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REVIEW ARTICLE

Presynaptic filament dynamics in homologous recombination and DNA repair

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Abstract

Homologous recombination (HR) is an essential genome stability mechanism used for high-fidelity repair of DNA double-strand breaks and for the recovery of stalled or collapsed DNA replication forks. The crucial homology search and DNA strand exchange steps of HR are catalyzed by *presynaptic filaments*—helical filaments of a recombinase enzyme bound to single-stranded DNA (ssDNA). Presynaptic filaments are fundamentally dynamic structures, the assembly, catalytic turnover, and disassembly of which must be closely coordinated with other elements of the DNA recombination, repair, and replication machinery in order for genome maintenance functions to be effective. Here, we reviewed the major dynamic elements controlling the assembly, activity, and disassembly of presynaptic filaments; some intrinsic such as recombinase ATP-binding and hydrolytic activities, others extrinsic such as ssDNA-binding proteins, mediator proteins, and DNA motor proteins. We examined dynamic behavior on multiple levels, including atomic- and filament-level structural changes associated with ATP binding and hydrolysis as evidenced in crystal structures, as well as subunit binding and dissociation events driven by intrinsic and extrinsic factors. We examined the biochemical properties of recombination proteins from four model systems (T4 phage, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Homo sapiens*), demonstrating how their properties are tailored for the context-specific requirements in these diverse species. We proposed that the presynaptic filament has evolved to rely on multiple external factors for increased multilevel regulation of HR processes in genomes with greater structural and sequence complexity.

Keywords: Recombinase, RecA, Rad51, BRCA2, mediator proteins, SSB, helicase, translocase

Introduction

Genomic integrity and stability are the foundation of life and are critical for human health. To maintain a genome—millions or billions of DNA base pairs depending on the organism—is not a trivial task. DNA is under constant threat of damage resulting from endogenous and exogenous challenges such as replication fork collapse, oxidative stress, and exposure to ionizing or ultraviolet radiation. DNA double-strand breaks (DSB) represent one of the most dangerous forms of damage since the continuity of both strands and therefore the ability of a chromosome to serve as a template for its own repair are lost. Homologous recombination (HR) is a high-fidelity DNA repair pathway that repairs double-strand breaks and supports the recovery of DNA replication forks stalled at template lesions. The hallmark of HR is that it allows compromised or lost genetic information to be re-acquired from either the sister chromatid or the homologous chromosome. In eukaryotes, the role of HR is especially important for the rescue of stalled/collapsed DNA replication forks that occur regularly in S-phase as well as for telomere maintenance. HR is also critical to cope with aberrant DNA structures including DNA inter-strand cross-links, based on the observation that deletion of the key recombinase RAD51 results in early embryonic lethality with characteristic chromosomal rearrangements and breaks (Lim and Hasty 1996; Tsuzuki et al. 1996; Sonoda et al. 1998).

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Abbreviations

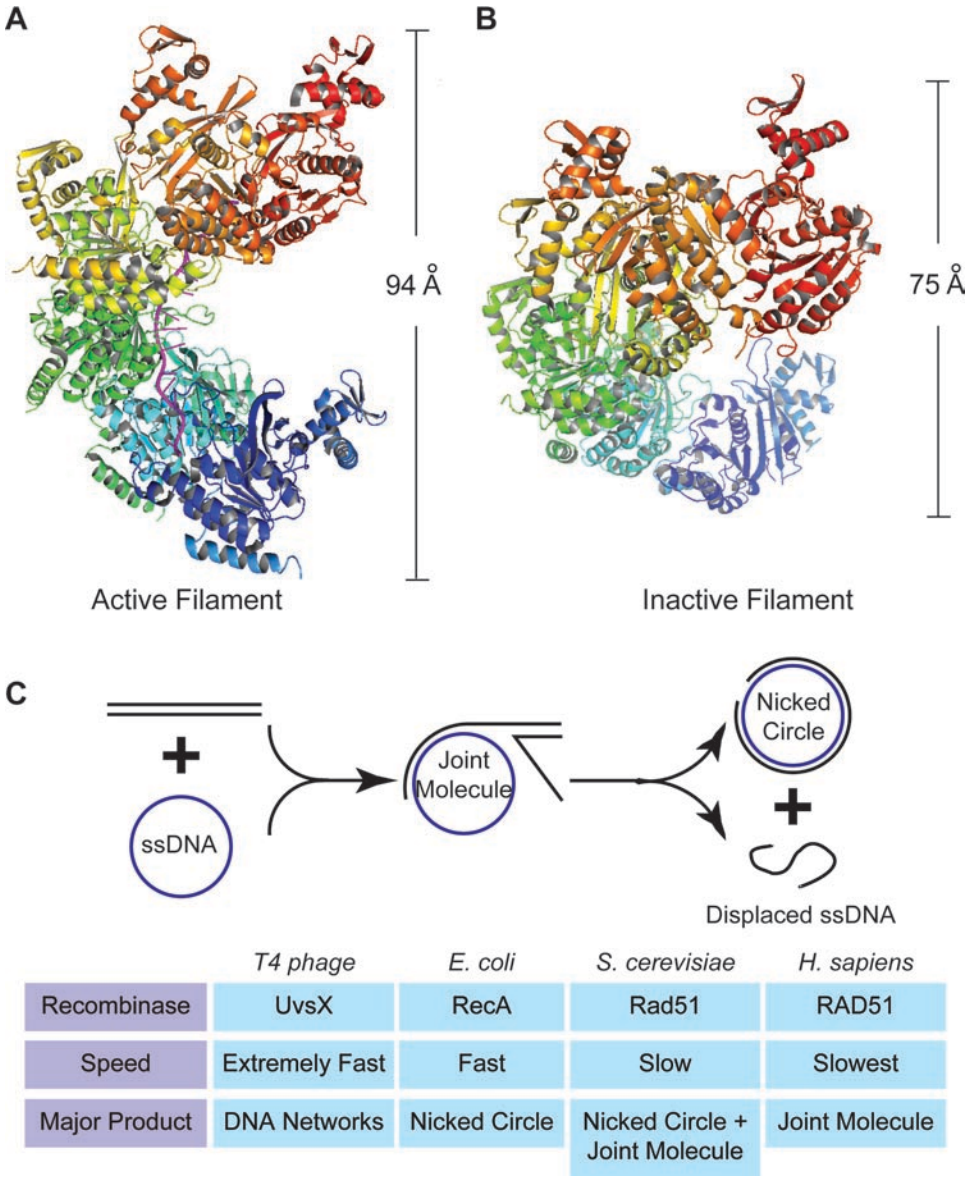
ssDNA, single-stranded DNA;
dsDNA, double-stranded DNA;
DSB, DNA double-strand break;
DSBR, DNA double-strand break repair;
HR, homologous recombination;
dHJ, double Holliday junction;
SDSA, synthesis-dependent strand annealing;
BIR, break-induced replication;
SSB, single-stranded DNA-binding protein;
RMP, recombination mediator protein;
ATPγS, adenosine-5′-(O)-(3-thio) triphosphate;
AMP-PNP, adenosine 5′(β, γ-imido) triphosphate;

Interaction between a compromised genetic sequence and its reference sequence in HR is accomplished during a stage of HR called synapsis, when DNA joint molecule intermediates are formed following homology search and DNA strand invasion. The presynaptic filament is the key intermediate that catalyzes homologous pairing and initiates DNA strand invasion. In the presynaptic filament, single-stranded DNA (ssDNA) generated by nucleolytic resection of a DSB, or by processing of a stalled replication fork, is coated with recombinase, a central enzyme that catalyzes homologous pairing and DNA strand exchange. Prototypical recombinases include bacteriophage T4 UvsX, *Escherichia coli* RecA, *Saccharomyces cerevisiae* Rad51, and human RAD51, all evolutionarily related and members of the RecA family of recombinases. The structures and functions of presynaptic filaments in different organisms are highly conserved. The transition of the presynaptic filament from a catalytically active to an inactive state is mainly controlled by the nucleoside triphosphate binding states of the recombinase. An "active" presynaptic filament (capable of catalyzing DNA strand exchange) is comprised primarily of ATP-bound recombinase monomers and is extended ~1.5-fold compared with normal B-form double-stranded DNA (dsDNA), into a right-handed helix with a helical pitch varying from 90 to 130 Å (Figure 1A). The "inactive" presynaptic filament is comprised primarily of ADP-bound recombinase monomers and is compressed relative to the active filament, with a helical pitch varying from 65 to 85 Å (Figure 1B). *In vivo*, we envision that the presynaptic filament exists primarily in an ATP-bound active form, especially at the initiation stage.

The large-scale changes in filament morphology that are tied to the binding and hydrolysis of nucleotide substrates must be accompanied at the atomic scale by rearrangements of the active site that couple DNA binding to catalysis. The structural basis for the activation of recombinase activities coupled to filament formation on DNA has recently been explored. The active, long-pitch filament forms of RecA, Rad51, and UvsX recombinases contain catalytic sites for ATP hydrolysis that are located at interfaces between subunits, with residues from both subunits participating in nucleotide binding and hydrolysis (Conway et al. 2004; Chen et al. 2008, 2010; Gajewski et al. 2011). The high-resolution crystal structure of a RecA–ssDNA complex reveals the coupling of DNA binding to ATP binding through a hydrogen bond network, linking DNA-binding residues (Arg196, and Ser172′ in the adjacent RecA molecule) to ATP-binding residues (Glu96, Gln194, and Lys250′ in the adjacent RecA molecule) (Chen et al. 2008). The coupling of nucleotide binding and ssDNA binding at the RecA–RecA monomer interface facilitates the allosteric activation of catalysis by DNA. Comparison of long-pitch filaments formed in the presence of DNA (*E. coli* RecA) and in the absence of DNA (*S. cerevisiae* Rad51) indicates that DNA binding is coupled to movements of residues that configure the ATPase active site for catalysis (Figure 2) (Chen et al. 2010). Most importantly, upon DNA binding the conserved catalytic glutamate residue Glu96 (Glu221 in Rad51) moves 1.25 Å closer to the γ-phosphate of ATP. This movement brings Glu96/221 into an optimal position to activate a water molecule for in-line nucleophilic attack on the γ-phosphate.

Since the presynaptic filament is the intermediate that defines HR as a homology search and strand exchange mechanism, its importance is manifested in a number of ways in all organisms. Aside from its fundamental role in HR, the presynaptic filament also functions in bacteria in the SOS response, a carefully regulated strategy to cope with high levels of DNA damage, as well as consequent DNA replication interruptions (Radman 1974). The RecA–ssDNA filament is the crucial signal to initiate inactivation of the LexA transcription repressor and the transcriptional activation of over 40 SOS genes involved in both non-mutagenic and mutagenic DNA repair pathways (Little 1984, 1991). Moreover, RecA has a role in the function of the specialized DNA polymerase V in mutagenesis (Patel et al. 2010).

In eukaryotes, the presynaptic filament also features in meiosis, a specialized cell division program that segregates homologous chromosomes to haploid gametes (Ehmsen and Heyer 2008). Although we do not explicitly discuss meiosis in this review, the underlying biochemical and biophysical principles we describe apply to the filament assembled during meiotic recombination as well, supplemented by an additional RecA homolog in most eukaryotes (Dmc1) and additional meiosis-specific cofactors (Sung and Klein 2006; San Filippo et al. 2008). In vertebrates, the fundamental biological role of the presynaptic filament is reflected by the observation that disruption of presynaptic filament assembly is associated with different cancer predisposition syndromes (Hoeijmakers 2001; Jasin 2002). *BRCA2*, a principal breast and ovarian cancer



	T4 phage	*E. coli*	*S. cerevisiae*	*H. sapiens*
Recombinase	UvsX	RecA	Rad51	RAD51
Speed	Extremely Fast	Fast	Slow	Slowest
Major Product	DNA Networks	Nicked Circle	Nicked Circle + Joint Molecule	Joint Molecule

Figure 1. Nucleoprotein filament properties. The interaction of recombinase monomers with ssDNA results in right-handed nucleoprotein filaments with structural properties that are conserved across all organisms. (A) In the filament form active for homology search and DNA strand invasion, represented by the *E. coli* RecA–ssDNA–ADP–AlF_4 structure (PDB code 3CMU), recombinase monomers are ATP-bound and DNA is extended. (B) In the inactive filament form, thought to resemble the *E. coli* RecA–ADP structure, recombinase monomers are AMP-PNP-bound (PDB code 3CMV) and the DNA is contracted relative to the extended form. (C) A DNA strand exchange assay demonstrates major differences in the biochemical properties of recombinases across bacteriophage T4, *E. coli*, *S. cerevisiae*, and *H. sapiens*.

susceptibility gene, functions as one of several mediator proteins that facilitate presynaptic filament formation in human recombinational DNA repair (Yuan et al. 1999; Davies et al. 2001; Moynahan et al. 2001; Jensen et al. 2010; Liu et al. 2010; Thorslund et al. 2010).

Like other kinds of biological filaments, such as actin filaments, presynaptic filaments assemble via nucleation and propagation processes, exhibiting dynamic instability of protomer binding, protein turnover, and disassembly, each of which may be a regulatory point of *trans*-acting factors (Pollard and Borisy 2003; Goley and Welch 2006). For all the RecA family proteins, the modulating factors fall into one of the following categories:

(1) *Nucleotides (ATP)*. The binding and hydrolysis of ATP transitions the recombinase monomers between high and low DNA-binding affinity (active and inactive) states.

(2) *ssDNA-binding proteins (SSBs)*. These are effectors with dual roles, since they facilitate presynaptic filament assembly by removing inhibitory ssDNA secondary structures and yet suppress assembly by competing with the recombinases for DNA binding.

(3) *Recombination mediator proteins (RMPs)/loading factors*. SSB competition for DNA-binding sites mandates that accessory proteins help to load recombinases onto ssDNA, and these proteins are called RMPs.

(4) *DNA helicases/translocases*. In all organisms, motor proteins modulate the DNA binding of recombinase, and can either inactivate a presynaptic filament by removing proteins from ssDNA, or prevent inappropriate assembly on dsDNA. Following strand invasion, motor proteins may also function to remove recombinase monomers from the heteroduplex product, transitioning the recombination transaction to subsequent stages in the pathway. In *E. coli*, a motor protein called RecBCD functions in an alternative pathway that loads RecA protein onto newly exposed ssDNA, simultaneously with dsDNA resection.

The purpose of this review is to explore the biochemical properties of protein factors and mechanistic themes in the assembly, dynamics, and turnover of presynaptic filaments in four systems (T4 phage, *E. coli*, *S. cerevisiae*, and *Homo sapiens*), and to demonstrate how the dynamic properties of the presynaptic filament may be tailored to suit applications of the HR pathway that may be specialized in different organisms. We focused on three model systems (T4 phage, *E. coli*, and *S. cerevisiae*) because they are well-characterized and represent a broad sample of presynaptic filaments across viruses, bacteria, and eukaryotes. We discuss *H. sapiens* when new protein factors appear and no orthologs have been found in the three model systems.

We suggest that although the presynaptic filament maintains its fundamental catalytic structure and dynamic nature in different organisms, recombinase properties (ATP hydrolysis and DNA binding) show an evolutionary trend toward increased reliance on external factors in organisms with greater genomic complexity. This is seen in an increased pool of facilitating and inhibitory protein components, to suit the demand for additional regulation of HR in genomes having greater structural and sequence complexity (Figure 3).

