Minireview

Rad51 Recombinase and Recombination Mediators*

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Prologue

Through pairing and shuffling of related DNA sequences, homologous recombination (HR) serves to create genetic diversity. In both mitotic and meiotic cells, HR is also an important mechanism for eliminating DNA double-stranded breaks (DSBs) (1, 2). Furthermore, HR is involved in restarting stalled DNA replication forks and provides a means for telomere length maintenance in cells lacking telomerase (3–6). Accordingly, defects in HR result in sensitivity to genotoxic agents, mitotic and meiotic chromosome aberrations, and destabilization of the genome (7, 8). Recent evidence points to a role of HR in cancer prevention via the tumor suppressors BRCA1 and BRCA2 (9).

At the core of all HR reactions lies the ability of the recombination machinery to utilize a ssDNA molecule, derived from the processing of DSBs or stalled DNA replication forks (1–3, 5, 6, 10), to invade a homologous duplex. The product of this DNA strand invasion reaction is a structure called D-loop, and the overall enzymological process is referred to as homologous DNA pairing and strand exchange (Fig. 1) (10, 11). Resolution of the D-loop is accomplished by one of a number of pathways (1, 2) to yield recombinants that either entail a reciprocal exchange of genetic information flanking the initiation site (crossover recombinants) or not (non-crossover recombinants).

The homologous DNA pairing and strand exchange reaction is mediated by a class of conserved recombinase enzymes: UvsX in bacteriophage T4, RecA in Escherichia coli, and Rad51 in eukaryotes (11, 12). Studies conducted in the past several years have helped define a set of operational principles for the Rad51 recombinase and have unveiled an array of ancillary factors of Rad51.

Recombination and the RAD51 Gene

Genetic analyses of DNA double-stranded break repair and mitotic and meiotic recombination in the budding yeast Saccharomyces cerevisiae have led to the identification of genes collectively known as the RAD52 epistasis group (1, 2). Among the members of the RAD52 group, RAD51 is, arguably, the most famed. The RAD51-encoded product has structural homology to E. coli RecA and T4 UvsX proteins and is highly conserved among eukaryotes (10, 11, 13). In many respects, Rad51 behaves like RecA in homologous DNA pairing and strand exchange. The recombinase activity of Rad51 can be described in three kinetic phases.

Rad51-mediated Homologous DNA Pairing and Strand Exchange

The Presynaptic Phase—Like RecA, Rad51 forms a right-handed helical filament on ssDNA in which the DNA is held in an extended state (10, 11, 14). The Rad51-ssDNA nucleoprotein filament, often referred to as the presynaptic filament, contains a binding site for dsDNA. The initiating ssDNA substrate is bound within the “primary” site of the presynaptic filament, and the homologous duplex resides within the “secondary” site (10, 11). The capability to hold two DNA molecules in close proximity underlies the ability of the presynaptic filament to form DNA joints between these molecules. Interestingly, even though Rad51 hydrolyzes ATP (15), presynaptic filament assembly requires only ATP binding (2, 10, 11, 16).

Rad51 nucleates onto ssDNA rather slowly, thus rendering presynaptic filament assembly vulnerable to competing factors. In fact, replication protein A (RPA), a classical ssDNA-binding protein, while playing an important role in Rad51-mediated recombination, also paradoxically competes with Rad51 for binding sites on the initiating ssDNA substrate. A number of accessory proteins, termed recombination mediators (10, 12) (see below), promote the nucleation of Rad51 onto ssDNA and in doing so help Rad51 overcome the inhibitory effect of RPA (Fig. 2) (2, 10, 12).

The Synaptic Phase—Herein, the incoming duplex is incorporated into the presynaptic filament through multiple contact points and then sampled for homology. Extensive studies with RecA have yielded no evidence for progressive scanning of the duplex in homology search. Rather, to test for homology, the incoming duplex is held transiently within the secondary binding site of the RecA presynaptic filament, and if homology is not found, the duplex is released. This cycle of binding and release of the duplex goes on until homology is located. Given its structural and functional similarities to RecA, it seems reasonable to assume that the Rad51 presynaptic filament also employs the same random collision mode of homology search (10, 11). However, Rad54 may endow the presynaptic filament with the ability to scan the incoming duplex molecule for homology (10, 17, 18) (see “Rad54, a DNA Translocase and Supercoiling Motor”).

