HHS Public Access

Author manuscript

Curr Opin Genet Dev. Author manuscript; available in PMC 2021 December 15.

Published in final edited form as:

Curr Opin Genet Dev. 2021 December ; 71: 120–128. doi:10.1016/j.gde.2021.06.016.

How strand exchange protein function benefits from ATP hydrolysis

Diedre Reitz[1], **Yuen-Ling Chan**[2], **Douglas K Bishop**[2,3]

[1]Department of Microbiology and Molecular Genetics, University of California, Davis, CA, USA

[2]Department of Radiation and Cellular Oncology, University of Chicago, IL, USA

[3]Department of Molecular Genetics and Cell Biology, University of Chicago, IL, USA

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

Corresponding author: Bishop, Douglas K (dbishop@uchicago.edu).

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 kcat of 25–30/min [2,3]. Rad51 and Dmc1 display a much lower level of DNA-dependent ATPase activity compared to RecA (kcat = 0.2–1 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 extention 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••]. Further support for this model is provided by the observations that ScRad54 strongly stimulates ScRad51-mediated D-loop formation *in vitro*, and 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••]. 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•]. 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••,31••]. 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••]. 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••]. 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 reponse 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 its 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, Ca^{2+} is well-known to support a higher level of recombination activity when used in place of Mg^{2+} *in vitro*. This is true for human Rad51 and human Dmc1 [46,47], budding yeast Dmc1 [4], and *Methanococcus voltae* RadA (*Mv*RadA; 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 Ca^{2++} [46,49]. 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 Ca^{2+} [46]. Interestingly, however, stimulation of Dmc1 by Ca^{2+} cannot be fully explained by inhibition of ATPase, because replacing Mg^{2+} with 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 Ca^{2+} likely has a positive allosteric effect distinct from that promoted by ATP binding alone. Consistent with this idea, crystal structures of *Mv*RadA show that the protein simultaneously binds two metals [48]. Furthermore, the amount of Ca^{2+} required to stimulate the activity of yeast Dmc1 is drastically reduced if Mg^{2+} is also present in the reaction [49]. The concentrations of Ca^{2+} routinely used to detect elevated activity are well above the physiological range. However, combining physiological levels of both Mg^{2+} and Ca^{2+} results in optimal Ca^{2+}-dependent stimulation. These results are consistent with cooperation of two metals rather than competition. Together, the results prompt speculation that 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 substates 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, ATPgS, 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 nitrocellulose filtering which is less sensitive than methods such as the 90º 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 stripase

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 in 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.

Acknowledgements

We thank Jennifer Mason for contributing Figure 3 and Mara Prentiss for approving Figure 1. DKB would also like to thank Steve Kowalczykowski for alerting us to the work of Campbell and Davis shortly after we proposed that Dmc1 displays off-pathway dsDNA binding. This paper was supported by the National Institutes of Health (grant # R35GM134942 to DKB and T32CA108459 to DR).

References and recommended reading

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

● of special interest

●● of outstanding interest

1. Kowalczykowski SC: An overview of the molecular mechanisms of recombinational DNA repair. Cold Spring Harb Perspect Biol 2015, 7:a016410. [PubMed: 26525148]
2. Brenner SL, Mitchell RS, Morrical SW, Neuendorf SK, Schutte BC, Cox MM: recA Protein-promoted ATP hydrolysis occurs throughout recA nucleoprotein filaments. J Biol Chem 1987, 262:4011–4016. [PubMed: 2951381]
3. Ogawa T, Wabiko H, Tsurimoto T, Horii T, Masukata H, Ogawa H: Characteristics of purified recA protein and the regulation of its synthesis in vivo. Cold Spring Harb Sym 1979, 43:909–915.
4. Hong EL, Shinohara A, Bishop DK: Saccharomyces cerevisiae Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous super-coiled duplex DNA. J Biol Chem 2001, 276:41906–41912. [PubMed: 11551925]
5. Sung P: Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science 1994, 265:1241–1243. [PubMed: 8066464]
6. Solinger JA, Kiianitsa K, Heyer W-D: Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. Mol Cell 2002, 10:1175–1188. [PubMed: 12453424]
7. Chi P, Kwon Y, Moses DN, Seong C, Sehorn MG, Singh AK, Tsubouchi H, Greene EC, Klein HL, Sung P: Functional interactions of meiotic recombination factors Rdh54 and Dmc1. DNA Repair 2009, 8:279–284. [PubMed: 19028606]
8. Cox MM: Motoring along with the bacterial RecA protein. Nat Rev Mol Cell Bio 2007, 8:127–138. [PubMed: 17228330]