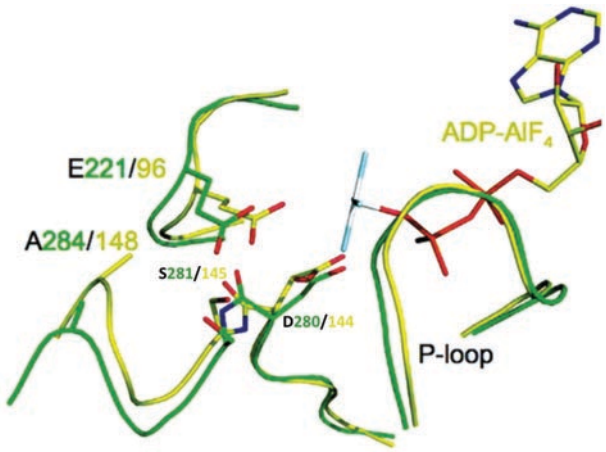


Figure 2. Activation of catalysis by DNA-dependent rearrangement of active site residues in RecA–Rad51 recombinases. The DNA-bound state is represented by the *E. coli* RecA–ssDNA–ADP–AlF_4 structure (PDB code 3CMU); the DNA-unbound state is represented by the *S. cerevisiae* Rad51–H352Y structure (PDB code 3LDA). DNA binding causes the movement of Ala284 (148 in *E. coli* RecA) toward the active site pocket, pushing the catalytic Glu221 (96 in *E. coli* RecA) 1.25 Å closer to the γ-phosphate position of ATP. Glu221/96 coordinates a water molecule (not shown) for in-line nucleophilic attack on the γ-phosphate. DNA-induced movements may be linked to a possible isomerization of *cis* peptide bond 280–281 (144–145 in *E. coli* RecA) to *trans*. The figure is a reproduction with permission from Chen et al. 2010.

Nucleation and propagation of presynaptic filaments on DNA

The assembly of catalytically active presynaptic filaments involves nucleation, propagation, and eventually dissociation events that fundamentally are driven by the DNA-binding properties of the recombinase, linked to the ATPase cycle and to functional interactions with

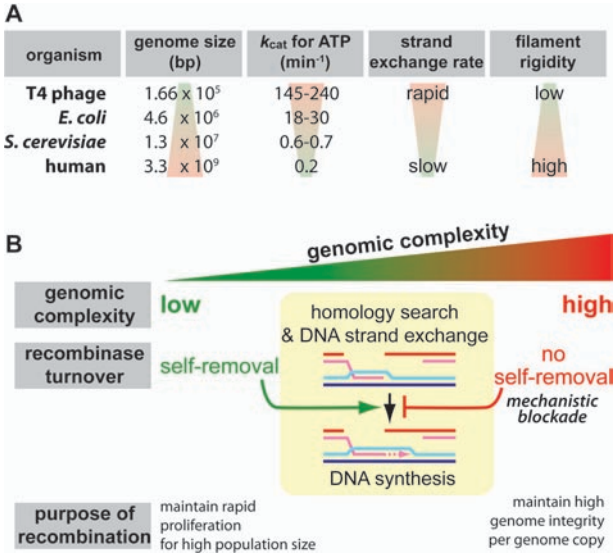


Figure 3. Correlation between genome complexity, recombinase ATP hydrolysis rates, filament rigidity, and ability to catalyze self-removal from dsDNA. Recombination is a DNA synthesis-based mechanism for recovery of genetic sequence from an intact, corresponding reference copy. (A) The larger a genome, the larger its degree of sequence complexity (repetitive sequences) and structural complexity (chromatin organization) that make the identification of the most appropriate reference copy more difficult. Although in T4 phage and *E. coli*, the recombinases UvsX and RecA have high rates of ATP turnover and catalyze self-removal from dsDNA, in eukaryotes with greater genomic sequence complexity, the corresponding Rad51 recombinases exhibit lower rates of ATP turnover, slower rates of DNA strand exchange, and greater filament rigidity, defined by its poor ability to catalyze self-removal from dsDNA. (B) The poor ability of eukaryotic Rad51 (associated with genomes of high sequence and structural complexity) to dissociate from dsDNA achieves a mechanistic blockade between homology search/DNA strand exchange and the transition to repair synthesis. This blockade can be managed by external factor(s) that must count on other inputs to determine whether an appropriate reference sequence has been identified by the recombinases, at which point their removal by an external ATPase can be effected to allow transition to DNA repair synthesis.

accessory proteins. Differences in the DNA-binding properties of the various recombinases correlate with differences in catalytic properties, which reflect differences in filament dynamics. Within this natural variation, common elements appear: each recombinase must assemble into filaments on ssDNA in a way that activates its ATPase and DNA strand exchange activities. The generation of active site asymmetry within filaments made up of identical subunits appears to be important for catalytic activation. Filament assembly must occur in the presence of SSB proteins that compete with recombinase for binding sites on ssDNA. In this section, we review findings from ensemble, single-molecule, and structural studies that have contributed to our knowledge of how presynaptic filament assembly occurs, and how similarities and differences between filament assembly processes in different systems contribute to differences in enzymatic and biological function.

Correlation of DNA-binding properties and enzymatic activities of RecA family recombinases

Even though presynaptic filaments across viruses, Archaea, Bacteria, and Eukarya share highly conserved structures and functions, individual recombinases display altered enzymatic activities, correlated with organismal genomic complexity. In this section, we explore the differences as well as important commonalities by examining the DNA binding, ATP hydrolysis, and DNA strand exchange activities of recombinases from T4 phage, *E. coli*, and *S. cerevisiae*.

DNA-binding properties

All three recombinases, T4 UvsX, *E. coli* RecA, and yeast Rad51, have binding-site sizes on ssDNA ranging from 3 to 4 nucleotides per monomer (Table 1) (Menetski and Kowalczykowski 1985; Morrical and Cox 1985; Lauder and Kowalczykowski 1991; Ando and Morrical 1998; Namsaraev and Berg 1998; Zaitseva et al. 1999). The intrinsic affinities (K_{ss}) of UvsX, RecA, and Rad51 toward ssDNA are very similar, about 10^4 to 10^5 M^{-1} under conditions that support DNA strand exchange (Menetski and Kowalczykowski 1985; Ando and Morrical 1998; Namsaraev and Berg 1998).

Recombinases bind cooperatively to DNA, consistent with their filament-forming properties. The measured cooperativity parameter, ω, equals 100 for UvsX–ssDNA interactions and 50 for RecA–ssDNA interactions, indicating that these enzymes bind to ssDNA with moderate or "limited" cooperativity (Menetski and Kowalczykowski 1985; Ando and Morrical 1998). (For comparison, $\omega = 1$ would indicate non-cooperative binding, whereas $\omega = 1000$ would indicate unlimited, essentially "all-or-none" binding.) These ω values may underestimate the true cooperativity, as they do not agree well with EM structures showing that RecA and UvsX form large clusters on DNA (Flory and Radding 1982). Free RecA protein forms oligomeric structures in solution, which can affect estimates of ω by distorting the quantitative analysis (Takahashi et al. 1986). RecA forms helical filaments on DNA and is capable of forming helical filaments off of the DNA under certain conditions (Stasiak and Di Capua 1982; Register and Griffith 1985a). However, light scattering studies demonstrate that these ssDNA-free RecA filaments, which are unstable in the presence of salt or ATP, are not direct intermediates in the ssDNA-binding pathway (Morrical and Cox 1985). Eukaryotic Rad51 proteins also appear to bind DNA with limited cooperativity, since human RAD51 nucleates on dsDNA frequently but forms clusters with limited size, leaving unbound DNA regions as kinks with less rigidity (Modesti et al. 2007b; Hilario et al. 2009).

Interestingly, nucleotides modulate presynaptic filament formation quite differently in these three model organisms. UvsX binds ssDNA well even in the absence of nucleotides, and the binding equilibrium is not affected by either of the two products of ATP hydrolysis, ADP or AMP (Ando and Morrical 1998; Liu et al. 2006a). The ATP substrate increases UvsX–ssDNA affinity transiently due to rapid turnover (Liu et al. 2006b). However, adenosine-5′-(*O*)-(3-thio) triphosphate (ATPγS), a slowly hydrolyzable ATP analog, stabilizes the high-affinity conformation of UvsX, consistent with the stabilizing effect of ATPγS on *E. coli* RecA–ssDNA filaments (Menetski and Kowalczykowski 1985; Ando and Morrical 1998; Liu et al. 2006a). Quite differently, RecA–ssDNA is partly stabilized by ATP at steady state and destabilized by ADP (Menetski and Kowalczykowski 1985). RecA readily hydrolyzes ATP when bound to ssDNA and dissociates in its ADP-bound state. Unlike UvsX and RecA proteins, however, yeast Rad51 binds to ssDNA only in the presence of ATP, and no DNA binding is observed in the presence of ADP, ATPγS, or AMP–PNP at neutral pH (Namsaraev and Berg 1998; Zaitseva et al. 1999).

Compared with its binding to ssDNA, RecA displays a significant delay in binding to dsDNA at neutral pH, demonstrating a kinetic barrier defined most likely by the nucleation step on dsDNA (Kowalczykowski et al. 1987a; Pugh and Cox 1987). This slow nucleation can be circumvented by reduced pH, which significantly enhances RecA–dsDNA complex formation (McEntee et al. 1981; Pugh and Cox 1987). This bias toward binding ssDNA probably plays a role in assembling RecA–ssDNA filaments efficiently on 3′-tailed ssDNA and gapped substrates rather than on nonspecific chromosomal DNA. Surprisingly, T4 UvsX has higher intrinsic affinity for dsDNA than for ssDNA (R. Maher, H. Xu, and S. Morrical, unpublished results), whereas Rad51 shows similar binding toward both ssDNA and dsDNA without apparent kinetic delay (Namsaraev and Berg 1998; Zaitseva et al. 1999). Thus, nucleotide binding and additional protein factors are needed to modulate the ssDNA–dsDNA-binding behavior of T4 UvsX and *S. cerevisiae* Rad51 to prevent nonspecific loss of these proteins to dsDNA binding. For example, UvsX–ssDNA interactions are selectively stabilized by nucleoside triphosphates (ATP, dATP, or

Table 1. Comparison of presynaptic filament properties in representative systems.

		T4 UvsX	*E. coli* RecA	*S. cerevisiae* Rad51	Human RAD51	References
Physical properties						
MW (kDa)		44	38	43	37	
Oligomerization		Yes	Yes	Yes	Yes	(Morrical and Cox 1985; Donovan et al. 1994; Bleuit et al. 2001; Yoshioka et al. 2003)
Presynaptic filament properties						
Nucleotide effectors	Required	No	No	Yes	No	(Menetski and Kowalczykowski 1985; Ando and Morrical 1998; Namsaraev and Berg 1998; De Zutter and Knight 1999; Zaitseva et al. 1999; Tombline et al. 2002; Bugreev and Mazin 2004; Hilario et al. 2009)
	Stabilize	ATPγS	ATPγS > ATP	ATP > ATPγS	Ca^{2+}–ATP > Mg^{2+}–ATP	
	Destabilize	None	ADP	N.A.[a]	N.A.[a]	
Active protein filament	Filament pitch	~95 Å[a]	~95 Å[b]	~99 Å[a]	~99 Å[b]	(Griffith and Formosa 1985; Ogawa et al. 1993; Yu and Egelman 1993; Yang et al. 2001; Yu et al. 2001)
	Nucleotide present	ATP–AlF_4^-, ATPγS	ATPγS	ATP–AlF_4^-	ADP–AlF_4^-	
Inactive protein filament	Filament pitch	~70 Å[a]	~75 Å	N.D.	~76 Å[b]	(Yu and Egelman 1992, 1993; Yang et al. 2001; Yu et al. 2001)
	Nucleotide present	ADP	ADP	N.D.	ATPγS	
DNA-binding parameters						
ssDNA	Binding-site size (nt)	4	3	3	3	(Menetski and Kowalczykowski 1985; Menetski et al. 1988; Lauder and Kowalczykowski 1993; Ando and Morrical 1998; Namsaraev and Berg 1998; De Zutter and Knight 1999; Zaitseva et al. 1999)
	Cooperative	Yes	Yes	Yes	Yes	
	Salt-back mid-dissociation point (mM NaCl)[c]	180	420–700	550	N.D.	
dsDNA	Cooperative	Yes	Yes	Yes	Yes	(Yonesaki and Minagawa 1985; Namsaraev and Berg 1998; Zaitsev and Kowalczykowski 1998; Zaitseva et al. 1999; Hilario et al. 2009)
	Bipolar growth	N.D.	Yes	N.D.	Yes	(Galletto et al. 2006; Hilario et al. 2009)
ATPase	k_{cat} on ssDNA (min^{-1})	145–240	18–30	0.6-0.7	0.2	(Weinstock et al. 1981; Formosa and Alberts 1986a,b; Sung 1994; Tombline and Fishel 2002)
	Cooperative	Yes	Yes	Yes	Yes	
	Hydrolysis products	ADP, AMP	ADP	ADP	ADP	
Recombinase	Joint molecule formation	Yes	Yes	Yes	Yes	(McEntee et al. 1979; Shibata et al. 1979; Yonesaki and Minagawa 1985; Formosa and Alberts 1986a,b; Sung 1994; Baumann et al. 1996; New et al. 1998; Shinohara and Ogawa 1998)
	Strand exchange	Yes	Yes	Yes	Yes	
	Relative activity	High	Medium	Low	Low	
	Polarity	5′ → 3′	5′ → 3′	3′ → 5′	N.D.	(Kahn et al. 1981; Cox and Lehman 1981; Kodadek et al. 1988; Mazin et al. 2000)
	ATP binding required	Yes	Yes	Yes	Yes	(Kodadek et al. 1988; Kowalczykowski and Krupp 1995; Sung and Stratton 1996; Chi et al. 2006)
	ATP hydrolysis required	No	No	No	No	

[a]Filament formed on dsDNA.
[b]Filament formed on ssDNA.
[c]Salt concentration at which 50% of protein–ssDNA complexes are dissociated.
N.D.: Not Determined.

analogs such as ATPγS) and by UvsY protein (Ando and Morrical 1998; Liu et al. 2006a). Nucleotide-binding effects on ssDNA–dsDNA binding and the role of external protein factors in modulating DNA-binding behavior of recombinase will be discussed further in the sections "Proteins that promote presynaptic filament assembly" and "Filament dynamics: factors that promote presynaptic filament turnover."

ATP hydrolysis and DNA strand exchange activities

All three representative recombinases show a characteristic ATP hydrolysis activity, greatly stimulated by the presence of ssDNA. T4 UvsX protein rapidly catalyzes ATP hydrolysis once bound to ssDNA, and the turnover rate varies from 150 to 500 min^{-1}, depending on the buffer conditions (Kodadek et al. 1989). *E. coli* RecA hydrolyzes ATP approximately 5–25-fold slower than UvsX, with an ATP turnover rate of about 20 min^{-1} (Ogawa et al. 1979; Roberts et al. 1979; McEntee et al. 1981). Eukaryotic Rad51 is characterized by a very low rate of ATP hydrolysis on ssDNA, and *S. cerevisiae* Rad51 only turns over 0.6–0.7 ATP per minute, ~30 times slower than RecA and 150–750 times slower than UvsX (Sung and Robberson 1995; Zaitseva et al. 1999).

Correspondingly, the ability to catalyze a three-strand DNA exchange reaction *in vitro* varies significantly from T4 UvsX to yeast Rad51, as shown in Figure 1 and Table 1. For eukaryotic Rad51 proteins, the order in which protein factors and DNA substrates are added to a reaction must be staged carefully to achieve an efficient three-strand DNA strand exchange reaction. *In vitro*, when *S. cerevisiae* Rad51 is incubated with ssDNA before the addition of RPA, Rad51 cannot form a saturated nucleoprotein filament because of inhibitory secondary structures in ssDNA. The subsequent addition of RPA to partially formed Rad51–ssDNA filaments promotes full coverage of Rad51 on ssDNA via the slow displacement of RPA by Rad51, which leads to efficient product formation. However, if RPA is allowed to bind ssDNA first, the strong and cooperative binding of RPA on ssDNA inhibits Rad51–ssDNA binding, resulting in very slow kinetics of presynaptic filament assembly and subsequent DNA strand exchange. Similar SSB/recombinase "order of addition" effects are observed in all recombination systems including T4 and *E. coli*, illustrating the competing stimulatory and inhibitory effects of SSBs in recombinase-catalyzed strand exchange reactions (Harris and Griffith 1989; Yonesaki and Minagawa 1989; Umezu et al. 1993; Umezu and Kolodner 1994; Sung 1997b; New et al. 1998; Shinohara and Ogawa 1998). Furthermore, dsDNA must be added after the Rad51–ssDNA filament has had the opportunity to assemble; otherwise, a form of substrate inhibition known as "dsDNA inhibition" occurs, in which untimely binding of Rad51 to dsDNA interferes with presynaptic filament assembly and inhibits strand exchange (Ogawa et al. 1993; Benson et al. 1994; Sung and Robberson 1995; Solinger et al. 2002). In contrast, UvsX and RecA appear to be less susceptible to dsDNA inhibition than Rad51. In the case of RecA, this is due to kinetic effects that favor RecA assembly on ssDNA rather than dsDNA at neutral pH (Pugh and Cox 1987). This kinetic partition apparently does not occur with Rad51. In further contrast with Rad51, both RecA and UvsX are able to catalyze DNA strand exchange alone without SSB protein, even when incubated with both ssDNA and dsDNA substrates simultaneously (Cox and Lehman 1981; Kahn et al. 1981; West et al. 1981; Formosa and Alberts 1986b).

