Right or left turn? RecA family protein filaments promote homologous recombination through clockwise axial rotation

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Summary

The RecA family proteins mediate homologous recombination, a ubiquitous mechanism for repairing DNA double-strand breaks (DSBs) and stalled replication forks. Members of this family include bacterial RecA, archaeal RadA and Rad51, and eukaryotic Rad51 and Dmc1. These proteins bind to single-stranded DNA at a DSB site to form a presynaptic nucleoprotein filament, align this presynaptic filament with homologous sequences in another double-stranded DNA segment, promote DNA strand exchange and then dissociate. It was generally accepted that RecA family proteins function throughout their catalytic cycles as right-handed helical filaments with six protomers per helical turn. However, we recently reported that archaeal RadA proteins can also form an extended right-handed filament with three monomers per helical turn and a left-handed protein filament with four monomers per helical turn. Subsequent structural and functional analyses suggest that RecA family protein filaments, similar to the F1-ATPase rotary motor, perform ATP-dependent clockwise axial rotation during their catalytic cycles. This new hypothesis has opened a new avenue for understanding the molecular mechanism of RecA family proteins in homologous recombination. ***BioEssays*** **30:48–56, 2008.**

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Abbreviations: HR, homologous recombination; DSB, double-strand break; ssDNA, single-stranded DNA; NTD, N-terminal domain; dsDNA, double-stranded DNA; CTD, C-terminal domain; HhH, helix-hairpin-helix; *Sso, Sulfolobus solfataricus*; AFM, atomic force microscopy; *Sc, Saccaromyces cerevisiae*; EM, electron microscopy; SRM, subunit rotation motif; TP, ATP-bound; DP, ADP + P_i- or ADP-bound; E, empty state; *Pf, Pyrococcus furiosus*; Mv, *Methanococcus voltae*; CAD, core ATPase domain.

Biological importance of homologous recombination

Homologous recombination (HR) involves the bringing together of two DNA molecules, a search for homologous sequences and exchange of DNA strands. It is an evolutionarily conserved mechanism for repairing DNA double-strand breaks (DSBs) and stalled DNA replication forks. The primary function of HR is to maintain genome stability during vegetative growth of prokaryotic cells or mitosis of eukaryotic cells. Loss of genome stability is common in cancerous cells. During meiosis, HR establishes a physical connection between homologous chromosomes to ensure their correct disjunction at the first meiotic division. Chromosome nondisjunction leads to aneuploidy in gametes, which can cause infertility, spontaneous abortion and developmental defects (e.g., Down syndrome).[1]

RecA family proteins and their architectures

RecA family proteins are the central recombinases for HR and recombinatorial DNA repair. The family includes prokaryotic RecA, archaeal RadA and Rad51, and eukaryotic Rad51 and Dmc1. While RecA-deficient *Escherichia coli* cells and Rad51-deficient yeast cells are viable, Rad51-deficient vertebrate cells are not. The latter accumulate chromosomal breaks before death.[2] Rad51 and its meiosis-specific homolog, Dmc1, are indispensable for meiosis.[3,4] Mammalian Rad51 and Dmc1 proteins interact with tumor suppressor proteins such as BRCA2.[5,6] RecA family proteins have important roles in cell proliferation, genome maintenance and genetic diversity, particularly in higher eukaryotes.

At the tertiary structural level, all RecA family proteins share a core ATPase domain containing two disordered loops for single-stranded DNA (ssDNA) binding (denoted the L1 and L2 motifs).[7] This core ATPase domain, often referred to as the

RecA fold,[8] is structurally and functionally similar to the ATPase domains of DNA/RNA helicases, F1 ATPases, chaperone-like ATPases and membrane transporters.[9] RadA, Rad51 and Dmc1 each has an additional N-terminal domain (NTD) that interacts with double-stranded DNA (dsDNA),[10,11] whereas RecA has a C-terminal domain (CTD) with a similar proposed function. The helix–hairpin–helix (HhH) motifs in the NTDs of RadA, Rad51 and Dmc1 mediate dsDNA binding.[10,11]