9. Heyer W-D, Li X, Rolfsmeier M, Zhang X-P: Rad54: the swiss army knife of homologous recombination? Nucleic Acids Res 2006, 34:4115–4125. [PubMed: 16935872]
10. Bell JC, Kowalczykowski SC: RecA: regulation and mechanism of a molecular search engine. Trends Biochem Sci 2016, 41:491–507. [PubMed: 27156117]
11. Prentiss M, Prévost C, Danilowicz C: Structure/function relationships in RecA protein-mediated homology recognition and strand exchange. Crit Rev Biochem Mol 2015, 50:453–476.
12. Cox MM, Lehman IR: Directionality and polarity in recA protein-promoted branch migration. Proc Natl Acad Sci U S A 1981, 78:6018–6022. [PubMed: 6273839]
13. Honigberg SM, Gonda DK, Flory J, Radding CM: The pairing activity of stable nucleoprotein filaments made from recA protein, single-stranded DNA, and adenosine 5´-(gamma-thio) triphosphate. J Biol Chem 1985, 260:11845–11851. [PubMed: 3840165]
14. Rosselli W, Stasiak A: Energetics of RecA-mediated recombination reactions without ATP hydrolysis RecA can mediate polar strand exchange but is unable to recycle. J Mol Biol 1990, 216:335–352. [PubMed: 2147722]
15. Menetski JP, Bear DG, Kowalczykowski SC: Stable DNA heteroduplex formation catalyzed by the *Escherichia coli* RecA protein in the absence of ATP hydrolysis. Proc Natl Acad Sci U S A 1990, 87:21–25. [PubMed: 2404275]
16. Vlassakis J, Feinstein E, Yang D, Tilloy A, Weiller D, Kates-Harbeck J, Coljee V, Prentiss M: Tension on dsDNA bound to ssDNA-RecA filaments may play an important role in driving efficient and accurate homology recognition and strand exchange. Phys Rev E 2013, 87:032702 ●● This paper provides a biophysical model for the homology search and strand exchange.
17. Yang D, Boyer B, Prévost C, Danilowicz C, Prentiss M: Integrating multi-scale data on homologous recombination into a new recognition mechanism based on simulations of the RecA-ssDNA/dsDNA structure. Nucleic Acids Res 2015, 43:10251–10263 [PubMed: 26384422] ●● This study uses molecular dynamic simulations to characterize the mechanism of homology recognition by RecA.
18. Sung P, Stratton SA: Yeast Rad51 recombinase mediates polar DNA strand exchange in the absence of ATP hydrolysis. J Biol Chem 1996, 271:27983–27986. [PubMed: 8910403]
19. Morrison C, Shinohara A, Sonoda E, Yamaguchi-Iwai Y, Takata M, Weichselbaum RR, Takeda S: The essential functions of human Rad51 are independent of ATP hydrolysis. Mol Cell Biol 1999, 19:6891–6897. [PubMed: 10490626]
20. Fung CW, Fortin GS, Peterson SE, Symington LS: The rad51-K191R ATPase-defective mutant is impaired for presynaptic filament formation. Mol Cell Biol 2006, 26:9544–9554. [PubMed: 17030607]
21. Reitz D, Grubb J, Bishop DK: A mutant form of Dmc1 that bypasses the requirement for accessory protein Mei5-Sae3 reveals independent activities of Mei5-Sae3 and Rad51 in Dmc1 filament stability. PLoS Genet 2019, 15:e1008217. [PubMed: 31790385]
22. Xu L, Marians KJ: A dynamic RecA filament permits DNA polymerase-catalyzed extension of the invading strand in recombination intermediates. J Biol Chem 2002, 277:14321–14328. [PubMed: 11832493]
23. Kim JI, Cox MM, Inman RB: On the role of ATP hydrolysis in RecA protein-mediated DNA strand exchange. I. Bypassing a short heterologous insert in one DNA substrate. J Biol Chem 1992, 267:16438–16443. [PubMed: 1644827]
24. Li X, Heyer W-D: RAD54 controls access to the invading 3′-OH end after RAD51-mediated DNA strand invasion in homologous recombination in Saccharomyces cerevisiae. Nucleic Acids Res 2009, 37:638–646. [PubMed: 19074197]
25. Wright WD, Heyer W-D: Rad54 functions as a heteroduplex DNA pump modulated by its DNA substrates and Rad51 during D loop formation. Mol Cell 2014, 53:420–432. [PubMed: 24486020]
26. Tavares EM, Wright WD, Heyer W-D, Cam EL, Dupaigne P: In vitro role of Rad54 in Rad51-ssDNA filament-dependent homology search and synaptic complexes formation. Nat Commun 2019, 10:4058 [PubMed: 31492866] ●● This article uses electron microscopy to visualize Rad51-mediated D-loops, and to show that Rad54 has critical functions in Rad51 homology search and D-loop formation.