From bacteriophage to bacteria to yeast to humans, the recombinases show a dramatic decrease in both ATP affinity and rates of hydrolysis (as defined by K_M and k_{cat}) and strand exchange (as defined by overall product yield). The ATP turnover rate of T4 UvsX is ~20-fold higher than that of *E. coli* RecA, ~300-fold higher than that of yeast Rad51, and ~1000-fold higher than that of human RAD51 (Table 1). The end products in DNA strand exchange reactions are quite different as well. UvsX catalyzes strand exchange through multiple invasions, leading to a rapid yield of large DNA networks as the final product (Kodadek et al. 1988). RecA produces high yields of nicked circles as final product (Cox and Lehman 1981). Yeast Rad51 accumulates joint molecule intermediates and fewer nicked circle products (Sung 1994). Finally, human RAD51 forms low levels of nicked circular products and joint molecules are the major product species (Baumann et al. 1996).

We propose that the decreasing activities of the recombinases are correlated to the different applications of HR in organisms having differing genomic complexity (Figure 3). Bacteriophage T4 uses HR primarily to support replication of its 166 kb, low-complexity (<3% repetitive sequences) genomic DNA, generating several hundred progeny particles in the 30-min lytic life cycle (Wood and Revel 1976). UvsX is engineered to achieve maximum speed and full capacity to serve this purpose. In bacteria, HR is primarily used in replication fork restart to process DNA damage to guarantee the continuation of DNA replication for a single circular chromosome (4.6×10^6 bp in *E. coli*). In eukaryotes, HR functions as a support mechanism for DNA replication and in DNA double-strand break repair (DSBR), in genomes of relatively large size and sequence complexity (3.3×10^9 bp in humans, 1.3×10^7 in *S. cerevisiae*). Repetitive sequences (dispersed gene families, rRNA and tRNA genes, pseudogenes, LINEs, SINEs, minisatellites, microsatellites, and telomeres) account for nearly 50% of the human genome (Lesk 2007). In mammals, *RAD51* is an essential gene and its deletion leads to embryonic lethality (Tsuzuki et al. 1996), which reflects the critical role of HR to recover stalled/collapsed replication forks during normal cell proliferation. The enrollment of multiple protein factors with different or overlapping activities provides an effective means for organisms with greater sequence complexity to modulate recombinase loading on ssDNA and dissociation from ssDNA and dsDNA, for the purpose of promoting finer regulation of whether HR is used and whether it is sanctioned to transition to repair synthesis at a particular

site of DNA strand exchange. Recombinases with lower intrinsic ATPase rates do not dissociate readily from dsDNA, imposing a greater requirement for an external factor to catalyze recombinase removal from the heteroduplex product of DNA strand exchange (Figure 3).

Active site asymmetry

Electron micrographs of presynaptic filaments typically show long tracts of recombinase subunits bound to ssDNA in a regular right-handed helical array, with apparently high symmetry (Griffith and Formosa 1985; Ogawa et al. 1993; Yu and Egelman 1993; Yu et al. 2001). Recombinases tend to crystallize in helical arrays in the absence of DNA, and most exhibit 6-fold screw symmetry ($P6_1$), with the protomer as the asymmetric unit (Story et al. 1992; Datta et al. 2003; Rajan and Bell 2004; Wu et al. 2004; Chen et al. 2010). Increasingly, however, biochemical and structural evidence indicates that presynaptic filaments can be asymmetric at the level of subunits and active sites, under conditions that promote enzymatic activity. For example, direct binding measurements demonstrate that *E. coli* RecA presynaptic filaments contain two classes of ATP-binding sites with different affinities for their nucleotide substrate (Lauder and Kowalczykowski 1991). In addition, steady-state kinetics studies demonstrate that presynaptic filaments of T4 UvsX contain two classes of ATPase active sites, one that generates ADP and one that generates AMP as product, each with distinct kinetic parameters (Farb and Morrical 2009a). Active site asymmetry appears to be important for DNA strand exchange activity, based on the observation that mutations that increase the ADP–AMP product ratio, that is, that decrease catalytic asymmetry within the UvsX–ssDNA filament, are defective in strand exchange (Farb and Morrical 2009a).

Structural evidence for active site asymmetry within recombinase filaments is provided by the X-ray crystallographic structure of the *S. cerevisiae* Rad51–I354T mutant (Conway et al. 2004). I345T is a gain-of-function mutant with enhanced affinity for ssDNA; it is competent for DNA strand exchange *in vitro* and *in vivo*, and it partially suppresses mutations in the recombination mediators Rad55 and Rad57 (Fortin and Symington 2002). The reported structure of Rad51–I345T is arranged as a filament of asymmetric dimers with 3-fold screw symmetry ($P3_1$). As a result, alternating protomers in the filament are in different conformations; this creates two classes of protomer–protomer interfaces that alternate along the filament (Figure 4). Since the ATPase active site is at the filament interface, there are also two classes of active sites that alternate along the filament (Conway et al. 2004). Interestingly, the crystal structure of the I345T mutant actually contains six independent protomers arranged as three distinct filaments, designated A–D, F–E, and B–C (Conway et al. 2004; Chen et al. 2010). The A–D and F–E filaments are both 3_1 helices comprised of alternating protomers, whereas the B–C filament is nearly a symmetric 6_1 helix. Based on the configurations of active site residues in the three filaments, it was proposed that the A–D and F–E filaments represent stable intermediates in the recombination pathway, whereas the B–C filament represents a transient intermediate between the two (Chen et al. 2010). The obvious implication is that conformational flexibility, including active site asymmetry, is important for the normal catalytic function of Rad51.

The B–C filament of I345T resembles the filament structure of Rad51–H352Y, which is a true, symmetric 6_1 helix (Chen et al. 2010). The latter mutation affects the protomer–protomer interface and ATPase active site of Rad51. Although it binds tightly to ssDNA, H352Y is incapable of catalysis beyond the first turnover of ATP hydrolysis (Chen et al. 2010). The H352Y mutation therefore stabilizes a filament conformation that cannot complete the catalytic cycle (ADP release and exchange for ATP).

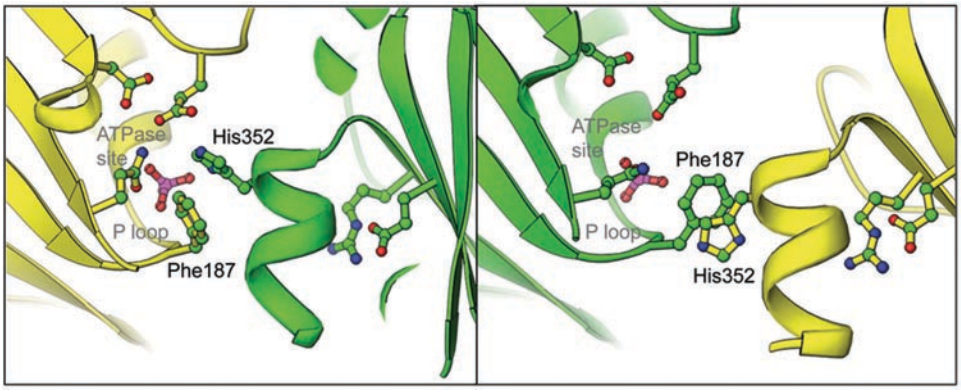


Figure 4. Two types of protomer–protomer interactions and active sites within the Rad51 filament. In the yeast Rad51–I345T structure (1SZP), His352 adopts two different configurations in alternating protomer–protomer interfaces that differ in structure. Left, His352 from the neighboring protomer stacks directly over the ATPase site of the other protomer. Right, access of His352 to the adjacent ATPase site is blocked by Phe187 from that protomer. The conservation of active site asymmetry in the recombinase family is implied by the observation that the helix containing His352 is aligned with a highly conserved glutamate–arginine salt bridge in both RecA and in this structure. All of the conserved residues involved in ATP binding and hydrolysis, as well as the sulfate in the active pocket, are highlighted. Figure was a reproduction with permission from Conway et al. 2004.

The fact that H352Y has identical active sites throughout the filament suggests that the ability to enter into an asymmetric state is important for catalytic turnover within the Rad51 filament.

The observation of active site asymmetry in presynaptic filaments from diverse organisms suggests that it is a common property of recombinase function. Indeed, active site asymmetry is expected given that recombinases are allosteric enzymes exhibiting cooperative binding to ATP and other non-Michaelis-Menten behavior. Crystal structures provide snapshots of individual filament states, which are reference points for understanding the conformational dynamics that must occur during filament assembly, catalysis, and turnover.

Kinetic and single-molecule studies of filament formation

The assembly of the presynaptic filament takes place in a similar manner to other biopolymers, such as SSB proteins, on nucleic acid lattices, composed basically of two steps: (1) nucleation, where initial protein–DNA binding creates isolated nucleation sites and (2) propagation, where additional protein monomers are recruited to the nucleation site and extend the filament's coverage on the DNA (Lohman and Kowalczykowski 1981; Pörschke and Rauh 1983). Ensemble RecA–dsDNA-binding data suggest that the nucleation phase is the rate-limiting step, whereas the propagation of the protein on dsDNA is fast (Pugh and Cox 1987). A kinetic time course of RecA assembly on poly(dεA) (poly-1,N^6-etheno-2′-deoxyadenylate, an etheno-modified ssDNA lattice lacking secondary structure) exhibits a lag phase and a slow binding phase, characteristic of a nucleation–propagation mechanism (Chabbert et al. 1987). The use of short oligonucleotide substrates permits finer dissection of the nucleation phase and reveals two kinetically distinct steps: the rapid formation of a stable intermediate and a slow isomerization to final complex formation (Cazaux et al. 1994).

Single-molecule studies greatly advance our understanding of presynaptic filament formation, because they allow direct visualization. Single-molecule studies have so far examined filament assembly on dsDNA, even though the physiological substrate for filament formation is ssDNA. Nevertheless, monitoring of filament assembly on dsDNA illuminates what is likely to be a similar assembly behavior on ssDNA (McEntee et al. 1981; Pörschke and Rauh 1983; Chabbert et al. 1987; Pugh and Cox 1987, 1988). These studies confirm the two-step nucleation–propagation model obtained from ensemble studies and also reveal several novel features of filament assembly:

Multiple nucleation centers result in discontinuous filaments

RecA forms multiple nucleation clusters on dsDNA and the average number of clusters formed depends on the salt concentration, the type of nucleoside triphosphate, and RecA protein concentration (Galletto et al. 2006). Continuous clusters can grow from each nucleation center with a linear growth rate until the entire dsDNA lattice is fully coated with RecA protein (Galletto et al. 2006). Unlike RecA, human RAD51 forms multiple nucleation centers but these do not propagate extensively. The final filament can therefore be discontinuous, composed of many tightly associated segments with small gaps in between (Modesti et al. 2007b; Hilario et al. 2009).

Nucleation is salt-sensitive

RecA filaments can initiate from multiple nucleation sites and the frequency of nucleation is strongly dependent on the salt concentration (ionic strength). The calculated average rate of RecA cluster formation decreases 8-fold when there is a moderate increase in [NaCl] from 20 to 80 mM (Galletto et al. 2006). At higher salt concentration, the nucleation step is rate-limiting, as ensemble biochemical studies suggested. It takes three times longer to detect a comparable number of RecA clusters formed on dsDNA when salt was increased from 66 to 80 mM NaCl (Galletto et al. 2006). Thus, the frequency of successful establishment of nucleation centers decreases dramatically with increasing ionic strength.

Filament propagation is bipolar

Ensemble studies have revealed that RecA polymerizes on both ssDNA and dsDNA with a net polarity from 5′ to 3′ (Register and Griffith 1985b; Shaner et al. 1987; Shaner and Radding 1987). Nonetheless, RecA assembles bidirectionally on polynucleotides, as observed directly in single-molecule studies, since the associated growth rate of bidirectional clusters is higher compared with that of unidirectional clusters (Galletto et al. 2006). The bipolar growth of both RecA and Rad51 on DNA might account for the difference between these two proteins in net filament growth polarity observed *in vitro* (Register and Griffith 1985b; Sung and Robberson 1995; Namsaraev and Berg 1998). Consistently, atomic force microscopy (AFM) studies on human RAD51–DNA filament formation revealed the existence of "kinks," protein-free regions with higher flexibility in the filament (Modesti et al. 2007b). These small gaps might be formed when two growing clusters with opposite polarity are about to collide. However, complete filament coverage can still occur when bipolar growth from multiple nucleation sites amalgamates the protein clusters (Hilario et al. 2009).

Filament assembly on SSB-covered ssDNA

Competitive nature of SSB– versus recombinase–ssDNA interactions

SSBs are a class of proteins that bind preferentially to ssDNA in a nonspecific manner and possess no other enzymatic activity. They are abundant *in vivo* and are involved in many critical physiological functions in DNA metabolism, in all organisms ranging from bacteriophage to humans. In HR, SSB plays an essential role in protecting the newly exposed ssDNA immediately after DSB resection, which is vulnerable to nuclease digestion and oxidative damage in the cell. This protection mechanism demands that SSB binds ssDNA selectively

and rapidly without obvious sequence specificity, and typical SSB proteins such as T4 Gp32 coat ssDNA with high affinity and cooperativity to ensure full coverage in a short amount of time. Binding also removes secondary structures in DNA that may be inhibitory to the binding of an enzyme that acts subsequently during the DNA transaction.

During presynaptic filament assembly, SSBs directly inhibit the loading of recombinases by occupying newly exposed ssDNA. The competitive nature of SSB proteins and recombinases in ssDNA binding is vividly illustrated by the "order of addition" effect on DNA strand exchange reactions mentioned earlier (Harris and Griffith 1989; Yonesaki and Minagawa 1989; Umezu et al. 1993; Umezu and Kolodner 1994; Sung 1997b; New et al. 1998; Shinohara and Ogawa 1998).

Evidence for SSB displacement during presynaptic filament assembly

Direct examination of the recombinase- and SSB structures on ssDNA across organisms indicates that these two classes of proteins typically compete for binding sites on ssDNA. For the phage proteins, when UvsX and Gp32 proteins were incubated with M13mp7 ssDNA together in a buffer containing ATP but no salt, roughly half of the ssDNA was bound exclusively by UvsX, and the other half was bound exclusively by Gp32. EM showed non-overlapping long tracts of either UvsX or Gp32 nucleoprotein filaments with distinguishable morphologies (Griffith and Formosa 1985). Kinetic studies confirm that UvsX filament assembly on ssDNA occurs concurrently with Gp32 displacement (Liu et al. 2006b). In the case of *E. coli* RecA and SSB proteins, EM images of mixed complexes on ssDNA are consistent with competitive binding (Register and Griffith 1985b). Results of DNA-binding studies are consistent with this model (Kowalczykowski et al. 1987b), although others have argued that non-competitive binding of RecA and SSB occurs under some conditions (Morrical et al. 1986). The different findings could be due to different binding modes of SSB (Lohman and Overman 1985; Bujalowski and Lohman 1986; Lohman et al. 1986), which differentially affect the biochemical activities of RecA (Morrical and Cox 1990).

