was used to provide evidence that ATP hydrolysis can increase the rate of release of RecA-ssDNA filaments from metastable search intermediates at sites of microhomology [29<sup>●●</sup>]. A similar process could enhance the efficiency of the homology search *in vivo*. However, it is worth noting that the substrates used in these experiments, very long (7 kb) ssDNAs with very short (70 bp) dsDNA donor molecules, differ considerably from *in vivo* substrates. Another study using short oligo-nucleotides supported the conclusion that fully homologous hybrid joints of up to 75 bp, formed by RecA, can undergo a reverse strand exchange reaction if hydrolysis is allowed to occur, but that joints of the same length are irreversible without hydrolysis [30<sup>●</sup>]. In this study, the reverse reaction appeared to be enhanced by addition of a heterologous tail to one substrate. The authors hypothesize that ATP hydrolysis-dependent reverse strand exchange suppresses ectopic recombination between repeated DNA; this hypothesis remains to be tested *in vivo*.

ATP hydrolysis by translocases may likewise contribute to Rad51-mediated homology search [26<sup>●●</sup>,31<sup>●●</sup>]. Electron microscopy showed more heterologous pairings form when Rad51 D-loop reactions are supported by ATPase-defective mutant version of Rad54 (K341R) compared to those supported by the wild-type protein [26<sup>●●</sup>]. This result suggests that, like RecA's ATPase activity, Rad54's ATPase-dependent translocase activity may disrupt metastable heterologous interactions.

A single-molecule study argued for a different role for translocases in the search [31<sup>●●</sup>]. In this study, Rad54's ATPase activity was found to promote one dimensional (1D) scanning of a dsDNA molecule by Rad51, prompting a model in which Rad54 separates the two strands of the dsDNA in a migrating bubble to aid Rad51 homology search. However, the translocation velocity using a 1000 nt substrate was only ~590 bp/s with homology recognition occurring in just 1 of 3 encounters. If these properties were similar to those *in vivo*, genome-wide homology searches would take ~15 hours on average, suggesting that either the single molecule system is inefficient, or the *in vivo* search mechanism differs in a more qualitative way from 1D scanning.

*In vivo* studies of the role of Rad54's ATPase in Rad51-mediated homology search are conflicting. Live cell imaging of a DSB-proximal fluorescent chromosome landmark showed DSB induction increases movement of the landmark, which may reflect active homology search [32]. This DSB-dependent movement depended on Rad54's ATPase. Similar findings were reported when using chromatin immunoprecipitation (ChIP) to

analyze ScRad51-mediated homology search in response to an inducible DSB in a system lacking a homologous donor [33]. Association of Rad51 with chromosomal sites distant from the DSB was observed, and control experiments indicated distant binding reflected the homology search. In this system, Rad54 was found to be required for distant binding and hence interpreted as required for searching. Two other ChIP systems, both of which included a homologous donor (target) locus, supported the opposite conclusion as the two studies previously mentioned that lacked a donor locus. In both studies that used a donor locus, Rad54 was found to be fully dispensable for capture of the donor locus by Rad51 filaments, with evidence suggesting that the captured locus was not converted to a mature D-loop [34,35].

There is relatively little analysis of the function of RecA's ATPase *in vivo*, in part because ATPase mutants are highly toxic (see below). However, one important study avoided the problem of toxicity using a transient recombination assay [36]. This approach showed RecA-E96D, an ATPase mutant, displays only a minor (threefold) defect in the ability to form recombinants compared to wild-type. Thus, while it is possible ATP hydrolysis improves the efficiency of homology search in cells, it is clearly not essential for that process *in vivo* or *in vitro*. The recombination proficiency of RecA-E96D also emphasizes the need for further *in vivo* studies of RecA-ATPase function. In summary, while there is robust evidence that ATP hydrolysis can enhance RecA and/or Rad51 homology search in biochemical systems, the extent to which RecA's intrinsic ATPase or the ATPase of the Rad54 family of translocases contributes to searching in cells is less definitely demonstrated.