27. Petukhova G, Stratton S, Sung P: Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature 1998, 393:91–94. [PubMed: 9590697]
28. Piazza A, Shah SS, Wright WD, Gore SK, Koszul R, Heyer W-D: Dynamic processing of displacement loops during recombinational DNA repair. Mol Cell 2019, 73:1255–1266.e4. [PubMed: 30737186]
29. Lee JY, Qi Z, Greene EC: ATP hydrolysis promotes duplex DNA release by the RecA presynaptic complex. J Biol Chem 2016, 291:22218–22230 [PubMed: 27587394] ●● Single molecule experiments show the ATPase activity of RecA can prevent stalling of the homology search at sites of microhomology.
30. Danilowicz C, Hermans L, Coljee V, Prévost C, Prentiss M: ATP hydrolysis provides functions that promote rejection of pairings between different copies of long repeated sequences. Nucleic Acids Res 2017, 45:gkx582 ● This work provides evidence that hybrid DNA tracts of up to 75 bp can be converted back to substrates by reverse strand exchange.
31. Crickard JB, Moevus CJ, Kwon Y, Sung P, Greene EC: Rad54 drives ATP hydrolysis-dependent DNA sequence alignment during homologous recombination. Cell 2020, 181:1380–1394. e18 [PubMed: 32502392] ●● Rad54 is shown to drive a 1D homology search along individual dsDNA molecules.
32. Dion V, Kalck V, Horigome C, Towbin BD, Gasser SM: Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. Nat Cell Biol 2012, 14:502–509. [PubMed: 22484486]
33. Renkawitz J, Lademann CA, Kalocsay M, Jentsch S: Monitoring homology search during DNA double-strand break repair in vivo. Mol Cell 2013, 50:261–272. [PubMed: 23523370]
34. Sugawara N, Wang X, Haber JE: In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol Cell 2003, 12:209–219. [PubMed: 12887906]
35. Wolner B, Peterson CL: ATP-dependent and ATP-independent roles for the Rad54 chromatin remodeling enzyme during recombinational repair of a DNA double strand break. J Biol Chem 2005, 280:10855–10860. [PubMed: 15653683]
36. Campbell MJ, Davis RW: Toxic mutations in the recA gene of *E. coli* prevent proper chromosome segregation. J Mol Biol 1999, 286:417–435. [PubMed: 9973561]
37. Hegner M, Smith SB, Bustamante C: Polymerization and mechanical properties of single RecA–DNA filaments. Proc Natl Acad Sci U S A 1999, 96:10109–10114. [PubMed: 10468570]
38. van Loenhout MTJ, van der Heijden T, Kanaar R, Wyman C, Dekker C: Dynamics of RecA filaments on single-stranded DNA. Nucleic Acids Res 2009, 37:4089–4099. [PubMed: 19429893]
39. Kim SH, Ahn T, Cui TJ, Chauhan S, Sung J, Joo C, Kim D: RecA filament maintains structural integrity using ATP-driven internal dynamics. Sci Adv 2017, 3:e1700676 [PubMed: 28913424] ● This article develops a single-molecule fluorescence assay to monitor RecA filament dynamics in the presence of ATP. Reorganization of individual RecA monomers into a continuous filament requires ATP hydrolysis and is influenced by the Chi sequence.
40. Kim SH, Park J, Joo C, Kim D, Ha T: Dynamic growth and shrinkage govern the pH dependence of RecA filament stability. PLoS One 2015, 10:e0115611. [PubMed: 25608006]
41. Zhao B, Zhang D, Li C, Yuan Z, Yu F, Zhong S, Jiang G, Yang Y-G, Le XC, Weinfeld M et al. : ATPase activity tightly regulates RecA nucleofilaments to promote homologous recombination. Cell Discov 2017, 3:16053 [PubMed: 28101376] ● Evidence that ATP hydrolysis limits RecA filament length in a manner that enhances function.
42. Kurokawa Y, Murayama Y, Haruta-Takahashi N, Urabe I, Iwasaki H: Reconstitution of DNA strand exchange mediated by Rhp51 recombinase and two mediators. PLoS Biol 2008, 6: e88. [PubMed: 18416603]
43. Su G-C, Chung C-I, Liao C-Y, Lin S-W, Tsai C-T, Huang T, Li H-W, Chi P: Enhancement of ADP release from the RAD51 presynaptic filament by the SWI5-SFR1 complex. Nucleic Acids Res 2014, 42:349–358. [PubMed: 24078249]
44. Haruta N, Kurokawa Y, Murayama Y, Akamatsu Y, Unzai S, Tsutsui Y, Iwasaki H: The Swi5–Sfr1 complex stimulates Rhp51/Rad51 - and Dmc1-mediated DNA strand exchange in vitro. Nat Struct Mol Biol 2006, 13:823–830. [PubMed: 16921379]