In eukaryotes, if RPA is pre-incubated with ssDNA first, Rad51 displaces RPA very slowly to form a filament on the DNA, resulting in a kinetic lag phase lasting about 20 min (Sugiyama and Kowalczykowski 2002). The biological importance of timely SSB displacement by the recombinase is demonstrated by the severe defects of an *rfa–t11* mutant in recombinational repair. The *rfa–t11* mutant strain carrying a point mutation in the RPA70 subunit displays a 1000-fold increased sensitivity to UV and to the alkylating agent MMS, compared with the wild type (Umezu et al. 1998). Purified RPA (*rfa–t11*) protein exhibits ssDNA-binding properties similar to the wild-type protein in binding-site size and salt sensitivity, but it is displaced by Rad51 more slowly than the wild type during presynaptic filament assembly (Kantake et al. 2003). The displacement defects of RPA (*rfa–t11*) in presynaptic filament assembly account for its severe sensitivity to DNA damage, supporting the physiological importance of SSB displacement during presynaptic filament assembly.

General principles of filament assembly

A general model of presynaptic filament assembly is shown in Figure 5. After resection, SSB binding protects the ssDNA from further degradation and removes inhibitory secondary structures, preparing the DNA substrate for recombinase loading. Mediator proteins can bind SSB-covered ssDNA and load recombinase onto it to form nucleation centers through a variety of mechanisms. Recombinase propagates from the individual nucleation site and assembles into the presynaptic filament, which initiates recombination by catalyzing homology search and strand exchange.

We highlight the following general features of presynaptic filament assembly:

(1) *Nucleation is salt-sensitive*. The source of the salt-sensitive nucleation stems from the electrostatic nature of the binding between the recombinases and DNA. It is vulnerable to increasing ionic strength, especially in a single isolated DNA-binding site where cooperativity does not apply.

(2) *Discontinuous short filaments are catalytically active*. In the bacteriophage T4 system, the early appearance of recombination intermediates in DNA strand exchange assays fits into the timeline of a partially assembled UvsX filament (Kodadek et al. 1988; Liu et al. 2006b). Human RAD51 propagates on DNA with limited length and frequently forms multiple small clusters under physiologically relevant conditions (Hilario et al. 2009). We propose that short, discontinuous filaments or segments, instead of a few long clusters, could be the preferred catalytic unit for initiation of homologous pairing and DNA strand exchange.

(3) *Additional protein factors modulate nucleation and cluster formation*. These modulating factors and their known associated mechanisms will be reviewed in the following section.

Proteins that promote presynaptic filament assembly

Recombination mediator proteins

The strong binding of SSBs on newly resected ssDNA poses a kinetic and thermodynamic barrier to the assembly of recombinases and the initiation of HR. Therefore, a special class of accessory proteins antagonizes the SSB barrier to facilitate the assembly of recombination proteins onto ssDNA. The concept of RMPs, or more simply mediators, was initially proposed to categorize these accessory proteins into a common group based on their ability to mediate the conflict between SSBs and recombinases for binding to ssDNA (Beernink and Morrical 1999). Recombination mediators promote presynaptic

filament formation by lifting the ssDNA-binding barrier and by loading recombinase proteins properly in a timely fashion (Sung and Klein 2006) (Table 2). In this section, a number of classic mediators and other recombinase-loading proteins will be discussed briefly, with particular emphasis on the mechanisms by which they promote assembly of an active filament functional for homology search and DNA strand exchange.

Bacteriophage: T4 UvsY protein

UvsY is the prototypical RMP, and extensive biochemical and biophysical characterizations of UvsY have significantly advanced our understanding of the mechanisms by which mediators promote presynaptic filament assembly (Beernink and Morrical 1999; Liu and Morrical 2010a,b). The 16-kDa UvsY protein exists as a stable 95-kDa hexamer in solution, and binds to ssDNA in this form (Beernink and Morrical 1998). It stimulates all activities of UvsX recombinase, including ATP hydrolysis, DNA strand exchange, and recombination-dependent replication (Morrical and Alberts 1990). It contributes fundamentally to the following known aspects of presynapsis:

UvsY orchestrates a handoff of the ssDNA from Gp32 to UvsX First, UvsY hexamers recognize and bind to Gp32–ssDNA and weaken Gp32–ssDNA interactions. Next, UvsY alters ssDNA structure in ways that destabilize Gp32–ssDNA interactions while promoting UvsX–ssDNA filament nucleation (Sweezy and Morrical 1999; Liu et al. 2006a; Pant et al. 2008; Farb and Morrical 2009b). The ssDNA structural transitions appear to involve different wrapped states of ssDNA around UvsY hexamers (Pant et al. 2008; Xu et al. 2010). Finally, UvsX is loaded onto a UvsY–Gp32–ssDNA intermediate with concomitant displacement of Gp32 (Liu et al. 2006b). Thus, presynaptic filament assembly involves a series of ssDNA handoff transactions that is best described by the progression Gp32–ssDNA + UvsY → UvsY–Gp32–ssDNA → UvsY–Gp32–ssDNA* + UvsX → UvsX–UvsY–ssDNA + Gp32, where UvsY–Gp32–ssDNA and UvsY–Gp32–ssDNA* represent different wrapped conformations of ssDNA (Farb and Morrical 2009b; Xu et al. 2010).

UvsY stabilizes UvsX–ssDNA clusters and filaments Indirect evidence for the UvsY-mediated stabilization of UvsX on DNA comes from the observation that sub-stoichiometric amounts of UvsY help UvsX to overcome various inhibitory effects *in vitro*, including those associated with high Gp32 concentrations, high salt, and/or low UvsX concentrations (Harris and Griffith 1989; Kodadek et al. 1989; Yonesaki and Minagawa 1989; Morrical and Alberts 1990; Morrical et al. 1991; Yassa et al. 1997). Direct evidence for this UvsY function comes from the observation that UvsY stabilizes UvsX–ssDNA complexes against high salt challenge, in synergy with the ATPγS-bound high-affinity form of UvsX (Liu et al. 2006a). Two UvsY point mutants (UvsY–K58A and UvsY–K58A, R60A) are significantly impaired in DNA binding, but retain normal self-association and heteroprotein associations, compared with wild-type UvsY. The ability of these two mutant proteins to stimulate DNA strand exchange catalyzed by subsaturating UvsX is dramatically decreased compared with wild-type UvsY, which suggests UvsY–ssDNA interactions are primarily responsible for the stabilization effect (Bleuit et al. 2004).

UvsY provides a ssDNA-binding bias for UvsX UvsX recombinase exhibits high affinity for dsDNA, which could interfere with its ability to locate and assemble on ssDNA under physiological conditions. In contrast, the affinity of UvsY for ssDNA is 2500-fold higher than that for dsDNA in buffer containing 200 mM NaCl, and 300-fold higher in buffer containing 250 mM NaCl, which suggests an important role that UvsY serves in presynaptic filament formation by loading UvsX preferentially onto ssDNA (Xu et al. 2010). The high affinity of UvsY for ssDNA is a direct result of ssDNA wrapping around UvsY hexamers (Pant et al. 2008; Xu et al. 2010). UvsY alone does not appear to recognize specific DNA structures such as ssDNA–dsDNA junctions; however, it does exhibit high affinity for mechanically stretched DNA (Pant et al. 2008). The latter activity may be important for the recognition and remodeling of Gp32–ssDNA complexes by UvsY.

UvsY, in concert with the T4 MR nuclease complex, may load UvsX directly onto resected DNA A complex formed by the T4 Gp47 (Mre11) and Gp46 (Rad50) proteins is orthologous to eukaryotic MR complexes required for nucleolytic resection of DSBs. The T4 MR complex exhibits exo- and endonuclease activities *in vitro* that are modulated by UvsY and Gp32 (Herdendorf et al. 2011). UvsY interacts specifically with both MR subunits (Bleuit et al. 2001), suggesting a model in which MR and UvsY together recruit UvsX directly onto a DSB undergoing resection, bypassing the competition with Gp32.

UvsY acts as a nucleotide exchange factor for UvsX UvsX generates both ADP and AMP as ATP hydrolysis products (Formosa and Alberts 1986b; Farb and Morrical 2009a), and UvsY specifically promotes ADP generation and suppresses further ADP hydrolysis to AMP (Farb and Morrical 2009b). Thus, it was proposed that UvsY could also enhance presynaptic filament stability by acting as an ADP–ATP exchange factor, which would increase the average lifetime of the high-affinity form of UvsX on the ssDNA (Farb and Morrical 2009b).

Mutations in a key allosteric switch residue of UvsX protein, UvsX–H195A and H195Q, display reduced ssDNA-binding and strand exchange activities (Farb and Morrical 2009b). Interestingly, UvsY mutants with reduced ssDNA-binding affinity partially rescue enzymatic activities of the UvsX mutants (Bleuit et al. 2001; Farb and Morrical 2009b). The functional complementation of these UvsX and UvsY mutants is at least partly due

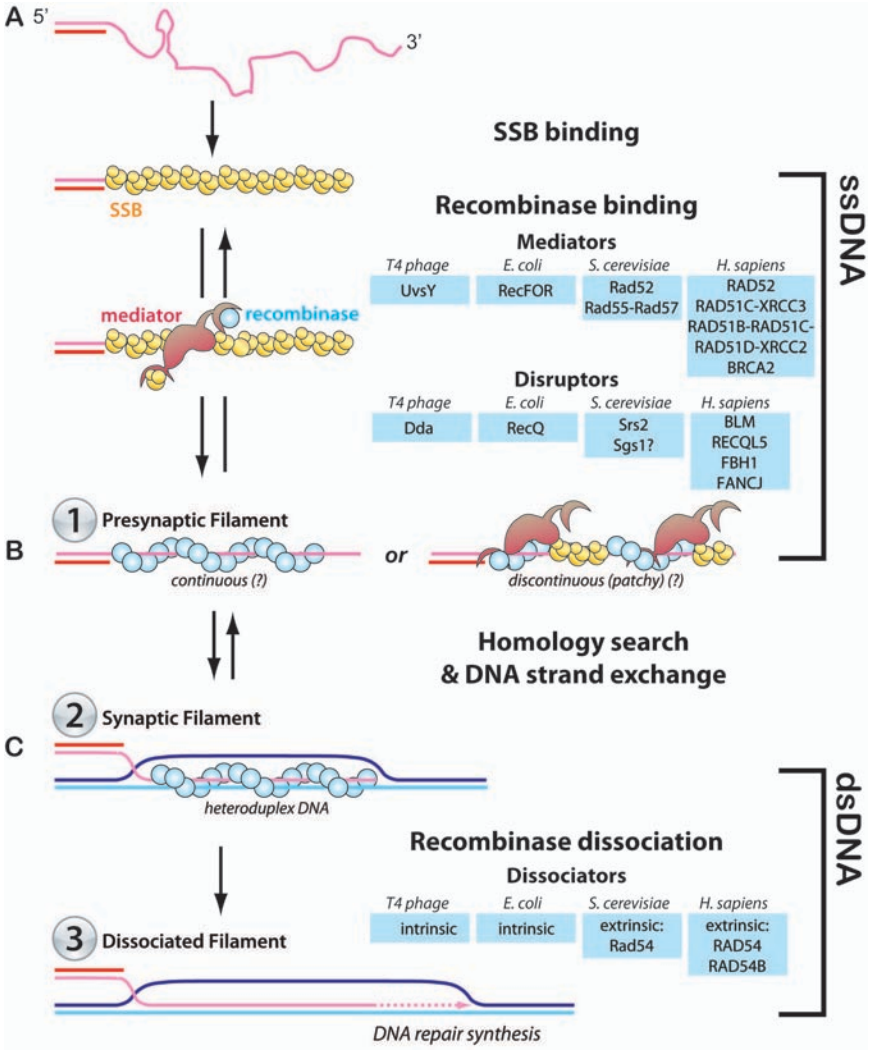


Figure 5. Principles of filament assembly and disassembly on ssDNA and dsDNA. The presynaptic filament is the intermediate that defines HR as a mechanism that can identify DNA sequence homology in a genome. Assembly of recombinase monomers on ssDNA establishes a "search engine" that uses the information in the ssDNA to direct pairing with homologous information already involved in stable base pairing in dsDNA, and to catalyze the exchange of base pairing between ssDNA in the filament and a complementary strand in the target dsDNA. (A) SSB binding to the ssDNA alleviates secondary structures that would otherwise be inhibitory to recombinase binding. *Mediators* catalyze the replacement of SSB by recombinase, whereas *disruptors* catalyze the removal of recombinase to favor rebinding of ssDNA by SSB. (B) Recombinase monomers on ssDNA form a *presynaptic filament* (1) that may be *continuous* or *discontinuous* (patchy), and whether mediator proteins remain components of the active filament during homology search and DNA strand exchange remains to be discerned. (C) Homology search and DNA strand exchange generate a displacement loop (D-loop) and initiate the stage at which recombinase is now bound to dsDNA (heteroduplex DNA) in the *synaptic filament* (2). Recombinase must dissociate from dsDNA to transition to DNA repair synthesis from the 3′-end of the *dissociated filament* (3). Dissociation from dsDNA is intrinsic to T4 phage UvsX and *E. coli* RecA, but requires the extrinsic ATPase Rad54 in eukaryotes.

to the need for a balance between the ssDNA-binding affinities of UvsX and UvsY in order to correctly perform ssDNA handoffs during filament assembly (Farb and Morrical 2009b). Complementation may also reflect the importance of correct protein–protein interactions. The heteroprotein associations such as between UvsX and UvsY, and between UvsY and the MR complex, might chaperone UvsX subunits into proper conformations and orientations such that synapsis between homologous DNA molecules can occur, which explains why protein interactions are required for efficient presynapsis and recombination (Jiang et al. 1993; Bleuit et al. 2004).

Bacteria: E. coli RecF/O/R proteins

Although in the T4 phage system one principal, multifunctional mediator oversees UvsX filament assembly, in *E. coli* three proteins collaborate to load RecA onto ssDNA. Genetic studies suggested that RecO, RecF, and RecR function as a heterocomplex in their presynaptic filament accessory role, independent of RecBCD exonuclease (Kowalczykowski et al. 1994). However, initial biochemical characterization of these proteins yielded somewhat confusing results. As early as 1980, RecF was proposed to stabilize the RecA–ssDNA nucleoprotein filament (Clark 1980). However, purified RecF protein inhibits, rather than stimulates, the ssDNA-dependent ATP hydrolysis

Table 2. Possible mechanisms of recombination mediator protein.

Mechanisms of action	Examples	References
Promote filament nucleation		
Stabilize salt-sensitive nucleation complexes	T4 UvsY	(Liu and Morrical 2010a)
Recruit recombinases onto specific DNA structures such as ssDNA–dsDNA junctions	*E. coli* RecFOR	(Morimatsu and Kowalczykowski 2003)
	U. maydis Brh2	(Yang et al. 2005)
Stabilize filaments		
Block dissociation by end-capping	*E. coli* RecFOR	(Webb et al. 1997)
	U. maydis Brh2	(Yang et al. 2005)
Induce high-affinity conformation	T4 UvsY	(Liu et al. 2006a)
Promote nucleotide exchange	T4 UvsY	(Farb and Morrical 2009a)
	Human XRCC2	Shim et al. 2004
Role of RMP–DNA interactions		
Induce DNA structural changes such as DNA wrapping	T4 UvsY	(Pant et al. 2008; Xu et al. 2010)
	Human Rad52	(Kagawa et al. 2002; Singleton et al. 2002)
Weaken SSB–ssDNA interactions	T4 UvsY	(Sweezy and Morrical 1999)
Provide ssDNA > dsDNA affinity bias	T4 UvsY	(Xu et al. 2010)
	Yeast Rad52	(Mortensen et al. 1996)
	Human BRCA2	(Jensen et al. 2010)
Provide ssDNA–dsDNA junction recognition	*E. coli* RecFOR	(Morimatsu and Kowalczykowski 2003
	U. maydis Brh2	(Yang et al. 2005)
Role of protein–protein interactions		
Load recombinase onto DNA directly	*E. coli* RecBCD	(Spies and Kowalczykowski 2006)
	BRCA2	(Jensen et al. 2010; Liu et al. 2010; Thorslund et al. 2010)
Chaperone recombinase into proper orientations for synapsis	Yeast Rad52 Yeast Rad54	(Sung 1997a; New et al. 1998; Shinohara and Ogawa 1998; Mazin et al. 2003)

and DNA strand exchange activities of RecA protein at high concentrations (Madiraju and Clark 1991). It was later revealed that the RecF inhibition of RecA filament assembly is relieved by the presence of RecO and RecR proteins in the system (Umezu et al. 1993). However, a subcomplex between RecO and RecR alone is sufficient to facilitate RecA replacement of SSB on SSB-covered ssDNA (Bork et al. 2001). The RecO–RecR complex also stimulates RecA enzymatic activities, particularly on a linear ssDNA substrate (Bork et al. 2001). When RecF is added in the presence of RecO–RecR, RecF competes with RecO for binding to RecR, and destabilizes RecA–DNA filaments formed in the presence of RecO–RecR (Bork et al. 2001). The apparent inconsistencies between genetic results that implied that RecF, RecO, and RecR function together, with the *in vitro* behaviors of RecF and RecO–RecR subcomplexes were resolved by the elegant biochemical demonstration that a complex of all three proteins—the RecFOR complex—collaborates to direct the loading of RecA protein specifically onto the ssDNA–dsDNA junctions of gapped DNA (Morimatsu and Kowalczykowski 2003). On these substrates, all three proteins are needed to relieve SSB inhibition of filament assembly and DNA strand exchange *in vitro*. RecFOR recognizes an exposed 5′ end at the dsDNA–ssDNA junction, and RecF in particular is responsible for the binding to this junction (Hegde et al. 1996; Morimatsu and Kowalczykowski 2003). RecFOR has therefore been proposed to function as a DNA structure-specific mediator at the initial steps in ssDNA gap repair and DSBR involving tailed substrates (Morimatsu and Kowalczykowski 2003). These three proteins serve as the first structure-specific mediator protein discovered to load recombinase at the ssDNA–dsDNA junction.