The crystal structures of several RecA family members reveal that a polymerization motif is responsible for the assembly of all known quaternary structures, including toroidal rings and right- and left-handed helical filaments.[7,12–19] The polymerization motif is located between the NTD and the core ATPase domain. A hydrophobic residue (phenylalanine in RadA, Rad51 and Dmc1; isoleucine in RecA) in the polymerization motif docks in a hydrophobic pocket on the neighboring core ATPase domain. This interaction was also observed in the structure of a fusion construct combining the human Rad51 monomer and a peptide from the human tumor suppressor protein BRCA2.[12,20]

Biochemical properties of the RecA family proteins

The catalytic functions of the RecA family proteins involve three steps. These proteins first load onto ssDNA (one monomer/three nucleotides) to form a 6_1 right-handed presynaptic nucleoprotein with six monomers per helical turn.[7] In the presence of ATP or its nonhydrolyzed analog (e.g. ATP-γS), ssDNAs within the nucleoprotein filaments are extended. For example, the axial rise/nucleotide of an *E. coli* RecA-ssDNA nucleoprotein filament (5.1 Å) is approximately 50% longer than that of a B-type dsDNA (3.4 Å). It is generally accepted that such presynaptic nucleoprotein filaments are the functional form of the RecA family proteins. Once homology is recognized, a synaptic complex consisting of three DNA strands in a protein filament is formed. The molecular mechanism behind the homology pairing process is not known. Eventually, DNA strands are exchanged, one of the original duplex strands is expelled and a new heteroduplex (or D-loop) is created.

Although members of the RecA protein family all have DNA-dependent ATPase activities and need ATP to make joints between recombinant DNA molecules, the functions of ATP hydrolysis are not fully understood. For example, it was reported that ATP hydrolysis mediated by *E. coli* RecA protein is crucial for ensuring effective recombinational DNA repair. Specifically, in the absence of ATP hydrolysis, hybrid DNAs formed before branch migration are blocked and therefore are shorter than those existent in the presence of ATP hydrolysis. ATP hydrolysis renders only a 5′ to 3′ direction (with respect to ssDNA) in the strand-exchange reaction catalyzed by *E. coli* RecA proteins.[21,22] In contrast, eukaryotic Rad51 proteins can promote ATP-dependent branch migration in either the 5′ to 3′ or 3′ to 5′ direction.[23–25] In addition, both *E. coli* RecA and eukaryotic Rad51 proteins have been found to promote DNA pairing and strand exchange without (or with very little) ATP hydrolysis.[21,26–30] ATP hydrolysis is generally accepted to be responsible for recycling RecA family protein monomers in nucleoprotein filaments after DNA strand-exchange reactions. ATPγS can block RecA filament disassembly, whereas ATP hydrolysis reduces the affinity of RecA protein for DNA.[31–33] The mechanism underlying the ATP-dependent disassembly of RecA family protein filaments is also unknown.

Current models of the function of RecA family proteins in HR

The biochemistry of the ATP-dependent strand-exchange reaction was recently reviewed in detail.[34] In some models, RecA family proteins function only as DNA-pairing enzymes, and ATP hydrolysis facilitates RecA dissociation and/or distribution along DNA substrates. These models do not explain the bypass of nonhomologous DNA sequences during homology pairing and strand-exchange reactions. Alternatively, the facilitated DNA rotation model proposes that RecA family proteins function as motor proteins to promote coordinated rotation between dsDNA and ssDNA (Fig. 1). This model seems compatible with all experimental results reported to date.[34] However, it is not without problems. Despite some mechanistic differences, all current models share the proposition that all RecA family proteins bind ssDNA and form 6_1 right-handed helical nucleoprotein filaments with six monomers per helical turn throughout their catalytic cycles, including homology pairing and strand-exchange reactions. This creates a problem for the facilitated DNA rotation model. Because all known DNA-binding motifs (i.e. L1, L2, HhH, or CTD) localize along or near the central axes of a 6_1 right-handed helical filament (Fig. 2),[7,14–16,35] the facilitated DNA rotation model implies that novel DNA-binding sites are located on the exterior of the right-handed helical filaments to facilitate DNA rotation.[34] To date, such novel DNA-binding sites have not yet been identified in any RecA family protein. Since RecA and other members of RecA family have been intensively studied for more than 15 years, we wonder if such novel DNA-binding sites indeed exist. An alternative possibility is that the RecA family proteins perform their catalytic functions by relocating their DNA-binding motifs from the central axis to the exterior of the filaments (see below). We speculate that these proteins may execute their catalytic functions by changing their quaternary structures from a 6_1 right-handed helical filament to other topological configurations.