Another aspect of the role of ATP hydrolysis is its ability to regulate presynaptic StrEx filament structure and continuity, and perhaps also filament length (Figure 2a). In the active, ATP-bound form, the DNA underlying the RecA/Rad51 filament is stretched ~1.5-fold relative to B-form DNA; the ADP-bound form of the filament is more compressed [1]. Moreover, ATP binding affects filament stiffness, with ADP-bound filaments being more flexible [37,38]. Single-molecule and biophysical studies have shown that ATP hydrolysis is necessary during RecA filament formation to promote assembly of continuous filaments [38,39]. RecA's ATPase may also enhance filament function by limiting filament length [40,41].

In eukaryotes, regulation of Rad51/Dmc1 filament length and ATP/ADP-binding state is likely controlled by a host of mediator proteins, including the Rad51 paralogs, BRCA2, and SWI5-SFR1 [1]. Extensive analysis of SWI5-MEI5/SFR1, an accessory factor to both Rad51 and Dmc1 in fission yeast, and a RAD51-specific accessory factor in mammals, indicates that it stabilizes Rad51/Dmc1 filaments by promoting ADP-ATP exchange, thereby maintaining the filament in the active form [42–44]. The budding yeast SWI5-MEI5/SFR1 homolog, Sae3-Mei5, is meiosis-specific, and is required for Dmc1 focus formation and strand exchange [45]. While little is known about the mechanism through which Sae3-Mei5 promotes Dmc1 filament formation, an ATPase mutant of Dmc1, Dmc1-E157D, bypasses the requirement for Sae3-Mei5 *in vivo*, suggesting that Sae3-Mei5's function is similar to that of the fission yeast and mammalian homologs [21].

In addition to the role of accessory proteins in enhancing the activity of StrEx proteins by stabilizing filaments via inhibiting the ATP hydrolytic cycle,  $\text{Ca}^{2+}$  is well-known to support a higher level of recombination activity when used in place of  $\text{Mg}^{2+}$  *in vitro*. This is true for human Rad51 and human Dmc1 [46,47], budding yeast Dmc1 [4], and *Methanococcus voltae* RadA (*MvRadA*; an archeal member of the RecA family) [48]. Among eukaryotic proteins, budding yeast Rad51 appears to be unusual in that its activity is not stimulated by  $\text{Ca}^{2+}$  [46,49].  $\text{Ca}^{2+}$  stimulates recombination reactions, at least in part, by inhibiting ATP hydrolysis which, as discussed previously, enhances the stability of the ATP-bound, active form of StrEx filaments. Indeed, inhibition of ATPase activity appears to fully explain the stimulation of human Rad51 by  $\text{Ca}^{2+}$  [46]. Interestingly, however, stimulation of Dmc1 by  $\text{Ca}^{2+}$  cannot be fully explained by inhibition of ATPase, because replacing  $\text{Mg}^{2+}$  with  $\text{Ca}^{2+}$  results in a higher level of activity even when the non-hydrolysable ATP analogue AMP-PNP is used in place of ATP as the nucleotide cofactor [47,50]. This finding suggests  $\text{Ca}^{2+}$  likely has a positive allosteric effect distinct from that promoted by ATP binding alone. Consistent with this idea, crystal structures of *MvRadA* show that the protein simultaneously binds two metals [48]. Furthermore, the amount of  $\text{Ca}^{2+}$  required to stimulate the activity of yeast Dmc1 is drastically reduced if  $\text{Mg}^{2+}$  is also present in the reaction [49]. The concentrations of  $\text{Ca}^{2+}$  routinely used to detect elevated activity are well above the physiological range. However, combining physiological levels of both  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  results in optimal  $\text{Ca}^{2+}$ -dependent stimulation. These results are consistent with cooperation of two metals rather than competition. Together, the results prompt speculation that  $\text{Ca}^{2+}$  is an important regulator of StrEx activity in cells. However, there is no data to support this hypothesis and further study is warranted.