45. Hayase A, Takagi M, Miyazaki T, Oshiumi H, Shinohara M, Shinohara A: A protein complex containing Mei5 and Sae3 promotes the assembly of the meiosis-specific RecA homolog Dmc1. Cell 2004, 119:927–940. [PubMed: 15620352]
46. Bugreev DV, Mazin AV: Ca^{2+} activates human homologous recombination protein Rad51 by modulating its ATPase activity. Proc Natl Acad Sci U S A 2004, 101:9988–9993. [PubMed: 15226506]
47. Bugreev DV, Golub EI, Stasiak AZ, Stasiak A, Mazin AV: Activation of human meiosis-specific recombinase Dmc1 by Ca^{2+}. J Biol Chem 2005, 280:26886–26895. [PubMed: 15917244]
48. Qian X, He Y, Ma X, Fodje MN, Grochulski P, Luo Y: Calcium stiffens archaeal Rad51 recombinase from *Methanococcus voltae* for homologous recombination. J Biol Chem 2006, 281:39380–39387. [PubMed: 17050545]
49. Chan Y-L, Brown MS, Qin D, Handa N, Bishop DK: The third exon of the budding yeast meiotic recombination gene HOP2 is required for calcium-dependent and recombinase Dmc1-specific stimulation of homologous strand assimilation. J Biol Chem 2014, 289:18076–18086. [PubMed: 24798326]
50. Lee M-H, Chang Y-C, Hong EL, Grubb J, Chang C-S, Bishop DK, Wang T-F: Calcium ion promotes yeast Dmc1 activity via formation of long and fine helical filaments with single-stranded DNA. J Biol Chem 2005, 280:40980–40984. [PubMed: 16204247]
51. Niu H, Klein HL: Multifunctional roles of *Saccharomyces cerevisiae* Srs2 protein in replication, recombination and repair. Fems Yeast Res 2017, 17:1–11 10.1093/femsyr/fow111.
52. Baumann P, Benson FE, West SC: Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. Cell 1996, 87:757–766. [PubMed: 8929543]
53. Zaitseva EM, Zaitsev EN, Kowalczykowski SC: The DNA binding properties of *Saccharomyces cerevisiae* Rad51 protein. J Biol Chem 1999, 274:2907–2915. [PubMed: 9915828]
54. Sung P, Robberson DL: DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. Cell 1995, 82:453–461. [PubMed: 7634335]
55. McEntee K, Weinstock GM, Lehman IR: Binding of the recA protein of *Escherichia coli* to single- and double-stranded DNA. J Biol Chem 1981, 256:8835–8844. [PubMed: 7021553]
56. Pugh BF, Cox MM: Stable binding of recA protein to duplex DNA. Unraveling a paradox. J Biol Chem 1987, 262:1326–1336. [PubMed: 3543002]
57. Capua ED, Engel A, Stasiak A, Koller Th: Characterization of complexes between recA protein and duplex DNA by electron microscopy. J Mol Biol 1982, 157:87–103. [PubMed: 7050394]
58. Pugh BF, Cox MM: recA protein binding to the heteroduplex product of DNA strand exchange. J Biol Chem 1987, 262:1337–1343. [PubMed: 3543003]
59. van der Heijden T, van Noort J, van Leest H, Kanaar R, Wyman C, Dekker N, Dekker C: Torque-limited RecA polymerization on dsDNA. Nucleic Acids Res 2005, 33:2099–2105. [PubMed: 15824062]
60. Kowalczykowski SC, Clow J, Krupp RA: Properties of the duplex DNA-dependent ATPase activity of *Escherichia coli* RecA protein and its role in branch migration. Proc Natl Acad Sci U S A 1987, 84:3127–3131. [PubMed: 3033635]
61. Gataulin DV, Carey JN, Li J, Shah P, Grubb JT, Bishop DK: The ATPase activity of *E. coli* RecA prevents accumulation of toxic complexes formed by erroneous binding to undamaged double stranded DNA. Nucleic Acids Res 2018, 46:gky748.
62. Lee AM, Wigle TJ, Singleton SF: A complementary pair of rapid molecular screening assays for RecA activities. Anal Biochem 2007, 367:247–258. [PubMed: 17601483]
63. Shinohara M, Gasior SL, Bishop DK, Shinohara A: Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination. Proc Natl Acad Sci U S A 2000, 97:10814–10819. [PubMed: 11005857]
64. Holzen TM, Shah PP, Olivares HA, Bishop DK: Tid1/Rdh54 promotes dissociation of Dmc1 from nonrecombinogenic sites on meiotic chromatin. Gene Dev 2006, 20:2593–2604. [PubMed: 16980587]
65. Shah PP, Zheng X, Epshtein A, Carey JN, Bishop DK, Klein HL: Swi2/Snf2-related translocases prevent accumulation of toxic Rad51 complexes during mitotic growth. Mol Cell 2010, 39:862–872. [PubMed: 20864034]