Newly recognized quaternary structures of the RecA family proteins

Although RecA family proteins are known to form 6_1 right-handed helical filaments[7,15,16,35] and toroidal rings,[12,13,36]

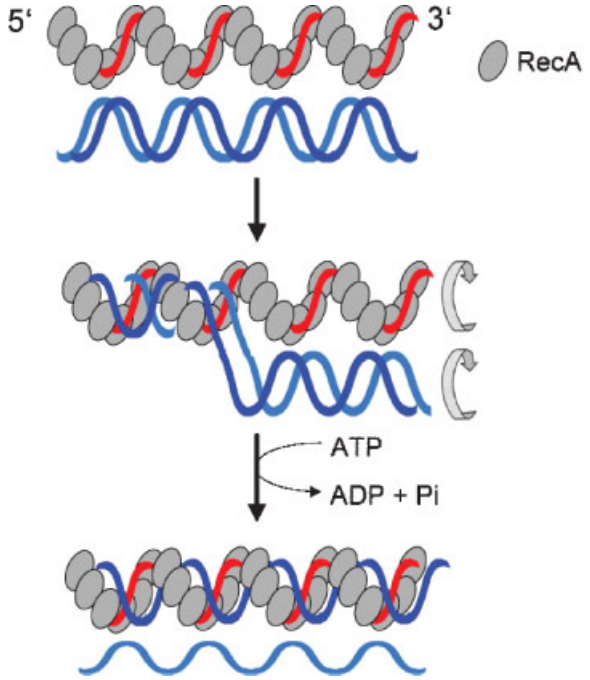

Figure 1. The facilitated DNA rotation model for RecA-mediated DNA strand exchange coupled to ATP hydrolysis. In this model, RecA acts as a motor protein to rotate one DNA molecule to align with another, with one DNA molecule inside the filament and the other on the exterior of the filament. DNA rotation could generate the torsional stress needed to unwind heterologous insertions so as to bypass them. This model assumes that the RecA protein filament remains a 6_1 right-handed helical filament throughout the homology pairing and strand-exchange reaction. Therefore, the model requires the presence of DNA-binding sites arranged longitudinally along the exterior of the filament.[34]

recent structural studies indicate that large structural variations of RecA family protein filaments can occur. For example, archaeal *Sulfolobus solfataricus* (*Sso*) RadA proteins can self-polymerize into a 3_1 right-handed filament with three monomers per helical turn and a 4_3 left-handed helical filament with four monomers per helical turn[17–19] (Fig. 2). RecA family protein filaments with left-handed helices were also observed using other ultrastructural techniques. Using atomic force microscopy (AFM) with carbon nanotube tips, we showed that *Sso*RadA[18,37] and *Saccaromyces cerevisiae* (*Sc*) Dmc1[18] could form left-handed helical filaments. Electron microscopy (EM) imaging analysis also revealed left-handed helical filaments of the *Sso*RadA[18] and *E. coli* RecA proteins (Chien-Der Lee, Yuan-Chih Chang and Ting-Fang Wang, unpublished results), respectively. Considering all these results together, we conclude that the left-handed helical filaments of RecA family proteins exist not only under protein crystallization conditions but also under neutral pH solutions. The ability to form right- and left-handed helical filaments is likely a general property of most, if not all, members of the RecA protein family.