Finally, another highly relevant mechanism of regulation of pre-synaptic filaments on ssDNA involves the activity of the ATP hydrolysis-dependent helicase Srs2 and its relatives. These proteins displace StrEx proteins from pre-synaptic ssDNA filaments, sometimes preventing homologous recombination. The functions of this important class of proteins has been reviewed previously [51].

We conclude that hydrolysis, by RecA's intrinsic ATPase, or by Rad54 family translocases, can play significant roles before, during, and after homology search and strand exchange. Before the search, ATPase dependent processes can contribute to regulation of the length, flexibility and integrity of pre-synaptic ssDNA filaments. During the search, ATP hydrolysis can prevent stalling at micro homologies *in vitro*, but it is less clear if expenditure of ATP improves the efficiency of the search *in vivo* or not. After the search, *in vitro* and *in vivo* results appear to agree that ATP hydrolysis strips StrEx proteins from D-loop intermediates.

## StrEx proteins bind directly to dsDNA

Early biochemical studies of eukaryotic strand exchange proteins revealed that they bind dsDNA efficiently, in spite of the fact that only filaments formed on ssDNA are functional for homology search and strand exchange [4,52,53]. The dsDNA binding activity of Rad51 and Dmc1 is so high that it is usually necessary to stage biochemical D-loop assays, adding ssDNA substrates to protein before adding dsDNA substrates in order to avoid inhibition of the reaction by direct protein binding to dsDNA [54].

The similar levels of ssDNA and dsDNA binding observed for Rad51 and Dmc1 were surprising in the context of earlier reports demonstrating preferential ssDNA binding of RecA protein. Early studies of dsDNA binding by RecA only detected appreciable binding at low pH (6.2–6.8), with little or no binding detected at more physiological pH levels [55,56]. However, ATP<sub>g</sub>S, a nonhydrolyzable analog of ATP, was known to support very stable dsDNA binding, a feature critical to the success of early studies of RecA filament structure [55,57]. Because DNA binding supported by ATP was more easily detected at acidic pH, a number of studies focused on characterizing binding under this condition [56,58,59]. These studies showed dsDNA binding occurs in two stages, rate limiting initiation and more rapid and cooperative filament elongation. The initiation step of dsDNA binding is pH dependent; but at the final binding equilibrium state, both binding and ATP hydrolysis of the RecA-ATP-dsDNA complexes become pH independent, and RecA-ATP binds dsDNA stably at pH 7.5 [56]. Thus, it was concluded that the low level of dsDNA binding seen in some experiments reflected slow initiation and not equilibrium binding [56,60]. A number of earlier experiments on RecA binding to dsDNA employed nitro-cellulose filtering which is less sensitive than methods such as the 90° light scattering [56] or fluorescence polarization [61,62]. Fluorescence polarization readily detects equilibrium binding of RecA to dsDNA at physiological pH 7.5, in the presence of ATP [61]. RecA was found to bind dsDNA with a  $K_D$  of ~400 nM, only about twofold weaker than the value obtained from similar experiments using a ssDNA substrate ( $K_D$  ~200 nM). Thus, the direct dsDNA binding activity of RecA is strong and expected to occur *in vivo*, as is the case for the eukaryotic StrEx proteins.

### **Evidence that StrEx proteins bind dsDNA erroneously, with ATP hydrolysis providing the energy required for error correction**

The discovery of Rad54's biochemical activity in displacement of Rad51 from dsDNA by Heyer *et al.* [6] prompted our group to re-consider interpretation of a phenotype associated with Rad54 family translocase mutants [63]. The high level of colocalization of Rad51 and Dmc1 immunostaining foci seen in spread nuclei from wild type cells is reduced in translocase mutants. This result had been interpreted to reflect a role for translocases in coordinating the assembly of the two StrEx proteins on tracts of ssDNA at sites of DSBs. The alternative explanation, suggested by Rad54's dsDNA-specific strippase activity, was that some of the Dmc1 foci observed in *rdh54* mutants, or *rdh54 rad54* double mutants, reflect off-pathway DSB-independent complexes formed by direct binding of Dmc1 to dsDNA (Figure 2b). This explanation proved true; a large fraction of the Dmc1 foci observed in translocase deficient cells are DSB independent, while those observed in translocase proficient cells are DSB dependent [64]. We further found via ChIP experiments that Dmc1 does not show preferential binding to DSB-proximal sequences in *rad54 rdh54* translocase double mutants, suggesting that Dmc1 is sequestered from ssDNA by random dsDNA binding in translocase mutants.