Eukaryotes: S. cerevisiae and human mediator proteins

In eukaryotes, HR pathways must face a number of challenges inherent to the chromatin architecture and sequence properties of a larger, more complex genome. Multiple levels of chromatin structure must be remodeled before a homologous dsDNA targeted by a presynaptic filament can be accessed. A number of non-allelic but similar (homologous) targets for strand exchange may be encountered and must be discriminated by a nucleoprotein filament before HR is sanctioned to be completed at a particular locus. One outcome of this genomic complexity is the introduction of a large variety of mediators that achieve multilevel regulation in eukaryotes. Here, we briefly review the presynaptic roles of four mediators that function in eukaryotic HR, including two from evolutionarily distinct protein families: (1) Rad52 (yeast) and (2) BRCA2 (humans) (Liu and Heyer 2011). Rad52 is a key member of the *RAD52* epistasis group in *S. cerevisiae*, along with Rad51 paralogs and Rad54 (Pâques and Haber 1999; Symington 2002). The Rad52 protein family is widely conserved among eukaryotes. BRCA2, on the other hand, does not appear to have orthologs in all eukaryotic genomes (Liu and Heyer 2011). *Sc*Rad52 and human BRCA2 appear to function as traditional recombination mediators to Rad51, in that their major role is to promote presynaptic filament assembly on RPA-covered ssDNA (Liu and Heyer 2011). Rad51 paralogs and Rad54 play more diverse roles, as we described in a later section.

S. cerevisiae Rad52 *RAD52* is the only gene that is essential and irreplaceable for HR in *S. cerevisiae*. In *S. cerevisiae*, *rad52* deletion mutants exhibit the most severe recombination defects of all *RAD52* epistasis group mutants, including extreme sensitivity to UV and γ-radiation and abrogated DSBR, break-induced replication (BIR), and single-strand annealing (SSA) (Symington 2002). The pre-eminent role of yeast Rad52 in HR is explained by two functions: (1) its mediator function in presynaptic filament assembly and (2) its ssDNA annealing activity in second-end capture and SSA (Sugiyama et al. 2006). Rad52 mediates strand annealing between complementary, RPA-coated ssDNA molecules (Mortensen et al. 1996; Sugiyama et al. 1998).

In vitro, purified Rad52 protein binds preferentially to ssDNA (Mortensen et al. 1996). Yeast Rad52 is a classical mediator in that it overcomes the inhibitory effect of RPA in Rad51-catalyzed DNA strand exchange (Sung 1997a; New et al. 1998; Shinohara and Ogawa 1998). Since Rad52 has been shown to interact with both Rad51 and RPA genetically and physically (Shinohara et al. 1992; Milne and Weaver 1993; Park et al. 1996; Sung 1997a), it is proposed that a pre-assembled Rad51–Rad52 complex establishes a nucleation site on RPA-covered ssDNA, to which additional Rad51 is recruited to replace RPA (Song and Sung 2000). Later, it was discovered that Rad52 participates in a tripartite complex consisting of Rad52–RPA–ssDNA, consistent with and analogous to T4 UvsY–Gp32–ssDNA and *E. coli* RecO–SSB–ssDNA complexes. This tripartite complex might serve as an intermediate that accelerates RPA displacement by Rad51 protein (Sugiyama and Kowalczykowski 2002), although unlike RecFOR, no specificity for a DNA structure has been observed. A second, independent function of Rad52 in HR concerns its promotion of ssDNA annealing in the presence of RPA, a role that is subsequent to DNA strand exchange and separate from its earlier mediator function in presynaptic filament assembly. Interestingly, this annealing function is the only biochemical activity observed for human RAD52, and it displays no mediator activity in RAD51 filament formation in the presence of RPA inhibition *in vitro* (Jensen et al. 2010). However, an *in vivo* function of RAD52 in HR was revealed recently by the observation that depletion of RAD52 in BRCA2-deficient cells causes further reduction in both spontaneous and DSB-induced HR events (Feng et al. 2011). Consistently, cells deficient in both BRCA2 and RAD52 show elevated genome fragility in both spontaneous and IR-induced chromosome breaks, telomere end associations, dicentrics, and radials (Feng et al. 2011), suggesting the existence of independent pathways represented by BRCA2 and RAD52 in mammalian HR (Liu and Heyer 2011).

Human BRCA2 Like yeast Rad52, BRCA2 is a classical mediator protein in HR, supplanting RAD52 in human cells as the principal mediator of RAD51 filament assembly on RPA-coated ssDNA. This explains why BRCA2 is an important tumor suppressor protein and individuals (in particular females) heterozygous for *BRCA2* hypomorphic or null mutations have highly elevated lifetime risks of breast and ovarian cancers (King et al. 2003). It also explains why *BRCA2* knockout mice exhibit early embryonic lethality with characteristic chromosomal rearrangements and breaks that are identical to the phenotypes of *RAD51* knockout mice (Lim and Hasty 1996; Tsuzuki et al. 1996; Yu et al. 2000), whereas *RAD52* knockout mice are viable and fertile without hypersensitivity to IR, UV, and DNA damage agents such as methyl methanesulfonate (Rijkers et al. 1998). Furthermore, RAD51 focus formation in response to DNA damage depends nearly entirely on functional BRCA2 (Yu et al. 2000; Tarsounas et al. 2003). Only in BRCA2-deficient cells, knockdown of RAD52 further reduces both spontaneous and IR-induced RAD51 foci formation about 2-fold (Feng et al. 2011).

The 384-kDa protein BRCA2 directly interacts with RAD51 (Scully and Puget 2002; Powell and Kachnic 2003) at multiple RAD51-binding sites: eight BRC repeats and a C-terminal site (Figure 6). Due to the technical difficulties associated with full-length BRCA2 protein purification, our knowledge of its mechanistic function built gradually on studies of either its *Ustilago maydis* homolog, Brh2, or fragments of BRCA2, such as different BRC repeats (San Filippo et al. 2008). The crystal structure of the DNA-binding domain of BRCA2, in complex with DSS1, reveals its unique structural features capable of binding both ssDNA and dsDNA (Yang et al. 2002). Indeed, the homolog Brh2 preferentially recruits Rad51 to a ssDNA–dsDNA junction and facilitates nucleation of the Rad51 filament in the presence of RPA (Yang et al. 2005). The crystal structure of a fusion protein linking a single BRC repeat (BRC4) and RAD51 core domain demonstrate that the BRC repeat can bind RAD51 by mimicking its oligomerization interface motif (Pellegrini et al. 2002). *In vitro*, this BRC4 repeat can promote RAD51–ssDNA formation and limit RAD51–dsDNA formation by eliminating nucleation (Carreira et al. 2009). Consistently, a fusion construct containing one single BRC repeat (BRC3 or BRC4) and the large subunit of human RPA protein increases DSB-induced HR and partially suppresses the mitomycin C and IR sensitivities of *BRCA2* mutant mammalian cells *in vivo* (Saeki et al. 2006). *In vitro*, a polypeptide containing human BRC3, BRC4, and a BRCA2 DNA-binding domain nucleates RAD51 onto ssDNA and overcomes RPA inhibition in RAD51-catalyzed DNA strand exchange (San Filippo et al. 2006). The C-terminal domain of BRCA2, containing a RAD51 interaction site distinct from the BRC repeats (Figure 6), can stabilize a RAD51 filament and protect it from disassembly by BRC repeats (Davies and Pellegrini 2007; Esashi et al. 2007). It is proposed that BRCA2 might recruit RAD51 through protein–protein interaction to the ssDNA substrate and stabilize RAD51 filament formation (Pellegrini et al. 2002; Yang et al. 2002; Shin et al. 2003; Kowalczykowski 2005; Davies and Pellegrini 2007; Esashi et al. 2007; Petalcorin et al. 2007; Carreira et al. 2009).

Recent reports characterized full-length, purified BRCA2 and its relationship with RAD51 (Jensen et al. 2010; Liu et al. 2010; Thorslund et al. 2010). One BRCA2 molecule interacts with about six RAD51 molecules, enough to initiate filament nucleation (Jensen et al. 2010; Liu et al. 2010). BRCA2 acts as a classical mediator protein, by facilitating RAD51 filament formation on RPA-coated ssDNA and thus stimulating RAD51-catalyzed DNA strand exchange (Jensen et al. 2010; Liu et al. 2010). BRCA2-stimulated loading of RAD51 onto RPA-covered ssDNA is further promoted by DSS1, a protein that interacts specifically with BRCA2 and that is required for homology-directed DNA repair and genome stability *in vivo* (Liu et al. 2010). Unlike Brh2, BRCA2 does not show strong preference toward the ssDNA–dsDNA junction and does not anneal complementary ssDNA in the presence of RPA (Mazloum et al. 2007; Jensen et al. 2010). The dramatic difference between Brh2 and BRCA2 may be a direct result of their structural differences: Brh2 only shares homology with the C-terminal DNA-binding domain of BRCA2 and contains one, instead of eight, BRC repeat (Figure 6). Furthermore, the lack of ssDNA annealing activity in the presence of RPA reinforces the functional divergence of BRCA2 and RAD52 in the human recombination system, with BRCA2 performing classical mediator functions, whereas RAD52 is specialized for second-end capture.

Eukaryotic Rad51 paralogs

Rad51 paralogs are a class of proteins related to Rad51 through gene duplication and divergence, sharing homology with the RecA core (and its Walker ATPase domains) but having diverged at their N- and C-termini (Thacker 2005). The paralogs cannot catalyze homologous pairing and DNA strand exchange, distinguishing themselves from the recombinases. Rad51 paralogs, especially *S. cerevisiae* Rad55–Rad57, are proposed to act as traditional mediator proteins (Sung 1997b). However, the mechanistic functions of the paralogs remain largely unknown and they are unique as a protein family in sequence conservation. Thus, the paralogs are discussed separately here.

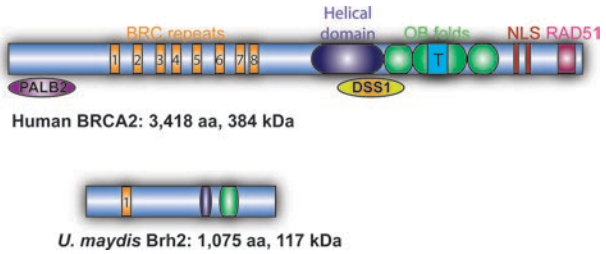


Figure 6. Schematic of human BRCA2 and *U. maydis* Brh2 proteins. The conserved motifs are labeled on top of the structural schematic, and the binding sites are shown for two interacting protein factors of human BRCA2, PALB2 and DSS1.

S. cerevisiae Rad55–Rad57 proteins

Cytological and genetic studies reveal a common role for Rad55 and Rad57 in promoting Rad51 filament formation and function. That Rad55 and Rad57 function exclusively as a heterodimer is supported by genetic observations that their mutants exhibit identical defects in DNA damage repair, recombination, and meiosis deficiency (Petes et al. 1991; Game 1993). The cold sensitivity of *rad55* and *rad57* null mutants suggests that they function in a higher-order multiprotein structure in HR, probably involved in Rad51 filament assembly/stabilization (Petes et al. 1991; Game 1993). Cytologically, Rad51 can be recruited to DSBs in vegetative cells in the absence of Rad55, although with slower kinetics and reduced intensity of focus formation (Sugawara et al. 2003; Lisby et al. 2004). Furthermore, overexpression of *RAD51* partially suppresses the radiation sensitivity of *rad55* or *rad57* mutants (Hays et al. 1995; Johnson and Symington 1995), suggesting that Rad55–Rad57 facilitates Rad51 function *in vivo*. Interestingly, Rad55–Rad57 is especially important in the recovery of stalled replication forks and may be less critical to DSBR (Mozlin et al. 2008). It is worthwhile to point out that the Rad55–Rad57 heterodimer plays only a minor and dispensable supporting role in yeast relative to the essential role of Rad52 (Pâques and Haber 1999). Biochemical studies of Rad55–Rad57 confirm that Rad55 and Rad57 form a stable heterodimeric complex in solution, and they relieve RPA inhibition to Rad51–ssDNA binding and stimulate Rad51-dependent homologous pairing and strand exchange (Sung 1997b). The observation that the recombination/repair deficiency of certain *rad55* and *rad57* mutants can be partially suppressed by expression of the gain-of-function mutant Rad51–I345T, a form of Rad51 recombinase with enhanced ssDNA-binding affinity (Fortin and Symington 2002), lends credence to the idea that the physiological role of Rad55–Rad57 is to nucleate or stabilize Rad51 presynaptic filaments. However, the precise mechanism by which Rad55–Rad57 promotes Rad51 filament formation and function remains unclear, however. Further investigations into the mechanism of how Rad55–Rad57 mediates Rad51 filament assembly and HR are needed. Note that additional paralogs may exist in budding yeast. Four proteins, Shu1, Shu2, Psy3, and Csm2, might function as a protein complex to facilitate Rad51 filament formation, although their mechanism of action remains unknown (Shor et al. 2005; Mankouri et al. 2007).

Human RAD51 paralogs

In humans, five RAD51 paralogs have been identified, with overall low sequence homology to RAD51 (~20%): RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 (Thacker 1999). All five paralogs are essential and non-redundant in HR, since mutations in any one paralog lead to severe phenotypes including spontaneous chromosomal aberrations, high sensitivity to cross-linking agents, and defective DNA repair (Johnson et al. 1999; Pierce et al. 1999; Takata et al. 2001; Godthelp et al. 2002).

In mammals, both RAD51 and RAD54 focus formation in response to DNA damage are significantly attenuated in paralog mutant cells, suggesting that RAD51 paralogs cooperate to support RAD51 assembly at DSB sites (van Veelen et al. 2005; Yonetani et al. 2005). The five paralogs compose two principal complexes: RAD51C–XRCC3 and RAD51B–RAD51C–RAD51D–XRCC2 (BCDX2) (Masson et al. 2001; Liu et al. 2002). The BCDX2 complex displays an apparent stoichiometry of about 1:1:1:1; Rad51C binds Rad51B and Rad51D, whereas Rad51D binds to XRCC2 (Schild et al. 2000; Liu et al. 2002; Miller et al. 2004).