Other investigators might have noted left-handed RecA family protein filaments before us. Yu and Egelman first reported in 1990 that *E. coli* RecA filaments that formed on a linear dsDNA in the presence of ATP and aluminum fluoride show both right- and left-handed helical pitches (Fig. 3, adapted from Fig. 1 in reference 38).[38] Aluminum fluoride can substitute for phosphate after ATP hydrolysis, and it was used to trap the ADP-Pi state of the RecA protein. However, after the first report of the crystal structure of a *E. coli* RecA 6_1 right-handed protein filament,[7] this filament form became generally accepted as the active form of the RecA protein. The left-handed helical filaments were presumed to be experimental artifacts due to the negative staining EM protocols. Specifically, it was postulated that deformation of flexible filaments during absorption to EM grids might yield the visual impression of left-handed filaments. Such deformations may break the axial symmetry and cause the superimposition of signals from the upper and lower parts of the filament, thus giving a visual impression of inclined striation. Although this interpretation seems reasonable, it has shortcomings. First, the interpretation arose from the preconception that RecA and RecA family proteins can only form helical filaments with right-handed pitches. Second, to our knowledge, it was not verified by other experimental approaches such as AFM or X-ray crystallography. As described above, we reported the crystal structure of the *Sso*RadA left-handed helical filament. AFM imaging analyses revealed that *Sso*RadA and *Sc*Dmc1 can form left-handed helical filaments, and EM imaging analyses demonstrated the same for *E. coli* RecA proteins. In addition, a recent AFM imaging study revealed a single RecA-dsDNA helical filament with not only right-handed but also left-handed helical pitches (see Fig. 1E in reference 39).[39] Seitz et al. might also have observed the left-handed helical filament of archaeal *Sso*RadA protein several years ago. The cover picture of the May 1, 1998 issue of *Genes and Development* shows an EM image of a left-handed helical filament made by the *Sso*RadA protein; however, the paper described only a right-handed filament.[40] It would be interesting to know if the image was inadvertently mirrored during the process of production.

Axial rotation controls both the quaternary structures and catalytic activities of RecA family proteins

New structural and mechanical insights have been achieved by comparing different archaeal RadA or Rad51 quaternary structures, including a heptameric protein ring,[12] a RadA-AMP-PNP 6_1 right-handed filament with six monomers per helical turn,[15] a 3_1 extended right-handed filament with three monomers per helical turn[17,19] and a 4_3 left-handed filament