Once homology is located, the initial capture of the duplex and its alignment with the ssDNA molecule are facilitated through DNA joints that are “paranemic” in nature. The paranemic joints, which occur away from a free DNA end, involve canonical Watson-Crick hydrogen bonding. The efficiency of paranemic joint formation is dictated by the ease of matching paired bases and the ease of forming auxiliary base pairs and helical distortions. The paranemic joints are collectively known as the “primary” site of the presynaptic filament (10, 11, 14). The capability to hold two DNA molecules in close proximity underlies the ability of the presynaptic filament to form DNA joints between these molecules. Interestingly, even though Rad51 hydrolyzes ATP (15), presynaptic filament assembly requires only ATP binding (2, 10, 11, 16).

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Although the paranemic linkage brings the two recombining molecules into homologous registry, it is transient (10, 11, 19).
strand exchange. The length of the DNA joint is extended by DNA
appropriately (10, 11). RPA promotes Rad51 presynaptic filament assembly by minimizing secondary structure in long ssDNA molecules (10, 11). In addition, by sequestering free ssDNA, RPA renders the already made DNA joints less prone to a reversal reaction (22). RPA also prevents ssDNA from precluding the entry of duplex DNA into the secondary binding site of the Rad51 presynaptic filament (23).

Because of the high affinity of RPA for ssDNA, when an excess of RPA is added either with or prior to Rad51 to the ssDNA, assembly of the presynaptic filament is hindered (2, 10, 24, 25). This paradoxical behavior of RPA has been used as the basis for uncovering recombination mediators (Fig. 2) (2, 10). Rad52 and Its Recombination Mediator Function—In S. cerevisiae, the suppression of Rad51 presynaptic filament assembly by RPA is overcome by Rad52. That Rad52 has recombination mediator activity is fully corroborated by cytological data (26, 27) and by chromatin immunoprecipitation experiments that examined the targeting of Rad51 to a site-specific DSB (28, 29). Rad52 is multimeric and a ring-shaped molecule (30). The three-dimensional reconstruction of the human Rad52 structure based on images from electron microscopy shows seven subunits in the protein ring with a central channel (31). The available evidence points to ssDNA being bound on the outer rim of the Rad52 ring (32, 33). The multimeric nature of Rad52 likely endows it with the ability to bind DNA cooperatively and maintain a stable nucleoprotein structure. S. cerevisiae Rad51 and Rad52 interact in the yeast two-hybrid system, co-immunoprecipitate, and form a stoichiometric complex. Complex formation between Rad51 and Rad52 is critical for the recombination mediator activity of the latter (2, 10, 34).

Rad52 binds ssDNA avidly but has lower affinity for dsDNA (10, 35). Recent crystallographic data on the N-terminal DNA binding/oligomerization domain of human Rad52 shows an undecameric (11-subunit) ring structure that possesses a deep groove on the outer surface. This groove is lined with basic and aromatic residues (32, 33) that are involved in binding DNA (33). Biochemical data suggest that binding of the Rad51-Rad52 complex to ssDNA provides nucleation sites for the recruitment of free Rad51 molecules to assemble the presynaptic filament (10, 36, 37). Rad52 also recognizes RPA-bound ssDNA. This property of Rad52 is thought to allow the Rad51-Rad52 complex to gain access to ssDNA covered with RPA (36). As alluded to above, the oligomeric structure of Rad52 and the Rad51-Rad52 complex is expected to enable them to make multiple contacts with ssDNA, likely to be critical for the avid and stable interaction of these protein species with the DNA. Furthermore, it is conceivable that the oligomeric nature of these protein species helps ensure that a single nucleation event will lead to the loading of multiple Rad51 molecules onto ssDNA for assembling a nascent Rad51 filament.

It is important to note that in S. cerevisiae, Rad52 is also indispensable for a subset of recombination and DNA repair events that still occur when Rad51 is absent, which could explain why rad52 mutants often exhibit a recombination/repair phenotype more severe than that of rad51 mutants (1, 2).