Rad51 exhibits behavior similar to Dmc1 in translocase-deficient cells [65]. Three partially redundant translocases, Rad54, Rdh54, and Uls1 function to prevent the accumulation of non-repair-associated Rad51 foci. Although Uls1 has not been reported to have strippase

function *in vitro*, the redundancy it displays with Rad54 and Rdh54 *in vivo* suggests it shares that function with the other two translocases. Accumulation of non-repair foci in translocase mutants was associated with reduced growth and chromosome segregation defects. These defects were rescued by mutational inactivation of Rad51 demonstrating that Rad51 acquires toxic activity when translocase activity is absent. Work in human tumor cells showed co-depletion of human Rad54L and Rad54B (Rad54 and Rdh54 orthologues respectively) results in accumulation of Rad51 foci without induced damage [66]. These Rad51 complexes blocked replication and chromosome segregation. Amusingly, the impact of translocase depletion could be dramatically enhanced by Rad51 overexpression, causing very long Rad51 fibers to accumulate (Figure 3a). These Rad51 structures were sometimes observed as components of long Rad51-DNA bridges between cells following defective cytokinesis (Figure 3b). Another study showed that RS-1, a small molecule that enhances Rad51's DNA binding activity, caused toxic Rad51 foci to accumulate in the absence of induced DNA damage [67]. Following RS-1 treatment, both Rad51 focus counts and toxicity were enhanced by translocase depletion, and suppressed by translocase overexpression. Together, these studies indicate a key function of Rad54-family translocases is to disassemble otherwise toxic dsDNA bound Rad51 complexes.

A key implication of our study of the role of Rad54 family translocases was that Rad51 and Dmc1 have considerable off-pathway, non-productive and even toxic activity caused by direct dsDNA binding. Evidence indicating that the ATPase activity of translocases is required for dissociation of StrEx proteins further implied that the cell expends considerable energy countering destructive StrEx protein activity. Why wouldn't evolution have selected against this toxic and energetically expensive misbehavior? Our preferred explanation for this conundrum derives from the mechanism of strand exchange. As mentioned above, the initiating ssDNA strand does not dissociate from the high affinity site I upon strand exchange and the product of exchange is a dsDNA bound at site I. As consequence of this mechanism, site I must be capable of binding dsDNA. Correspondingly, when StrEx proteins bind dsDNA directly, they bind via site I (Figure 1e; [68–70]). Thus, although the mechanism of strand exchange is efficient in catalyzing formation of the hybrid DNA tracts required for homologous recombination, it appears to have an unavoidable short-coming; erroneous dsDNA binding. Chemical energy from ATP hydrolysis is required to counteract this potentially detrimental binding via a type of kinetic proofreading [71]. The importance of this correction process is emphasized by the fact that ssDNA is at very low concentration in the cell relative to dsDNA.

Our interpretation of the function of Rad54 translocases in countering dsDNA binding led us to recognize that bacterial RecA was also likely to be saddled with off-pathway dsDNA binding activity in cells. Given that bacterial homologs of Rad54 family translocases had not been identified, we hypothesized that the intrinsic ATPase activity of RecA could prevent the accumulation of erroneous dsDNA-bound structures (Figure 2b). Prior identification of a toxic, ATPase-defective version of RecA, RecA-E96D, encouraged us to test this hypothesis [36,72]. We developed a method for immunostaining of spread bacterial nucleoids that allowed us to detect functional, damage-induced RecA complexes, as well as off-pathway DNA bound forms [61]. Our study provides evidence that the toxicity of ATPase-defective forms of RecA results from off-pathway dsDNA binding. Thus, it appears that, although



RecA is not an essential protein in *Escherichia coli*, its ATPase activity is essential to prevent the protein's erroneous behavior from killing the cell. Overall, the correction of erroneous StrEx protein binding to dsDNA is a critical process, carried out by different mechanisms in prokaryotes and eukaryotes. This process is just one of a multitude of mechanisms involving expenditure of ATP to enhance the fidelity of biochemical reactions [73●].