Recombinant RAD51B forms a stable subcomplex with RAD51C, displaying ssDNA-binding and ssDNA-stimulated ATP hydrolysis activities. The complex partially alleviates RPA inhibition in RAD51-catalyzed strand exchange (Sigurdsson et al. 2001). However, the mechanism of action for this mediator effect remains unknown. Human XRCC2 serves as the nucleotide exchange factor for RAD51 by stimulating ADP → ATP exchange, and thus enhances its DNA strand exchange activity in a concentration-dependent manner (Shim et al. 2004). In addition to functioning in presynaptic filament assembly, a late postsynaptic involvement of the RAD51 paralogs has been suggested. Both RAD51B protein and the BCDX2 complex preferentially bind synthetic Holliday junctions over other DNA substrates (Yokoyama et al. 2003, 2004). A physical interaction between BLM helicase and RAD51D protein has been mapped, and RAD51D–XRCC2 stimulates and modulates BLM helicase activity to disrupt synthetic Holliday junctions (Braybrooke et al. 2003). This cooperation between BLM and RAD51 paralogs was proposed to function in the recovery of stalled or collapsed replication forks (Braybrooke et al. 2003). Genetic studies supported a late role of RAD51C–XRCC3 in HR, since their mutants show altered processing of recombination intermediates (Brenneman et al. 2002; Nagaraju et al. 2006). However, such a genetic outcome could also be caused by defects in the formation or properties of the RAD51 presynaptic filament. Purified recombinant RAD51C–XRCC3 does not have intrinsic nuclease activity (Liu et al. 2007). More biochemical investigations are needed to clarify both the presynaptic and postsynaptic roles of RAD51 paralogs in HR.

Other proteins with recombinase-loading activity

In this section, we briefly discussed other proteins capable of loading recombinase monomers onto ssDNA. *E. coli* RecBCD and human RAD51AP1 and RAD51AP2 are recombination accessory proteins that are not categorized as traditional mediator proteins because their major function in presynaptic filament formation does not revolve around the relief of SSB inhibition. Instead, RecBCD functions as a nuclease that can also load RecA onto ssDNA, and RAD51AP1 and RAD51AP2 promote RAD51 filament assembly by mechanisms that remain to be determined.

E. coli RecBCD

The RecBCD pathway constitutes a major approach in *E. coli* by which a DSB is processed into a ssDNA substrate suitable for RecA filament assembly (Kowalczykowski et al. 1994). The 330-kDa RecBCD enzyme is composed of three subunits: RecB (a 3′ → 5′ helicase and multifunctional nuclease), RecC (a subunit on which duplex DNA is split, and that recognizes a DNA sequence called χ), and RecD (a 5′ → 3′ helicase) (Dillingham et al. 2003). *In vitro*, RecBCD binds blunt dsDNA ends with high affinity and then processively unwinds the duplex at a remarkably high unwinding rate of up to ~1000 bp/sec (Bianco et al. 2001). It translocates processively on the dsDNA in a 3′ → 5′ direction and preferentially degrades the 3′ end (Dixon and Kowalczykowski 1991, 1993; Bianco and Kowalczykowski 2000). When RecBCD encounters the eight-nucleotide DNA sequence called χ (5′-GCTGGTGG-3′), the holoenzyme pauses for about 5 sec and then continues to translocate at approximately half of the initial rate (Spies et al. 2003). χ recognition by the RecC subunit elicits a conformational change that modifies the holoenzyme activities (Handa et al. 2005). RecBCD switches the focus of its nucleolytic degradation from the 3′-tailed DNA strand to the complementary 5′-tailed strand (Dixon and Kowalczykowski 1991; Anderson and Kowalczykowski 1997a). As a result, after the RecBCD complex encounters the χ site, its continued translocation produces a 3′-tailed ssDNA, the substrate for HR. At this point, RecBCD also acquires the ability to load RecA onto the χ-terminating ssDNA to form a presynaptic filament (Dixon and Kowalczykowski 1991, 1995; Anderson and Kowalczykowski 1997b). It was proposed that encounter of the χ site by RecBCD functions as a "molecular throttle" that slows RecBCD translocation, allowing RecBCD to coordinate 3′-tailed ssDNA substrate generation with the slower kinetics of RecA loading and contiguous filament assembly (Spies et al. 2003). Besides the slower velocity that facilitates RecA loading onto ssDNA, RecB directly loads RecA through interaction with the RecA-fold (Spies and Kowalczykowski 2006). RecBCD serves as a prototypic recombination nuclease by rolling two critical functions into one protein complex, ssDNA production and RecA filament assembly, shedding light onto the potential mechanistic roles of other eukaryotic nucleases.

RAD51AP1 and RAD51AP2

Human RAD51AP1 (RAD51-associated protein 1) is highly expressed in aggressive lymphomas and carcinomas (Song et al. 2004; Henson et al. 2006). This protein, along with its murine homolog RAB22, was discovered through yeast two-hybrid assays for its novel interaction with RAD51 (Kovalenko et al. 1997; Mizuta et al. 1997). Another protein, RAD51AP2, was later also identified to interact with RAD51, sharing a similar RAD51-interaction motif with RAD51AP1 at the C-terminus. The RAD51AP1 protein was first proposed to be a factor in RAD51-mediated meiotic recombination, based on its

tissue-specific expression in adult testis and fetal ovary (Kovalenko et al. 1997; Mizuta et al. 1997). Later, two groups discovered that RAD51AP1-depleted cells also exhibit impaired repair of spontaneous or induced chromatid breaks. *In vitro*, RAD51AP1 stimulates RAD51-catalyzed D-loop formation (Modesti et al. 2007a; Wiese et al. 2007). It was reported to be a structure-specific DNA-binding protein, since it preferentially binds branched DNA structures. It was proposed that RAD51AP1 stimulates D-loop formation through a combination of physical interaction with RAD51 and binding to joint molecule intermediates, controlling the structural transitions of DNA in HR (Modesti et al. 2007a). A recent report shows that RAD51AP1 physically interacts with human DMC1 and stimulates its D-loop formation, suggesting its involvement in meiotic HR processes as well (Dray et al. 2011). Whether these are additional mediators that function to facilitate RAD51 binding to RPA-coated ssDNA remains to be determined. Moreover, whether the effect on D-loop formation is accomplished by interaction with the presynaptic filament needs further study.

Themes in mediator mechanisms of action

Mediator proteins play critical roles in the choreography of presynaptic filament assembly, and thus in HR and genome maintenance. The strong connection between BRCA2 protein dysfunction and cancer predisposition is a perfect illustration. Therefore, a deeper understanding of the mechanistic functions of mediators will help to clarify the management and regulation of DNA recombination and repair. In vertebrates, this extends to an understanding of how genetic changes that can lead to carcinogenesis are minimized. Based on mediator proteins from different organisms, we propose the following general mechanisms of action for mediators in HR (Figure 7), (Table 2):

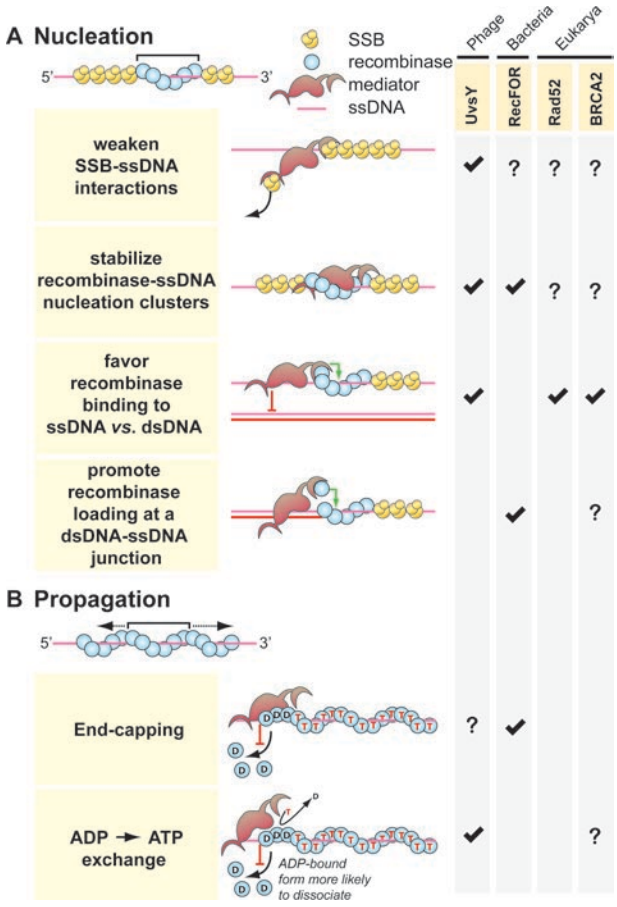


Figure 7. Mechanisms of classic mediators: general principles. Mediator proteins "mediate" the conflict between SSB and recombinase binding to ssDNA, and are therefore critical to the assembly of presynaptic filaments. They promote the (A) *nucleation* of recombination on SSB-coated ssDNA, and the (B) *propagation* of recombinase binding from nucleation clusters by a number of mechanisms.

Promotion of filament nucleation

Stabilization of salt-sensitive nucleation complexes Sequence nonspecific interactions of recombinases with DNA are strongly electrostatic in nature, and therefore salt-sensitive (Menetski and Kowalczykowski 1985; Ando and Morrical 1998; Zaitseva et al. 1999). Recent findings suggest that the most salt-sensitive step is filament nucleation (Galletto et al. 2006). Kinetic studies of T4 presynaptic filament assembly indicate that UvsY selectively stabilizes a salt-sensitive nucleation complex during UvsX filament assembly on Gp32-covered ssDNA (Liu et al. 2006b; Liu and Morrical 2010a). Further studies are needed to see if other mediators share this property.

Optimization of ssDNA conformation for filament nucleation Mediator proteins are either oligomeric, such as UvsY, RecFOR, Rad52, or have multiple DNA-binding domains per protomer, such as BRCA2. This suggests that a common theme of mediator activity could be structural changes in ssDNA induced by wrapping or bending of the strand around the protein oligomer. ssDNA wrapping can serve as a mechanism to weaken SSB–ssDNA interactions and to stabilize recombinase–ssDNA interactions (Figure 7), as deduced for UvsY protein. Similarly, crystal structures of the N-terminal domain of human RAD52 protein reveal a positively charged surface aligned outside of the undecameric ring, suitable for ssDNA phosphodiester backbone binding with the bases pointing away from the surface of the protein (Kagawa et al. 2002; Singleton et al. 2002). Mutational analysis of the basic and aromatic residues further demonstrated that these residues are primarily responsible for DNA binding (Kagawa et al. 2002). Wrapping of DNA around a positively charged ring structure could occur within the RAD52 heptameric ring as well, distorting and disrupting the high-affinity binding of RPA (Stasiak et al. 2000; Kagawa et al. 2002).

DNA structure specificity

The newly resected 3′-ssDNA end is the primary substrate for presynaptic filament assembly; either the ssDNA or the ssDNA–dsDNA junction can be the nucleation substrate to initiate the assembly process. Recombinases do not

show strong binding preference toward ssDNA, dsDNA, or ssDNA–dsDNA junctions, but specificity could be provided through structure-specific DNA-binding activities of mediator proteins.

ssDNA–dsDNA lattice discrimination How to specifically load recombinases onto ssDNA and to prevent protein unavailability through recombinase binding to chromosomal dsDNA in a cellular context are serious issues. Mediator proteins provide the ssDNA-binding preference that a typical recombinase lacks, based on their DNA-binding properties and unique protein interactions. In T4 phage, UvsY demonstrates a preferential binding with higher affinity toward ssDNA than dsDNA in equilibrium conditions. The lattice discrimination of UvsY enables a specific recruitment of UvsX protein onto ssDNA, preventing an unproductive dsDNA nucleoprotein filament formation at an early stage (Xu et al. 2010). Similarly, yeast Rad52 shares the same binding preference toward ssDNA (Mortensen et al. 1996). Additionally, yeast Rad52 establishes species-specific protein–protein interactions with both RPA and Rad51, which enables Rad51 to gain selective access to the ssDNA and to displace RPA from ssDNA (Sung 1997a; New et al. 1998; Shinohara and Ogawa 1998). Human RAD52 binds ssDNA with higher affinity than dsDNA, as well (Benson et al. 1998); however, this enhanced ssDNA binding does not support RAD52 in nucleating RAD51 filament formation but rather in annealing complementary ssDNA molecules (Grimme et al. 2010; Jensen et al. 2010). As expected, a similar ssDNA-binding preference is shared and utilized by human BRCA2 protein in loading RAD51 specifically onto ssDNA substrates to form the presynaptic filament (Jensen et al. 2010).

Recognition of ssDNA–dsDNA junctions A ssDNA–dsDNA junction can occur at a newly processed DSB end, and equally important, at a single-strand gap (Figure 7). During DNA replication, a replication fork can permit bypass of lesions on both leading and lagging strands, leaving gaps opposite to the DNA lesions to avoid fork stalling or collapse (Langston and O'Donnell 2006). HR and translesion synthesis (TLS) are the two major pathways that function to manage single-strand gaps. Both *E. coli* RecFOR and *U. maydis* BRCA2 homolog Brh2 proteins act as structure-specific mediator proteins to direct the assembly of a cognate recombinase filament initiated from the ssDNA–dsDNA junction, but human BRCA2 protein lacks this junction specificity (Morimatsu and Kowalczykowski 2003; Yang et al. 2005; Jensen et al. 2010; Liu et al. 2010). Additionally, the junction-specific RecA-loading activity of RecFOR proteins is supported by demonstrating that this complex is essential for active RecA filament formation to support DNA polymerase V (Pol V)-mediated TLS (Fujii et al. 2006). Thus the junction recognition of these structure-specific mediator proteins is critical for recombinational DNA repair processes.

Stabilization of filaments

In addition to nucleating the initial binding of recombinases onto ssDNA, mediators also stabilize a filament against premature dissociation. Several distinct mechanisms can be employed here:

Blockage of dissociation by filament end-capping RecF–RecR minimizes RecA filament extension from a gapped ssDNA region to neighboring dsDNA (Webb et al. 1997), which might reflect an end-capping mechanism of RecFOR to block the dissociation of RecA monomers at the filament end adjacent to the junction. Brh2 might use a similar mechanism to block the dissociation of Rad51 at one end, allowing filament growth by the addition of new monomers at the other end. The crystal structure of a RAD51–BRC complex also suggests that successive BRC repeat binding by RAD51 might spatially order RAD51 molecules to facilitate their loading onto ssDNA (Pellegrini et al. 2002), and BRCA2 can probably block Rad51–dsDNA complex formation by eliminating nucleation on the duplex side based on BRC4 peptide studies (Carreira et al. 2009).

Regulation of nucleotide turnover/exchange to maintain the recombinase in a high-affinity state for ssDNA An efficient synchronization of the binding and hydrolysis of triphosphate nucleotide between each monomer within the presynaptic filament is the key to homologous strand pairing and exchange (Kowalczykowski 1991; Cox 2007a,b). The ADP–ATP nucleotide exchange activity of UvsY protein suppresses further hydrolysis of ADP to AMP within the UvsX filament, which serves to stabilize filaments by keeping UvsX primarily in the ATP-bound, high-affinity state (Farb and Morrical 2009b). Similarly, human XRCC2 protein significantly enhances the ATP-processing activity of RAD51 by stimulating the ADP → ATP exchange rate, providing a regulatory mechanism in eukaryotic HR (Shim et al. 2004). Interestingly, both a peptide of BRCA2, BRC4, and full-length BRCA2 show inhibition of RAD51-catalyzed ATP hydrolysis, presumably keeping RAD51 in an ATP-bound, high-affinity state within the filament (Carreira et al. 2009; Jensen et al. 2010).

Cognate protein–protein interactions

Correct heteroprotein contacts might chaperone recombination proteins into proper conformations and orientations so that synapsis between homologous DNA molecules can occur efficiently. In T4 phage, studies of two UvsY mutant proteins with DNA-binding defects that retain normal self- and heteroprotein associations reveal that UvsX–UvsY protein interactions play a significant role in stabilizing the UvsX nucleoprotein complex (Liu et al. 2006a). Also, the loading of UvsX onto Gp32–ssDNA by UvsY protein requires specific contacts between UvsY and the C-terminal domain of Gp32 (Jiang, Giedroc et al. 1993). In *S. cerevisiae*, a Rad52Δ409–420 mutant protein completely abolishes its interaction with Rad51 protein, while maintaining similar levels of activities in DNA

binding, ssDNA annealing, and self-association, compared with wild-type Rad52 protein. The heteroprotein association between Rad51–Rad52 is essential for Rad52's mediator function since the Rad52 deletion mutant fails to stimulate Rad51-catalyzed DNA strand exchange in the presence of RPA (Krejci et al. 2002). The *in vivo* data further support the *in vitro* observation by demonstrating that a mutant strain carrying *rad52Δ409–420* displays a highly elevated sensitivity to ionizing radiation compared with the wild-type strain, but not as severe as a deletion mutant strain. Additionally, overexpression of Rad51 protein can fully complement the γ-ray sensitivity of the *rad52Δ409–420* strain.