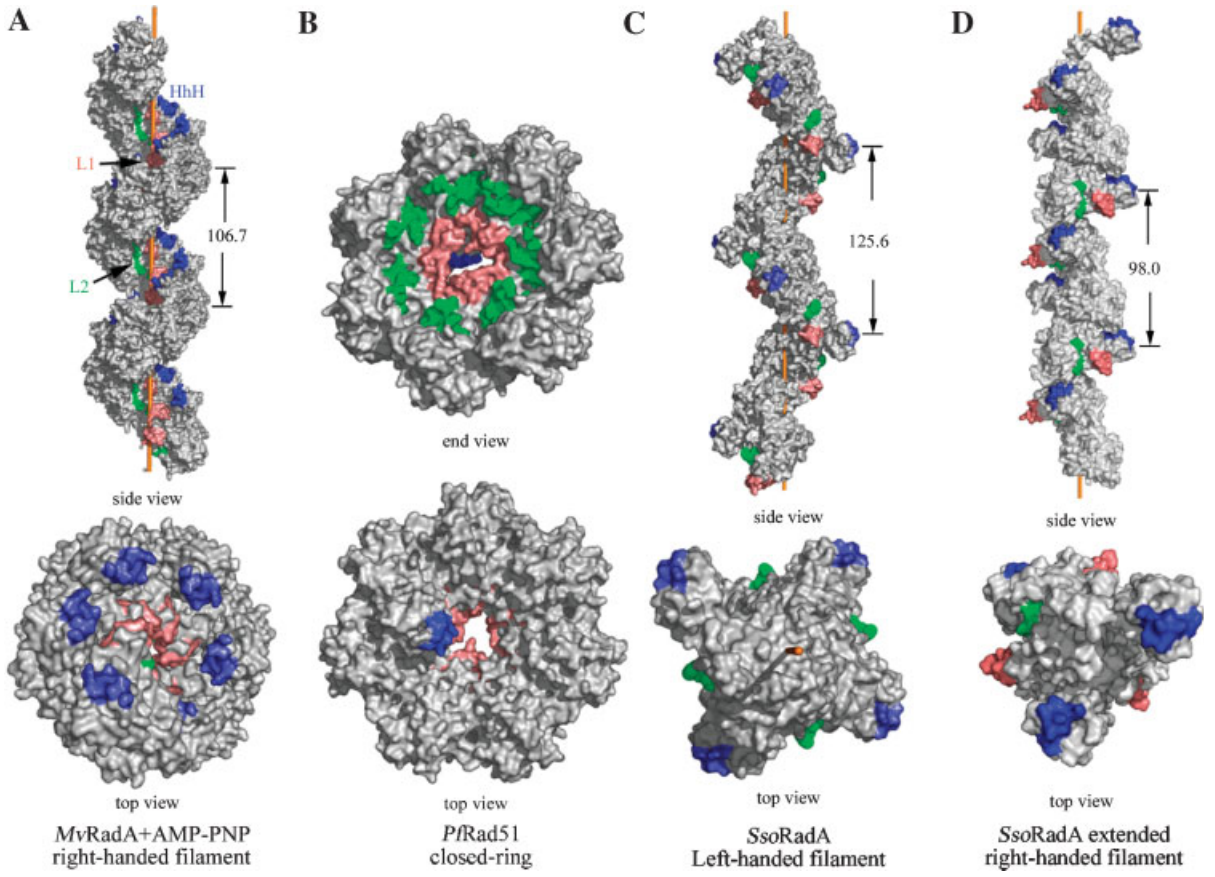

Figure 2. Quaternary structures of **A:** a *Methanococcus voltae (Mv)* RadA-AMP-PNP filament, **B:** a *Pyrococcus furiosus (Pf)* Rad51 ring, and **C,D:** two *S. solfataricus (Sso)* RadA filaments. Side and top views are shown. The helical pitches of the right-handed *Mv*RadA-AMP-PNP filament, the left-handed *Sso*RadA filament, and the overwound right-handed *Sso*RadA filament are 106.7 Å, 125.6 Å, and 98.0 Å, respectively. The putative dsDNA-binding HhH motifs are highlighted in blue. The ssDNA-binding L1 and L2 motifs are highlighted in pink and green, respectively. The helical filament axis is depicted as an orange rod running through the filament. This figure is reproduced from Fig. 3 of Chen LT, Ko TP, Chang YC, Lin KA, Chang CS et al. 2007 Nucleic Acids Res 35:1787–1801 with permission from *Nucleic Acids Research*.

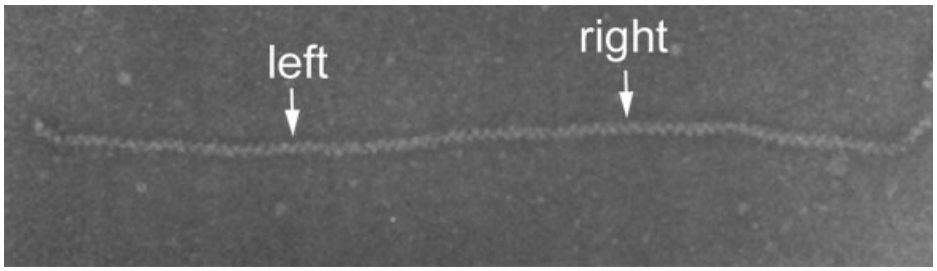

Figure 3. An electron micrograph of a negatively stained RecA filament formed on linear dsDNA in the presence of ATP and aluminum fluoride (from Fig. 1 in Ref. 38). The aluminum fluoride substitution for phosphate after hydrolysis and the complex of RecA–ADP–AlF_4^- are stable over time. Right-handed and left-handed helical pitches are indicated by the white arrows. This figure is reproduced with permission from Dr. Edward H. Egelman (University of Virginia, USA) and *The Biophysical Journal*.