66. Mason JM, Dusad K, Wright WD, Grubb J, Budke B, Heyer W-D, Connell PP, Weichselbaum RR, Bishop DK: RAD54 family translocases counter genotoxic effects of RAD51 in human tumor cells. Nucleic Acids Res 2015, 43:3180–3196. [PubMed: 25765654]

67. Mason JM, Logan HL, Budke B, Wu M, Pawlowski M, Weichselbaum RR, Kozikowski AP, Bishop DK, Connell PP: The RAD51-stimulatory compound RS-1 can exploit the RAD51 overexpression that exists in cancer cells and tumors. Cancer Res 2014, 74:3546–3555. [PubMed: 24753542]

68. Egelman E, Yu X: The location of DNA in RecA-DNA helical filaments. Science 1989, 245:404–407. [PubMed: 2667137]

69. Cloud V, Chan Y-L, Grubb J, Budke B, Bishop DK: Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. Science 2012, 337:1222–1225. [PubMed: 22955832]

70. Müller B, Koller T, Stasiak A: Characterization of the DNA binding activity of stable RecA-DNA complexes interaction between the two DNA binding sites within RecA helical filaments. J Mol Biol 1990, 212:97–112. [PubMed: 2319601]

71. Ninio J: Kinetic amplification of enzyme discrimination. Biochimie 1975, 57:587–595. [PubMed: 1182215]

72. Campbell MJ, Davis RW: On the in vivo function of the RecA ATPase. J Mol Biol 1999, 286:437–445. [PubMed: 9973562]

73. Boël G, Danot O, Lorenzo V, Danchin A: Omnipresent Maxwell's demons orchestrate information management in living cells. Microb Biotechnol 2019, 12:210242 ● An extensive compilation of biological mechanisms that use the energy from ATP hydrolysis to enhance the fidelity of biological mechanisms.