Rad55-Rad57 Complex and Other Rad51 Paralogs—Rad55 and Rad57 proteins exhibit some homology to Rad51 and are therefore regarded as paralogs of Rad51. These two proteins form a stable heterodimeric complex (2, 10). Like Rad52, Rad55-Rad57 heterodimer binds ssDNA and possesses a recombination mediator activity (24). In congruence with the biochemical data, the targeting of Rad51 to DSBs is impaired in rad55 and rad57 mutants, as revealed by cytological and chro-

**Factors That Influence the Presynaptic Stage**

RPA—Through its ability to protect and help remove secondary structure in ssDNA, RPA plays a key role in DNA metabolic pathways that entail the formation of a ssDNA intermediate. The involvement of RPA in recombination is multifaceted. It has both positive and negative effects on the recombination reaction. The stimulatory effect of RPA is seen most clearly when long DNA substrates are used (10, 11), whereas only a modest stimulatory effect of RPA is noted when oligonucleotides are employed as substrates (21). RPA promotes Rad51 presynaptic filament assembly by minimizing secondary structure in long ssDNA molecules (10, 11). In addition, by sequestering free ssDNA, RPA renders the already made DNA joints less prone to a reversal reaction (22). RPA also prevents ssDNA from precluding the entry of duplex DNA into the secondary binding site of the Rad51 presynaptic filament (23).

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matin immunoprecipitation experiments (28, 29, 38). An interaction between Rad55 and Rad51 is seen in the yeast two-hybrid assay (2), and purified Rad55-Rad57 complex binds Rad51 (24). A recent study suggests that Rad55-Rad57 also stabilizes the presynaptic filament (39).

Five Rad51 paralogs, XRC2, XRC3, Rad51B, Rad51C, and Rad51D, are found in vertebrates. These proteins dimerize among themselves to form three distinct complexes, XRC2-Rad51D, XRC3-Rad51C, and Rad51B-Rad51C, that have ssDNA binding activity (2). In addition, XRC2-Rad51D and Rad51B-Rad51C can form a tetrameric complex (2). The results from a number of cytological and genetic investigations are consistent with these paralog pairs acting as mediators of Rad51 presynaptic filament assembly (2, 4, 40). Biochemical evidence that Rad51B-Rad51C fulfills such a role has been provided (41). It is possible that XRC2-Rad51D and XRC3-Rad51C complexes also have a recombination mediator activity and cooperate with the Rad51B-Rad51C complex in the delivery of Rad51 to recombination substrates.

Presynaptic Role of Rad54—Recent biochemical studies have implicated S. cerevisiae Rad54 in promoting the assembly and stability of the presynaptic filament (29, 42). The presynaptic function of Rad54 is consistent with the observation that the appearance of Rad51 foci, believed to contain DNA-bound Rad51, is delayed in meiotic S. cerevisiae cells lacking Rad54 (43). Likewise, in mouse embryonic stem cells deleted for Rad54, the frequency of DNA damage-induced Rad51 foci is reduced (44). More recently, a defect in targeting Rad51 to a DSB has been demonstrated by chromatin immunoprecipitation in the S. cerevisiae rad54 mutant (29). Because Rad54 binds Rad51 and ssDNA avidly (10, 29), perhaps Rad54 directly loads Rad51 onto ssDNA to initiate presynaptic filament assembly.

BRCA2, a Probable Recombination Mediator—BRCA2 interacts with Rad51 through its C terminus and via several copies of a conserved motif called the BRC repeat (4, 45, 46). Recent biochemical and crystallographic studies have shown an ability of BRCA2 to bind ssDNA via three “OB” folds, DNA binding modules that are present in many single-strand DNA-binding proteins (47). It has been proposed that BRCA2 acts as a recombination mediator in Rad51-dependent events (46, 47).

Such a role would certainly explain the recently increasing efficiency of mutant BRCA2 cells and the greatly reduced ability of these cells to assemble DNA damage-induced Rad51 foci (4). If so, the presence of multiple Rad51 binding motifs in BRCA2 could ensure that a sufficient number of Rad51 molecules are delivered by BRCA2 to ssDNA to seed the assembly of the presynaptic filament.