## Concluding remarks

It has become increasingly clear that the principal function of ATP hydrolysis in the service of StrEx protein function lies in its ability to regulate binding to DNA as originally demonstrated by Kowalczykowski *et al.* In some cases, hydrolysis-dependent StrEx protein dissociation helps recombination events to progress from early to later stages. In other cases, ATP hydrolysis fuels the correction of DNA binding errors, including the particularly problematic errors resulting from binding to undamaged sites on chromosomes. Without this expenditure of energy, StrEx proteins do damage to cells rather than heal them.

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Papers of particular interest, published within the period of review, have been highlighted as:● of special interest

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- of outstanding interest

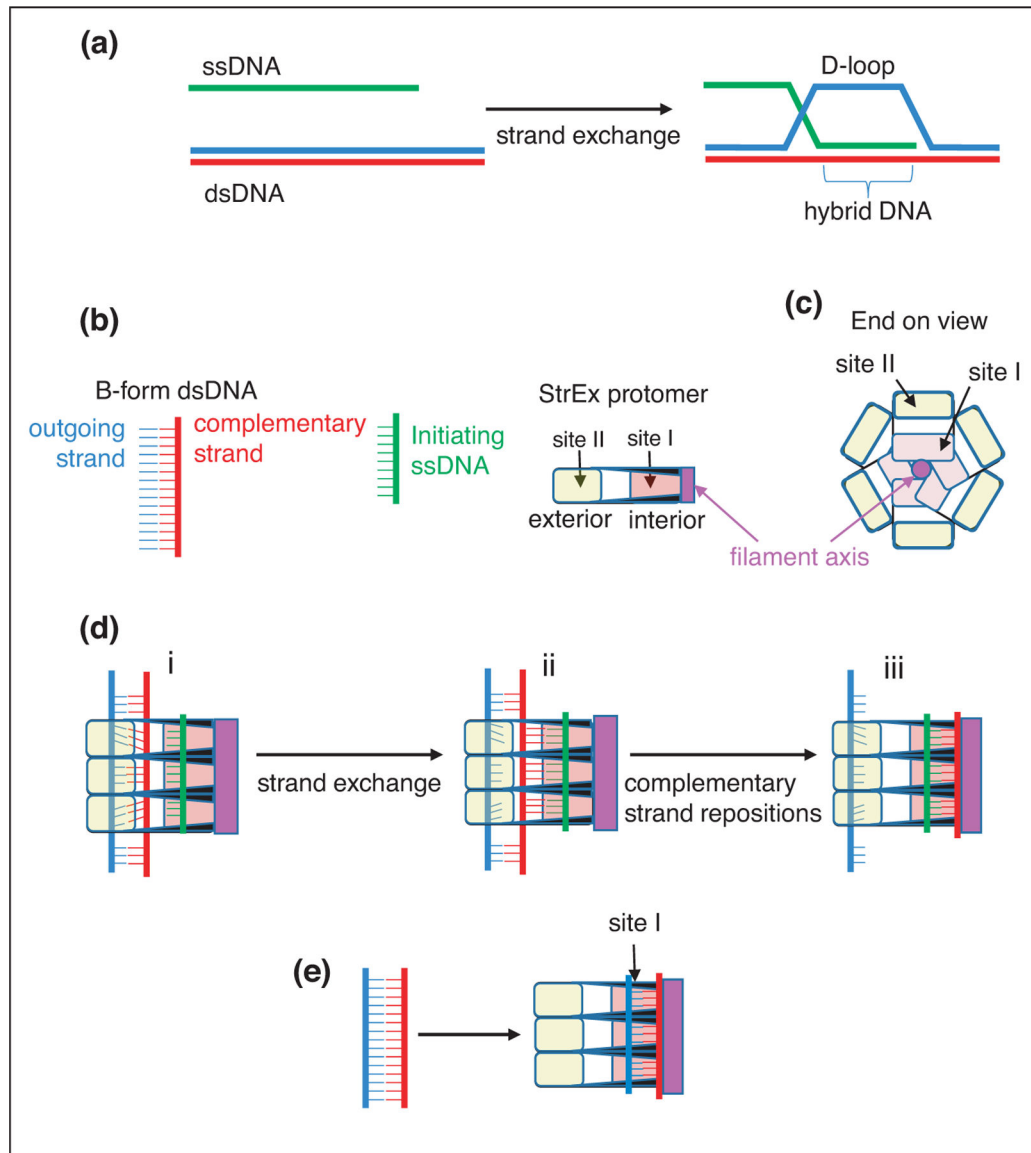
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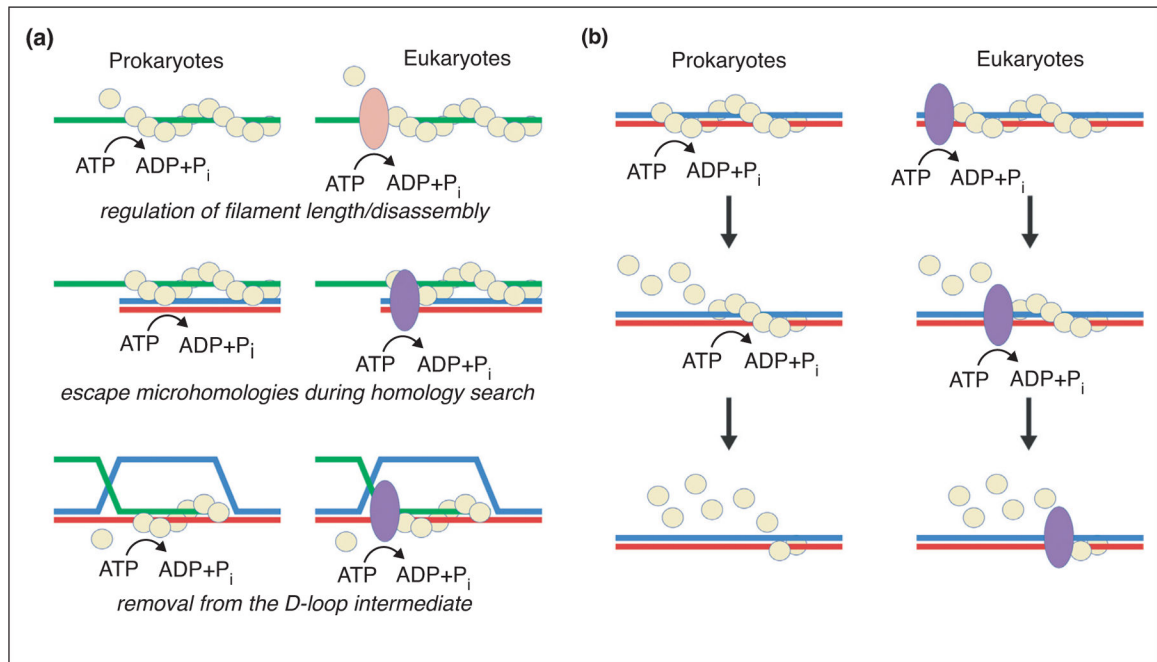
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**Figure 1. Homology recognition and strand Exchange without ATP hydrolysis.**

**(a)** Diagram of strand exchange between an initiating tract of ssDNA and a dsDNA 'donor' dsDNA molecule containing the same sequence as the initiating DNA. Strand exchange forms hybrid DNA and creates a D-loop intermediate. **(b)** Diagrams of components including dsDNA, ssDNA and a StrEx protein protomer. The positions of the high affinity DNA binding site (site I) and the low affinity DNA binding site (site II) relative to the helical axis (purple rectangle) are shown. **(c)** End on view of one turn (6 protomers) of the helical filament using the same color scheme as in (b); DNA is not shown. This view helps emphasize that site I lies closer to the filament axis than site II. A consequence of this geometry is that a dsDNA bound to site II must assume a more extended configuration relative to B-form DNA, than a corresponding dsDNA bound at site I. **(d)** The strand exchange mechanism proposed by Prentiss and Prévost based on theoretical modeling and molecular dynamic simulations [16<sup>●●</sup>, 17<sup>●●</sup>]. Three protomers are shown in a flattened