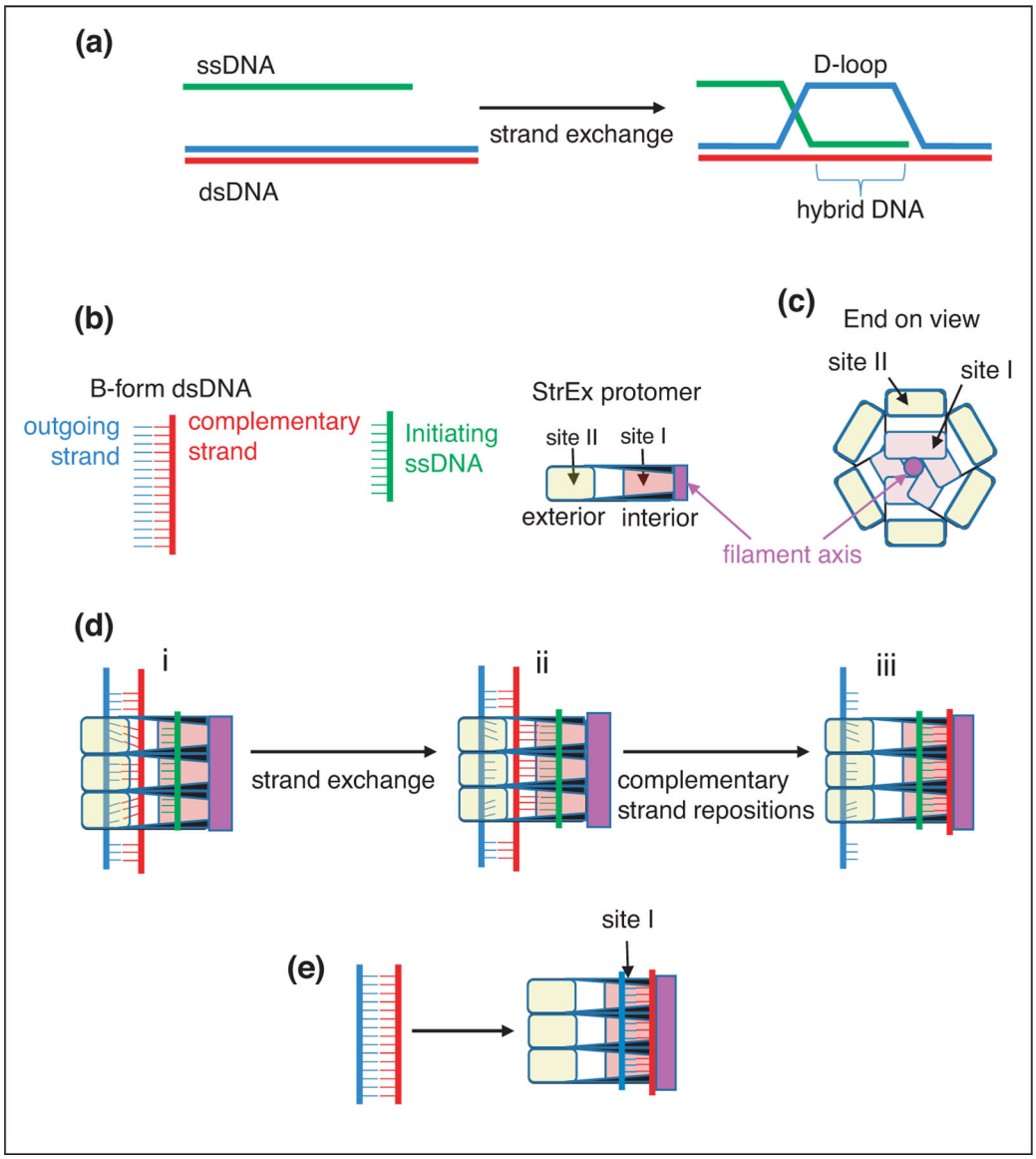

Figure 1. Homology recognition and strand Exchange without ATP hydrolysis.

(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●●,17●●]. 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●●] Figure 2).