Filament dynamics: factors that promote presynaptic filament turnover

A dynamic presynaptic filament, with protomers undergoing constant assembly and dissociation, is endowed with a catalytic activity appropriate for homology search and DNA strand exchange, but also with a capacity to disassemble under the appropriate circumstances. Recombinases in different organisms all exhibit DNA-stimulated ATP hydrolysis. Although ATP hydrolysis is not required for presynaptic filament formation, nucleoside triphosphate turnover affects the dynamics of recombinases on ssDNA and dsDNA. Evidence suggests that ATP hydrolysis and nucleotide exchange can occur throughout a presynaptic filament and that this usually does not require the dissociation of interior subunits (Brenner et al. 1987; Arenson et al. 1999). Instead, filaments continue to grow and dissociate at the two ends, coupled to the ATP hydrolytic cycle. Therefore in this section, we used the term "dynamics" to describe the phenomenon of binding and dissociation of protomers at the ends of recombinase–DNA filaments, and replacement of dissociated protomers by binding of new protomers.

Formosa and Alberts proposed a treadmilling model to explain how filament dynamics contribute to unidirectional branch migration during initial synapsis and subsequent bubble migration DNA synthesis reactions (Formosa and Alberts 1986a,b). DNA-dependent ATP hydrolysis provides individual recombinases with the ability to exist in conformational states compatible with nucleic acid association or dissociation. Thus, the recombinase can bind DNA with sufficient affinity to form an active filament and to perform enzymatic activities; however, it can also be released from the DNA for protein redistribution, to prevent filament discontinuities and to bypass sequence heterology (Menetski et al. 1990; Kowalczykowski 1991; Rehrauer and Kowalczykowski 1993; Kowalczykowski and Krupp 1995). For UvsX and RecA proteins with high ATP turnover rates, constant assembly/disassembly of the nucleoprotein filament is facilitated by the cycle of nucleotide cofactor binding and hydrolysis and by constant SSB competition for the ssDNA-binding sites (Liu et al. 2006b). For eukaryotic Rad51 proteins with slow ATP turnover, this process is facilitated by additional protein factors that confer dynamics to what might otherwise be a rigid and static nucleoprotein filament.

In eukaryotes, dynamic instability of presynaptic filaments affects the choice between synthesis-dependent strand annealing (SDSA) and double Holliday junction (dHJ) pathways, the two major subpathways of HR, and controls the crossover/non-crossover outcomes (Heyer et al. 2010). Both illegitimate recombination events and toxic recombination intermediates can be eliminated. The involvement of anti-recombinational translocases/helicases provides additional posttranslational modification targets to initiate HR in a cell cycle-controlled and context-controlled manner (e.g. replication forks vs. DSBs) (Heyer et al. 2010).

The dynamics of recombinase–dsDNA filaments are also important for recombination. Prior to synapsis, recombinase–dsDNA filaments must be disrupted to avoid dsDNA inhibition of DNA strand exchange. Following synapsis, filament disassembly from heteroduplex DNA allows the filament to turnover without temporally or spatially hindering the next enzymatic steps in HR. This is particularly relevant in the transition to DNA repair synthesis from the 3′-invasion end in heteroduplex DNA (Li and Heyer 2009). Furthermore, filament disassembly from ssDNA allows HR to be aborted when recombinase binding to ssDNA was initiated but HR is no longer sanctioned (Heyer et al. 2010). In this section, we discuss three factors that contribute to presynaptic/synaptic filament dynamics: (1) the ATP- versus ADP-bound state of recombinase monomers, (2) competition with SSB for DNA binding in the case of ssDNA, and (3) helicase/translocase activities that can remove recombinase from ssDNA or dsDNA (Figure 8).

Nucleotide binding and hydrolysis

Relationship between bound nucleotide and DNA-binding affinity

Electron microscopic studies of different presynaptic filaments from various organisms demonstrate that the active filament forms can be differentiated from inactive filament forms by characteristic morphological changes, the transition between which is controlled by the nucleotide cofactor binding (ATP, ADP, or AMP) (VanLoock et al. 2003). The binding of RecA stretches the dsDNA from an axial rise of 3.4 Å per base pair in B-form DNA to about 5.1 Å, and untwists the double helix from ~10 bp per turn to ~19 bp per turn (Stasiak and Di Capua 1982). Recombinases, from archaeal RadA, bacteriophage T4 UvsX, budding yeast Rad51, to human RAD51, show similar extended filamentous structures on DNA, suggesting an evolutionarily conserved mechanism of DNA stretching and unwinding to destabilize the substrate dsDNA and to expose the nucleobases for homology search (Ogawa et al. 1993; Seitz et al. 1998; Yang et al. 2001; Chen et al. 2008). These filaments exhibit an extended helical pitch of about 95 Å when the recombinases are locked into high-affinity

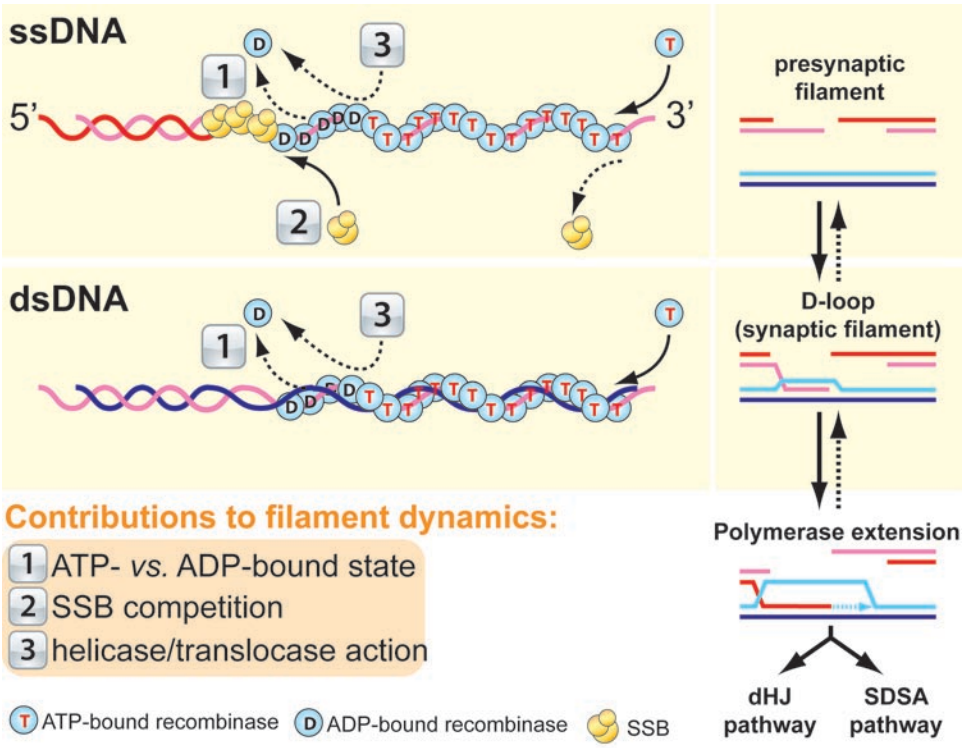


Figure 8. Factors that influence filament dynamics on ssDNA and dsDNA. Dynamic binding and dissociation of recombinase from ssDNA and dsDNA is relevant to presynaptic filament behavior in HR, and is influenced by factors including: (1) the ATP- versus ADP-bound state of recombinase monomers (on ssDNA and dsDNA), (2) competition with SSB (on ssDNA), and (3) external helicase/translocase activities that can dissociate recombinase monomers from ssDNA (such as Srs2 removal of Rad51 in *S. cerevisiae*) or dsDNA (such as Rad54 removal of Rad51 in *S. cerevisiae*). Filament dynamics on ssDNA allow reversibility before HR completion (filament dissociation) or recombinase turnover to promote registry of independently nucleated recombinase patches (monomer dissociation and rebinding), and filament dynamics on dsDNA allow transition to DNA repair synthesis (the filament on heteroduplex DNA can be dissociated to allow 3′-OH access to polymerase).

DNA-binding states using different non- or slowly hydrolyzable ATP analogs. The filaments show a relatively compressed helical pitch of about 65–85 Å, when the recombinases are saturated with ADP and kept in low-affinity DNA-binding states.

Beyond modifying filament morphology, the ATP hydrolytic cycle also modulates the stability of the recombinase complex in solution. The salt titration mid-point (STM; the salt concentration where 50% of pre-formed nucleoprotein filament disassembles when challenged by salt), serves as a biochemical parameter by which the stabilities of filaments under different conditions can be compared *in vitro*. The rapid ATP turnover of T4 phage UvsX results in a highly dynamic filament, with an STM of 180 mM NaCl (Ando and Morrical 1998). In contrast, the slow ATP turnover of *S. cerevisiae* Rad51 leads to a stable filament on ssDNA, and the STM is much higher at 550 mM NaCl (Zaitseva et al. 1999). The stable and nearly rigid Rad51 filament does not allow Rad51 dissociation from DNA either before or after synapsis, and therefore requires additional factors to facilitate protein turnover. In the following sections, we discuss the biological importance of a dynamic filament, and the factors that contribute to recombinase turnover.

The importance of a dynamic filament capable of ATP turnover

Filaments that are unable to accomplish ATP turnover are defective for DNA strand exchange, as demonstrated by assays using a slowly hydrolyzable ATP analog such as ATPγS, or by mutants in the Walker A nucleotide-binding motif. ATPγS-bound UvsX catalyzes branch migration at a transiently faster rate than ATP-bound UvsX, leading to an increase in the initial yield of nicked circular product in DNA strand exchange assays. However, the final product yield is greatly decreased and the reaction efficiency is significantly compromised when ATPγS instead of ATP is the available nucleotide cofactor (Kodadek et al. 1988). The situation is similar for *E. coli* RecA. Although ATPγS binding is sufficient for RecA to catalyze homologous pairing and strand exchange (Menetski et al. 1990; Rehrauer and Kowalczykowski 1993; Kowalczykowski and Krupp 1995; Shan et al. 1996), strand exchange over longer extents, bypass of mismatches in homologous DNA, and new DNA synthesis as well all require ATP and its hydrolysis (Rosselli and Stasiak 1991; Kim et al. 1992; Shan et al. 1996). A catalytically compromised RecA mutant defective in its Walker A box (RecA–K72R) binds ATP but its rate of ATP hydrolysis is reduced ~600-fold relative to wild type. *In vitro*, both the ssDNA binding and DNA strand exchange activity of this mutant are compromised

(Rehrauer and Kowalczykowski 1993; Shan et al. 1996). Furthermore, this mutant cannot complement a *recA* deletion strain for UV sensitivity and fails to induce the SOS response after UV irradiation (Konola et al. 1994; Renzette and Sandler 2008).

In *S. cerevisiae* Rad51, mutations at filament interface/active site residue His352 have been investigated. The Rad51–H352Y mutant has very low ATPase activity that is essentially limited to a single turnover due to defective ADP–ATP exchange (Chen et al. 2010). This mutant fails to catalyze DNA strand exchange reactions using either short oligonucleotide or long M13-derived DNA substrates (Grigorescu et al. 2009; Chen et al. 2010). In addition, Rad51–H352Y fails to displace RPA from ssDNA in the presence of ATP even though it has higher than wild-type affinity for ssDNA (Chen et al. 2010). This finding suggests that multiple ATP turnovers (hydrolysis + exchange) are necessary for RPA displacement. In contrast, the Rad51–H352A mutant exhibits weak ssDNA binding but forms catalytically competent filaments on ssDNA under low salt conditions. These filaments are capable of multi-turnover ATP hydrolysis, DNA strand exchange, and RPA displacement at low salt (Chen et al. 2010). Therefore, DNA strand exchange activity clearly correlates with ATP turnover.

Two Rad51 Walker A motif mutants have been extensively characterized, Rad51–K191A and Rad51–K191R in *S. cerevisiae*, and RAD51–K133A and RAD51–K133R in humans. *S. cerevisiae* Rad51–K191A and human RAD51–K133A mutants cannot hydrolyze ATP and do not support DNA strand exchange, conferring a dominant negative phenotype *in vivo* (Sung and Stratton 1996; Morrison et al. 1999; Morgan et al. 2002; Stark et al. 2002; Chi et al. 2006). *S. cerevisiae* Rad51–K191R (human RAD51–K133R) can bind DNA and catalyzes DNA strand exchange, despite low levels of ATP hydrolysis (Sung and Stratton 1996; Morrison et al. 1999; Chi et al. 2006). *In vitro*, *S. cerevisiae* Rad51–K191R protein has decreased affinity for both ATP and DNA binding; however, once the filament on DNA is formed, it is more stable than wild-type Rad51–DNA filaments (Li et al. 2007). The increased stability on DNA caused by the lack of ATP turnover decreases the efficiency of Rad51–K191R removal from dsDNA by Rad54 (Li et al. 2007). Thus, *S. cerevisiae rad51–K191R* haploid cells are sensitive to radiation *in vivo*, consistent with the ATP hydrolysis and DNA-binding defects (Morgan et al. 2002). Similarly, human *RAD51–K133R* mutant cells exhibit hypersensitivity to DNA cross-linking agents and to ionizing radiation, decreased rates of spontaneous sister chromatid exchange, and abrogation of homology-directed repair of an induced chromosomal DSB (Stark et al. 2002). Clearly, ATP hydrolysis is imperative for the normal function of recombinases *in vivo*. Precisely how ATP hydrolysis is important may be indicated from observations that overexpression of either of two DNA translocases can rescue the defects of a yeast *rad51–K191R* mutant strain (Fung et al. 2006). Both Srs2 and Rad54 modulate Rad51 filament turnover; Srs2 is specialized for Rad51 disassociation from ssDNA, whereas Rad54 is specialized for Rad51 dissociation from dsDNA. Both are discussed in the section "DNA helicases and translocases."

Competition with SSBs

Competition between recombinases and SSBs for access to ssDNA, and the role of RMPs in mediating recombinase nucleation on SSB-coated DNA, was discussed in the sections "Nucleation and propagation of presynaptic filaments on DNA" and "Proteins that promote presynaptic filament assembly." Competition for ssDNA binding from SSB proteins also contributes to the dynamics of presynaptic filaments, as demonstrated by the T4 phage reconstituted recombination system (Liu et al. 2006b). In a kinetic competition setup, UvsX and Gp32 are allowed to compete for ssDNA binding in the presence of UvsY and various nucleotides. Gp32 binds first to ssDNA, and then only the ATP- or ATPγS-bound high-affinity form of UvsX, facilitated by UvsY, can displace Gp32 to assemble into a filament. Once formed, however, the filament undergoes constant assembly and disassembly (collapse), tightly linked to the ATP hydrolytic cycle. Gp32 readily displaces the ADP- or AMP-bound low-affinity forms of UvsX and rebinds ssDNA, even in the presence of the mediator protein UvsY. Differential competition between Gp32 and high- versus low-affinity forms of UvsX for binding sites on ssDNA could lead to vectorial movement of presynaptic filaments (Liu et al. 2006b; Liu and Morrical 2010a,b). New ATP-bound subunits with high affinity for ssDNA would add to one end of a filament while displacing Gp32, which would extend the ATP-capped filament end. This is presumed to occur preferentially at the 3′ end since UvsX filaments exhibit net growth in the 5′ → 3′ direction on naked ssDNA (Griffith and Formosa 1985). Meanwhile, previously bound subunits undergo "aging" via ATP hydrolysis, so that the 5′ end of the filament is likely to be ADP- or AMP-capped. Gp32 readily displaces subunits at this end due to their lower affinity for ssDNA. The net result would be treadmilling of the filament in the 5′ → 3′ direction that depends both on the ATPase cycle and on SSB competition (Liu et al. 2006b). Treadmilling could hypothetically be coupled to mechanical processes such as polar branch migration or to the joining up of short filaments into longer filaments.