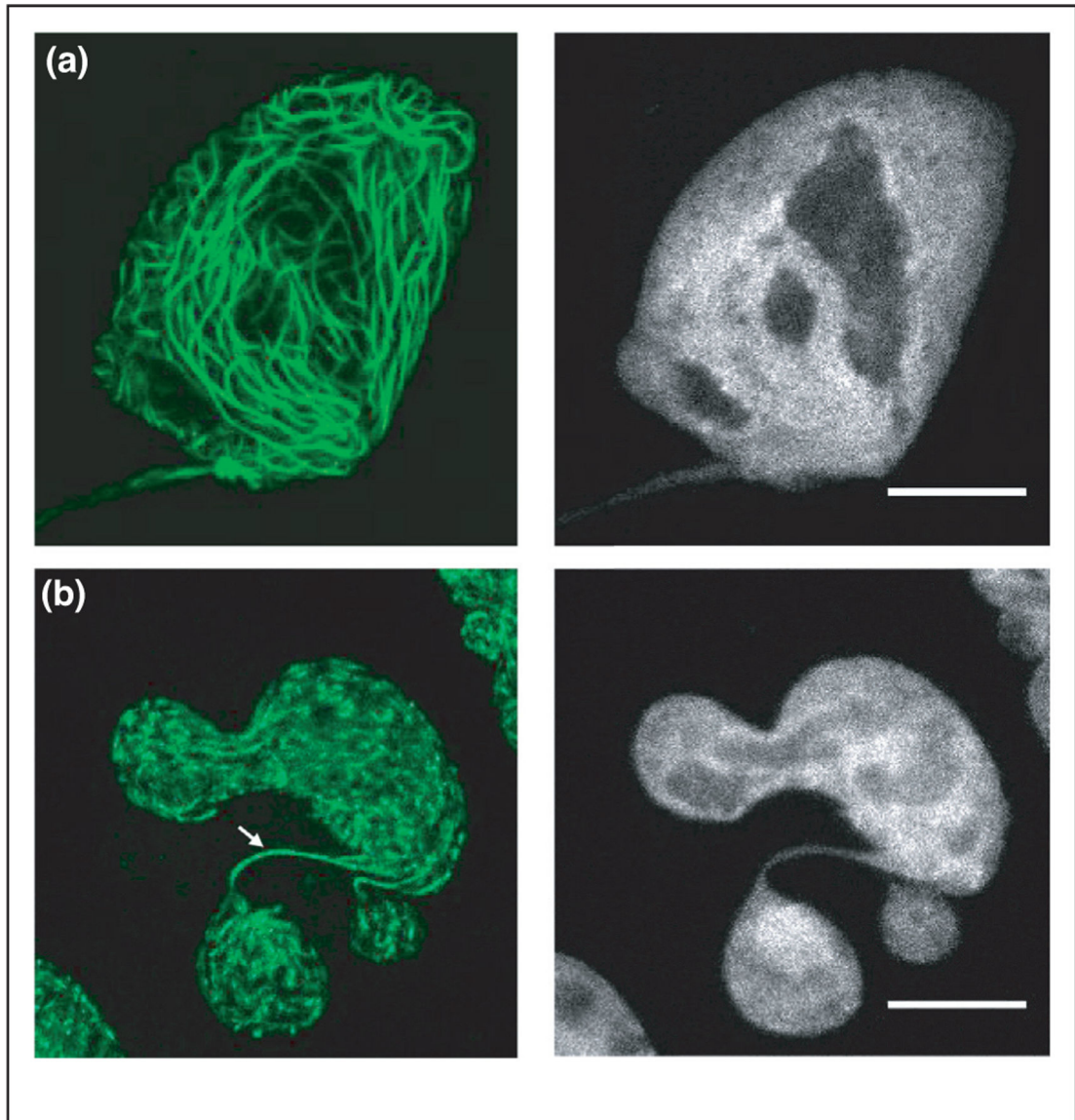
projection representing the interior surface of one half of a turn of the helical filament. **(i)** Configuration of bound DNA before strand exchange. The binding of the outgoing strand backbone to site II causes severe backbone extension which unstacks bases in the duplex. The complementary strand is also distorted via base pairing to the outgoing strand even though it has very few direct protein interactions. The extension of the backbone unstacks bases of the complementary strand allowing them to flip away from their initial base pairing partners on the outgoing strand, thereby testing for homology via attempted pairing with bases from the initiating ssDNA bound to site I. The initiating strand is also in an extended conformation owing to intercalation of site I residues that separates triplets of stacked bases. If the sequence of the DNA bound to site II does not match the ssDNA in site I, the dsDNA will rapidly dissociate allowing the homology search to continue. If the sequence matches, strand exchange is energetically favorable, and occurs without the need for ATP hydrolysis. **(ii)** The initial product of strand exchange in which complementary strand bases are paired with bases from the initiating strand. The complementary strand backbone remains near its starting location. **(iii)** The complementary strand backbone undergoes a structural rearrangement positioning it closer to the filament axis in a less extended configuration. The product of strand exchange is a dsDNA tightly bound at site I. **(e)** Direct binding of dsDNA to StrEx proteins occurs via binding to site I. Parts B and D of this Figure represent a simplified version of parts of a previously published figure ([16<sup>●●</sup>] Figure 2).