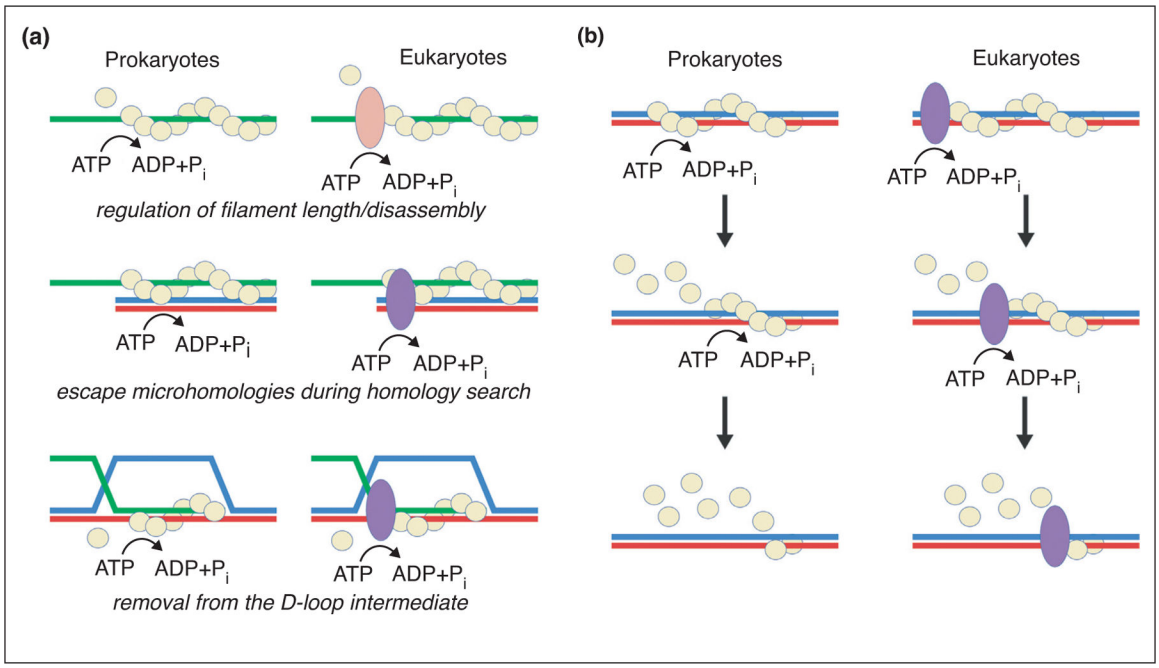

Figure 2. On-pathway and off-pathway ATP consumption by the StrEx proteins. ATP regulates StrEx protein affinity for DNA.

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 (left), removes the StrEx proteins from dsDNA.