DNA helicases and translocases

In this section, we examine some of the DNA motor proteins, for example, DNA helicases and translocases, which are involved in HR. Some DNA motors catalyze branch migration and therefore participate directly either in the formation or resolution of heteroduplex DNA, or in the temporary relief of topological constraints to facilitate DNA strand exchange and repair synthesis (Colavito et al. 2010). Others regulate recombinase–DNA interactions and therefore play important roles in filament dynamics. Here, we focus on two classes of DNA motors that regulate HR either by disrupting recombinase–dsDNA filaments or by disrupting recombinase–ssDNA filaments.

Rad54 and the dissociation of recombinase filaments from dsDNA

Recombinases such as Rad51 do not discriminate effectively between dsDNA and ssDNA lattices, for which they have nearly equal affinity. Therefore, excess dsDNA inhibits strand exchange. In addition to mediator proteins recruiting Rad51 to ssDNA (see above), DNA motor proteins such as Rad54 help overcome dsDNA inhibition.

Rad54 is a eukaryotic DNA translocase belonging to the Swi2/Snf2 family of DNA-dependent ATPases (Eisen et al. 1995). On protein-free dsDNA molecules, Rad54 acts as a motor protein and translocates at a speed of 300 bp/sec, catalyzing hydrolysis of up to 800 ATPs per minute (Petukhova et al. 1998; Swagemakers et al. 1998; Amitani et al. 2006). Rad54 strongly stimulates the rate of Rad51-catalyzed DNA strand exchange by disrupting Rad51–dsDNA filaments that form inappropriately prior to synapsis (Petukhova et al. 1998; Solinger et al. 2002). This turnover of Rad51–dsDNA filaments catalyzed by Rad54 maintains a pool of recombinase subunits that are available for presynaptic filament formation on ssDNA. Rad54 further promotes presynapsis by interacting with and stabilizing the Rad51–ssDNA filament, in a manner independent of ATP hydrolysis (Solinger et al. 2002; Mazin et al. 2003; Wolner and Peterson 2005; Agarwal et al. 2011), a function shared by classic RMPs (Beernink and Morrical 1999). This physical association with the Rad51 filament might also target Rad54 to the homologous pairing locus, providing transient dsDNA unwinding that facilitates the homology search (Mazin et al. 2000; Van Komen et al. 2000; Solinger et al. 2002).

Following synapsis, the dissociation of recombinase from the heteroduplex product is important for loading of a DNA polymerase and other downstream events in DSB repair (Xu and Marians 2002; Li et al. 2009). As illustrated in Figure 5, self-dissociation of UvsX- and RecA–ssDNA filaments is facilitated by their relatively fast ATPase cycles, but the slow ATP turnover by eukaryotic Rad51 leads to a relatively rigid filament even after synapsis, calling for extrinsic factors to catalyze filament disassembly and the loading of DNA polymerase and its associated factors. During postsynapsis, Rad54 facilitates the dissociation of Rad51 from the heteroduplex product of DNA strand exchange to expose the 3′-OH end for DNA polymerase δ and its loading/processivity factors RFC/PCNA (Bugreev et al. 2007a; Li and Heyer 2009; Li et al. 2009). In addition to Rad51 release from heteroduplex, Rad54 performs other postsynaptic roles in HR. Rad54 stimulates heteroduplex DNA extension after joint molecule formation in Rad51-catalyzed strand exchange (Solinger and Heyer 2001). Finally, human RAD54 binds model Holliday junction structures with high specificity *in vitro* and promotes ATP-dependent branch migration, dissociating DNA structural intermediates associated with HR (Bugreev et al. 2006, 2007a).

"Anti-recombination" helicases that disrupt recombinase–ssDNA interactions

HR is the preferred DNA repair pathway to rescue stalled/collapsed replication forks and to repair DSBs. A homologous sequence, usually the sister chromatid, serves as the template for repair. However, loss of heterozygosity and genomic rearrangements, precursors to carcinogenesis in vertebrates, can occur when the homologous chromosome or an ectopic (non-allelic) sequence is used as template. For instance, recombination-induced mutation rates can be up to 1400 times greater than spontaneous mutation rates in budding yeast (Strathern et al. 1995; Hicks et al. 2010). Thus, special mechanisms exist to suppress inappropriate recombination. A number of ATP-dependent helicases converge on the presynaptic filament, using physical disruption of the filament to regulate whether HR is sanctioned to proceed.

S. cerevisiae Srs2 helicase Genetic studies have suggested that Srs2 can suppress HR at an early stage by eliminating inappropriate presynaptic filament formation and reversing recombination intermediates (Aguilera and Klein 1988; Aboussekhra et al. 1992; Kaytor et al. 1995; Milne et al. 1995; Schild 1995; Chanet et al. 1996). Biochemical studies directly demonstrate that Srs2 dismantles Rad51–ssDNA filaments and inhibits Rad51-mediated DNA strand exchange reactions (Krejci et al. 2003; Veaute et al. 2003). The 3′ to 5′ DNA helicase activity of Srs2 is suggested to dissociate Rad51 from the 3′ invading end (Rong and Klein 1993). The majority of Rad51 dissociation from ssDNA is actually caused by its own ATP hydrolysis activity, triggered by Srs2 through the protein–protein interaction (Antony et al. 2009). Although genetic studies have indicated a positive role of Srs2 in recombination by promoting non-crossover product formation in the SDSA pathway (Aylon et al. 2003; Ira et al. 2003), a biochemical mechanism for this effect remains to be demonstrated. Interestingly, Srs2 helicase activity is significantly stimulated by the Rad51–dsDNA complex, which suggests a potential role of Srs2 in promoting the SDSA pathway (Dupaigne et al. 2008).

The bacterial homolog of Srs2 is the UvrD protein, which also disrupts RecA–ssDNA filament formation and supports proper progression of replication forks in *E. coli* (Rong and Klein 1993; Veaute et al. 2005). No ortholog of Srs2 has been found in mammals, but FBH1 might be the functional homolog in fission yeast and humans, sharing an identical role in HR suppression (Chiolo et al. 2007; Lorenz et al. 2009). Overexpression of human FBH1 decreases RAD51 recruitment to ssDNA, and conversely, depletion of FBH1 increases sister chromatid exchange events in human cells (Fugger et al. 2009).

Eukaryotic RecQ helicases The RecQ helicase family includes only one helicase in budding yeast (Sgs1), but five helicases in humans (BLM, WRN, RECQ1, RECQ4, and RECQ5). Sgs1 has multiple roles in recombination, since

sgs1 mutants show hyper-recombination rates in spontaneous heteroallelic recombination and hypo-recombination rates in damage-induced recombination involving a homologous chromosome (Gangloff et al. 2000; Onoda et al. 2001). It is predicted that Sgs1 suppresses recombination through the same mechanism as Srs2 by disrupting the Rad51–ssDNA filament, based on the hyper-recombination phenotype. However, direct biochemical support for this suggestion comes from two human homologs, RECQ5 and BLM. *In vitro*, RECQ5 inhibits D-loop formation by displacing RAD51 from ssDNA, and *in vivo*, its deletion in mice elevates frequencies of spontaneous DSBs and HR between direct repeats (Hu et al. 2007). Similarly, BLM can also disrupt RAD51–ssDNA filaments *in vitro* (Bugreev et al. 2007b). Furthermore, both RECQ1 and BLM catalyze D-loop dissociation and branch migration to facilitate joint reversion and Holliday junction dissolution at the postsynaptic stage (Bachrati et al. 2006; Ralf et al. 2006; Bugreev et al. 2008). It is worthwhile to point out that FANCJ, a component in the Fanconi Anemia protein group, displays inactive ADP-bound RAD51 from ssDNA. FANCJ is not a RecQ helicase and the biological meaning of its RAD51-ssDNA filament disruption remains unknown (Sommers et al. 2009).

Bacteriophage T4 Dda helicase T4 phage Dda protein is an ATP-dependent DNA helicase in the SF1 helicase superfamily (Jongeneel et al. 1984). It loads onto a 5′-tailed ssDNA adjacent to a duplex region and unwinds DNA with a 5′ → 3′ polarity (Jongeneel et al. 1984). Dda was the first helicase identified to stimulate recombinase-catalyzed DNA strand exchange, when added to the reconstituted reaction at time points after synapsis (Kodadek and Alberts 1987). Dda increases the rate of branch migration >4-fold, from ~15 to ~70 bp/sec, and this stimulation requires protein–protein interactions between Dda and UvsX (Formosa and Alberts 1984; Kodadek and Alberts 1987). A plausible mechanism for this stimulation is that as Dda translocates the three-stranded junction in the 5′ → 3′ direction, it simultaneously recruits new UvsX subunits, thereby promoting filament treadmilling on the displaced strand (Kodadek and Alberts 1987). In contrast, when Dda is added to the ssDNA substrate simultaneously with UvsX, it inhibits the DNA strand exchange reaction. This may be explained by Dda translocase activity destabilizing the UvsX–ssDNA filament (Kodadek 1991), but this has yet to be formally demonstrated. The translocase activity of Dda also allows it to facilitate bypass of a protein-bound template at a replication fork and to rescue stalled replication forks through a template switching mechanism in collaboration with UvsX (Bedinger et al. 1983; Barry and Alberts 1994; Gauss et al. 1994; Kadyrov and Drake 2004; Nelson and Benkovic 2010). It remains unknown how Dda coordinates its contribution to protein turnover during presynapsis and branch migration activity during postsynapsis, differentially exerting negative or positive HR regulation through its translocation activity on DNA.

Overview and perspectives

To perform their essential functions in HR and homology-directed DNA repair, presynaptic filaments engage in dynamic behavior at multiple levels. At the atomic level, rearrangements of active site residues occur in response to DNA binding in order to activate catalysis of ATP hydrolysis, homology search, and DNA strand exchange reactions (Figures 2 and 4). Within each filament, allosteric communication occurs between active sites that span the protomer–protomer interface. At the molecular level, filament dynamics is governed by both intrinsic factors (linkage of catalytic turnover to changes in DNA-binding affinity) and extrinsic factors (ssDNA binding, mediator, and motor proteins, as well as DNA structure). The dynamic behavior of presynaptic filaments must be fine-tuned to meet the genomic challenges and physiological demands of each species (San Filippo et al. 2008).

In this review, we proposed that the decreasing ATPase activity of the central recombinases, and their self-removal rates from ssDNA and in particular from dsDNA, are correlated with increasing genomic complexity as observed across bacteriophage T4, *E. coli*, yeast, and human systems (Figure 3). Since ATP hydrolysis by a recombinase is associated with a conformational state amenable to dissociation from ssDNA or dsDNA, the decreased ATPase rates across these systems represents a reduced ability of recombinase to effect its own removal from DNA. The result is a reliance on external factors to modulate recombinase behavior on ssDNA and dsDNA, suiting the purpose of greater control and tighter regulation of HR initiation on ssDNA and HR progression to repair synthesis at dsDNA target sites. In eukaryotes, more protein factors are employed to modulate presynaptic filament formation and to regulate the dynamics of monomer association and dissociation in an active filament (Figure 5). By examining the biochemical mechanisms of different classic mediator proteins, we summarized some common strategies that mediator proteins employ to relieve SSB competition and to enhance recombinase assembly on ssDNA (Figure 7).

The mediator proteins facilitate the loading of recombinases onto SSB-covered ssDNA and thus perform key roles in the formation of presynaptic filaments, by promoting filament nucleation and propagation. It remains unknown whether mediator proteins continue to associate with the filament once recombinase has assembled, because no observations have been reported demonstrating the dissociation of mediator proteins from the recombinase–ssDNA filament before synapsis. There is evidence, however, that the T4 UvsY mediator remains associated with UvsX–ssDNA filaments following assembly (Jiang et al. 1997; Liu et al. 2006a). Thus, how accessory proteins are dissociated remains a key question to address. We envision that the formation of heteroduplex DNA after synapsis might trigger the motor proteins to remove both the recombinase and its associated mediator proteins away from ssDNA, clearing the road for

polymerase loading and DNA synthesis. Alternatively, handoff mechanisms could transfer mediator proteins to the second end to participate in annealing and second-end capture. The physical maturation of DNA structures during HR may serve as transition points that provide the necessary signals.

In humans, additional accessory proteins exist at two levels: (1) a new protein family (BRCA2) with homologs apparently absent in some microbial model systems (Brh2 identified in *U. maydis*, but no homolog identified in *S. cerevisiae*, for example) and (2) additional paralogs in the same protein family with different but similar cellular functions. The paralogs of greatest relevance to understanding presynaptic filament construction and behavior fall primarily into two families: the Rad51 paralogs and the RecQ-like homologs. Although associations among the five human Rad51 paralogs have been detailed, their mechanistic contributions to presynaptic filament dynamics have yet to be explained. As for the RecQ-like proteins, whereas there is only one RecQ helicase in both *E. coli* and *S. cerevisiae* (RecQ and Sgs1), five different RecQ helicases function in human cells (BLM, WRN, RECQ1, RECQ4, and RECQ5). Perhaps a general function in *E. coli* and *S. cerevisiae* has been more discretely separated into several different functions assigned to specific paralogs in humans. This may allow specialized responses to specific cellular contexts, which are fulfilled by different protein factors with similar enzymatic activity. On the other hand, the emerging number of different protein interaction partners might act as a means to modulate a target protein by providing substrate specificity (such as DNA structure specificity), or may contribute to protein stability or folding.

Also relevant to an understanding of presynaptic filament accessory factors in eukaryotes will be a rationale for the apparently different roles of Rad52 in yeast and humans, presumably related to the presence or absence of a BRCA2 ortholog in the same genome. Rad52 is an essential protein for both HR and the SSA pathway in budding yeast, but BRCA2 supplants RAD52 as the critical mediator protein in human presynaptic filament formation. However, RAD52 shows an *in vivo* role in facilitating RAD51 filament formation but lacks a mediator activity *in vitro* (Jensen et al. 2010; Feng et al. 2011; Liu and Heyer 2011). On the other hand, human BRCA2 cannot anneal complementary ssDNA in the presence of RPA, an activity expected for second-end annealing in both SDSA and dHJ formation (Jensen et al. 2010; Liu and Heyer 2011). As proposed in this review, it seems likely that in humans a division of labor has evolved between BRCA2 and RAD52, with the former specialized for presynaptic functions and the latter specialized for postsynaptic functions such as second-end capture. In addition, BRCA2 has multiple interaction partners (Figure 6), which might modulate its activities, perhaps allowing it to participate in other stages of recombination such as annealing and second-end capture (Liu and Heyer 2011).

In summary, the presynaptic filament is a remarkable search engine that can use any stretch of ssDNA sequence on which it assembles to define the identification of a homologous target in a genome. This behavior is achieved by dynamic behavior on multiple levels: (1) at the atomic level, where DNA binding reorders the active site to facilitate catalysis; (2) at the subunit level, where conformational changes during the ATP hydrolytic cycle modulate affinity for ssDNA and dsDNA; (3) at the intersubunit level, where allosteric effects promote cooperative binding of DNA and nucleotide substrates and establish active site asymmetry; (4) at the filament level, including changes in filament morphology (compressed vs. extended forms) that are associated with homology search and DNA strand exchange mechanisms; and (5) at the level of subunit exchange, where recombinase association and dissociation with the ssDNA substrate and heteroduplex product determine the frequencies of recombination events and of the transition to DNA synthesis. In genomes with greater sequence complexity, the recombinases have co-evolved with a set of external factors that relieve the recombinase of the capacity to regulate its own dynamics on ssDNA and dsDNA. We propose that the explanation for outsourcing control of these dynamics to other factors lies in greater regulatory control over homologous target site choice and transition to DNA synthesis. We expected that additional mechanisms for the regulation and dynamics of presynaptic filaments remain to be revealed, in particular through continued studies of helicases, translocases, and mediator proteins with their associated protein factors.

Declaration of interest

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