**Figure 2. On-pathway and off-pathway ATP consumption by the StrEx proteins. ATP regulates StrEx protein affinity for DNA.**

Prokaryotic RecA has robust ATPase activity, whereas eukaryotic Rad51 and Dmc1 have reduced rates of ATP hydrolysis. Functions accomplished by RecA ATP consumption in prokaryotes are replaced in eukaryotes by ATP hydrolysis of accessory proteins such as the Rad54 family of translocases (shown in purple) and the Srs2 family of helicases (shown in pink). **(a) On-pathway functions of ATP hydrolysis. Top:** Following DNA damage, the StrEx proteins form helical filaments on ssDNA. In prokaryotes (left), ATP hydrolysis may limit filament length or promote filament disassembly to allow for repair via an alternative DNA repair pathway. RecA filaments can also be disassembled by the UvrD helicase. In eukaryotes (right) filament disassembly is driven by the UvrD homolog Srs2. **Middle:** During homology search, ATP hydrolysis by RecA allows it to escape persistent associations with microhomologies (left). Eukaryotic Rad51 relies on ATP expenditure by Rad54 (right) for this same function. **Bottom:** Following strand exchange, RecA ATP hydrolysis promotes its dissociation from the 3 end of the broken strand to allow for extension of the D-loop by a DNA polymerase. In eukaryotes, Rad54 removes Rad51 from the D-loop. **(b) Off-pathway functions of ATP hydrolysis.** StrEx proteins form off-pathway complexes on dsDNA. Intrinsic ATP hydrolysis by RecA (left), or ATP expenditure by the Rad54 family of translocases (right), removes the StrEx proteins from dsDNA.





**Figure 3. Toxic RAD51 complexes in RAD51 overexpressing HT1080 cells.**

RAD51 overexpression was induced in HT1080 cells containing RAD51 under the control of a doxycycline repressible promoter after transfection with siRNAs targeting RAD54L and RAD54B. Cells were stained with RAD51 antibodies (shown in green). DNA was stained with 4',6-diamidino-2-phenylindole (DAPI, shown in white). Representative images depict cells showing (a) extensive elongated RAD51 fibers and (b) a nucleus containing a DAPI-staining DNA bridge that co-localizes with a RAD51 fiber. The bridge is marked with an arrow. Scale bar = 10  $\mu$ m. Figure kindly provided by Jennifer Mason (Clemson University).