Proteins That Work in the Synaptic and Postsynaptic Phases

Rad54, a DNA Translocase and Supercoiling Motor—Although Rad51 has only a modest ability to make a D-loop, addition of a catalytic amount of Rad54 renders D-loop formation highly robust (2, 10, 48). Rad54, a member of the Swi2/Snf2 protein family, has a robust ATPase function that is specifically dependent on dsDNA for activation. The free energy from ATP hydrolysis fuels the translocation of Rad54 on duplex DNA, which generates positive supercoils ahead of protein movement and negative supercoils trailing it (Fig. 3) (10, 17, 18). The negative supercoils produced by Rad54 lead to transient opening of the DNA strands (18). As expected, mutations that inactivate the ATPase activity of Rad54 abolish the DNA translocase and supercoiling functions and impair recombination (2, 10). The ATPase and DNA supercoiling activities of Rad54 are strongly stimulated by Rad51 (2, 10). Although the Rad51 presynaptic filament likely utilizes a random collision mechanism to search for homology in the incoming duplex molecule, Rad54 may endow the presynaptic filament with the ability to actively scan the duplex for homology. The negative supercoils generated by Rad54 probably facilitate both parame- ric and plectonemic DNA joint formation (Fig. 3).

Aside from promoting the homologous pairing reaction, Rad54 also enhances the rate of DNA branch migration severalfold (20). When translocating on duplex DNA, Rad54 removes bound Rad51 from its path. This property of Rad54 may be germane for releasing Rad51 from newly made DNA joints, hence promoting the recycling of Rad51 and facilitating DNA branch migration (49).

Rdh54/Tid1 and Rad54B—A Rad54-like protein has been described in S. cerevisiae (Rdh54/Tid1) and vertebrates (Rad54B) (10). Rdh54/Tid1 has a robust dsDNA-activated ATPase function that fuels its translocation on duplex DNA to produce unconstrained supercoils in the DNA, much like Rad54. In addition, Rdh54/Tid1 binds Rad51 and stimulates the Rad51-mediated D-loop reaction (2, 10, 50). Whether Rdh54/Tid1 can promote DNA branch migration and dissociate Rad51 from duplex DNA is not known. Rdh54/Tid1 helps mediate a subset of mitotic recombination events and has a major influence on meiotic recombination (2, 51). Rad54B possesses a dsDNA-dependent ATPase activity (52) but remains poorly characterized overall.

Chromatin Remodeling by Rad54—A number of Swi2/Snf2 family members are involved in chromatin remodeling during transcription and DNA repair (53). For this reason, several laboratories have investigated whether Rad54, being a canonical member of the Swi2/Snf2 protein family, also has a chromatin remodeling activity. In these studies, using assays that measure mononucleosome redistribution (54) and the accessibility of DNA packaged into chromatin to restriction enzymes...
(55, 56), it was shown that Rad54 from S. cerevisiae and Droso-
phila melanogaster indeed possesses the ability to remodel chromatin (54–56). Furthermore, chromatin protects S. cerevisiae Rad54 against thermal denaturation (55). The chromatin remodeling function of Rad54 is stimulated by Rad51 and requires ATP hydrolysis by Rad54 (54–56).

In two of the published studies, Rad54 cooperated with the Rad51 presynaptic filament in D-loop reactions involving chromatinized duplex DNA. In fact, a more robust D-loop reaction was seen when such a template was used, leading to the suggestion that the eukaryotic recombination factors have evolved to function specifically with chromatin (55, 56). The ability of Rad54 to remodel chromatin is likely linked to its DNA translocase and supercoiling functions (55), as has been similarly suggested for the SWI/SNF from yeast complex (54, 55).

Epilogue

HR mechanisms are highly intricate and subject to multiple layers of control. Studies in the past few years have helped define the functions of many core recombination factors. However, several major questions remain unanswered. In particular, the functional relationship among the recombination mediators, i.e. whether they act synergistically or in a defined temporal sequence, remains to be deciphered. The manner in which BRCA1 affects HR efficiency is not yet understood. Gene regulatory factors, which BRCA1 affects, have evolved to function specifically with chromatin (55, 56). Whether Rad54 acts alone or in conjunction with other factors (e.g. Radh54/Tid1) in chromatin remodeling during HR, above all, it can be stated with some confidence that the knowledge gained from studying the basic mechanism of HR will find practical applications in cancer prevention, diagnosis, and treatment.

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