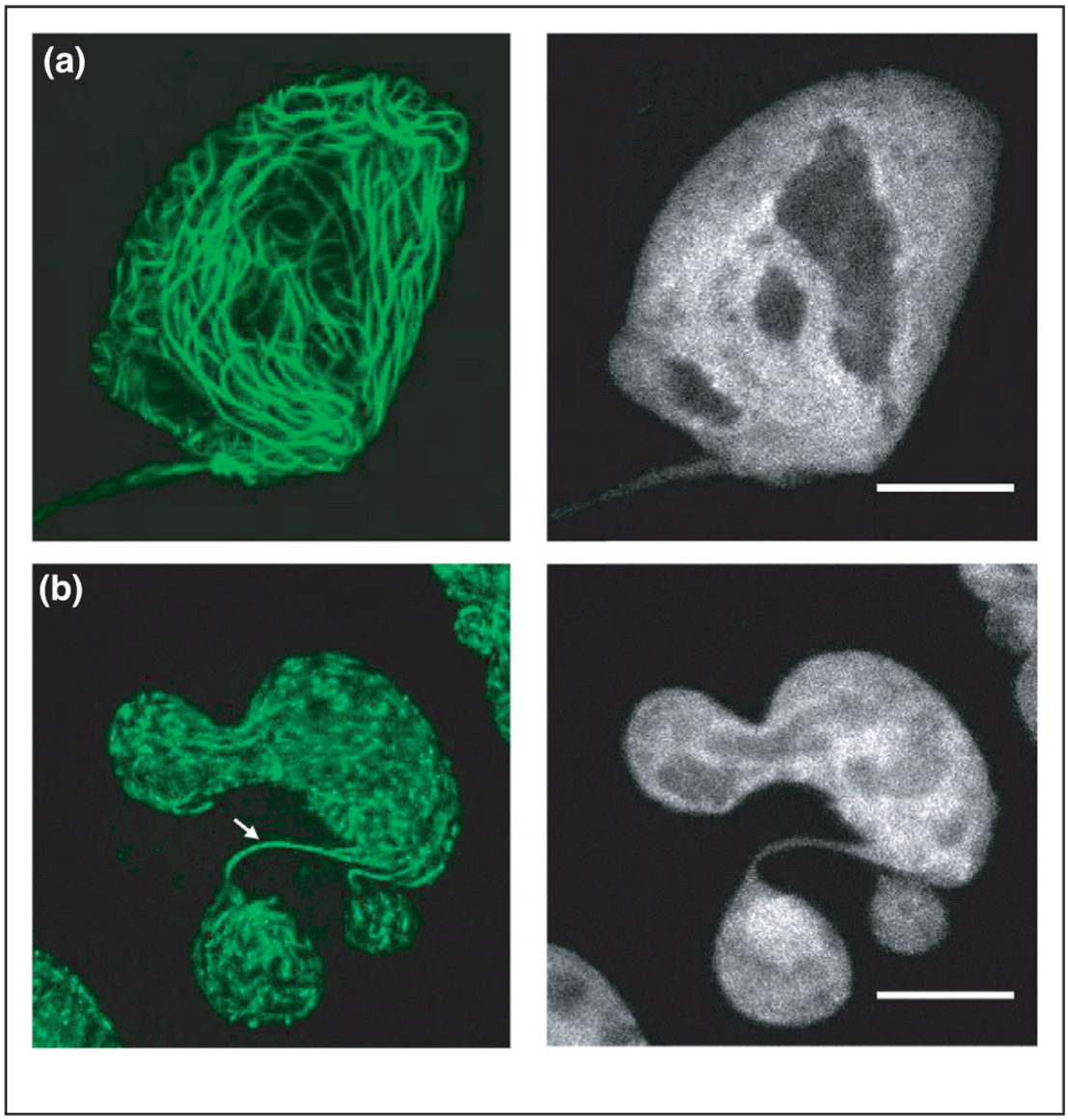

Figure 3. Toxic RAD51 complexes in RAD51 overexpressing HT1080 cells.

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 μm. Figure kindly provided by Jennifer Mason (Clemson University).