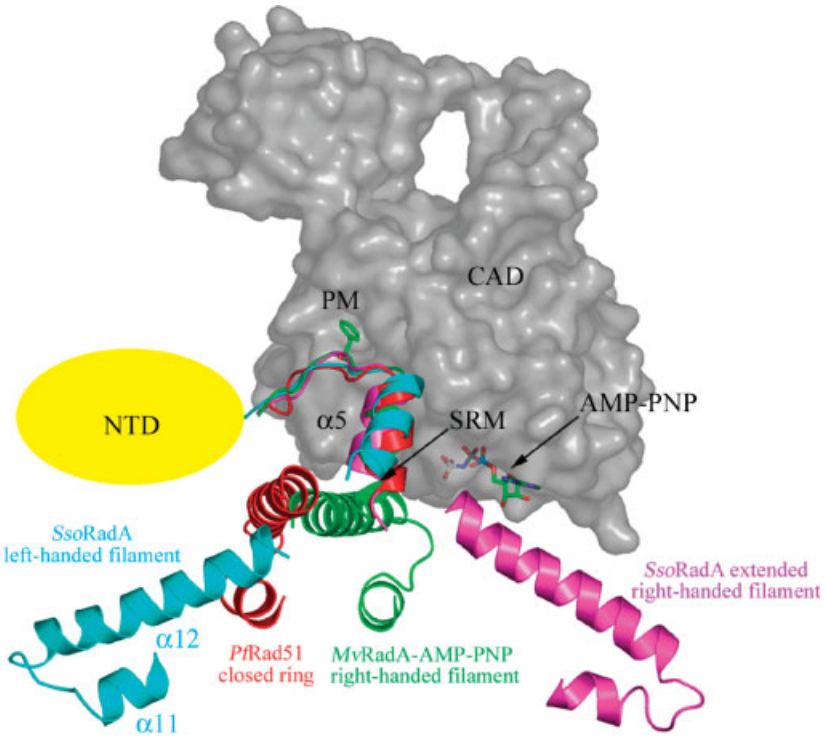

Figure 4. Subunit rotation influences the quaternary structures of archaeal RadA and Rad51 proteins. The structures of the left-handed *Sso*RadA helical filament (cyan), the *Pf*Rad51 closed ring (red), the right-handed *Mv*RadA-AMP-PNP helical filament (green), and the extended or overwound right-handed *Sso*RadA helical filament (pink) are superimposed by fixing the N-terminal domain (NTD, yellow) and polymerization motif (PM). The side chain of the phenylalanine residue of the PM is indicated in green. The core ATPase domain (CAD) of neighboring protomers is indicated in grey. The subunit rotation motif (SRM) is located immediately after the α5 helix. The location of AMP-PNP in the right-handed *Mv*RadA-AMP-PNP helical filament is indicated. The positions of adjacent α11 and α12 helices are shown to illustrate subunit rotation. This figure is reproduced from Chen LT, Ko TP, Chang YC, Lin KA, Chang CS et al. 2007 Nucleic Acids Res 35:1787–1801 with permission from *Nucleic Acids Research*.

with four monomers per helical turn[18] (Fig. 2). First, all four quaternary structures use the same phenylalanine in the polymerization motif. Second, the monomers in these four different polymers exhibit almost identical overall secondary structures, except that their subunit rotation motifs (SRMs) have different Φ or Ψ dihedral angles (see Fig. 4 in Ref. 18). The SRM is located between the polymerization motif and the core ATPase domain and uses the polymerization motif as a fulcrum to produce rotation along the central axis of the protein polymer. Accordingly, a progressive clockwise axial rotation can account for the structural transition from a protein ring to a 6_1 right-handed filament, then to a 3_1 overwound right-handed filament, and, finally, to a 4_3 left-handed filament (Fig. 4; adapted from Fig. 5 in Ref. 18).

Third, we also found that axial rotation accompanies the migration of the L1, L2 and HhH DNA-binding motifs from the interior to the exterior of the overall protein structures, that is, from the inner circle of the toroidal ring to the central axis of a 6_1 right-handed filament, then to the exterior of a 3_1 extended right-handed filament, and, finally, to the outermost surface of a 4_3 left-handed filament (Fig. 2).[18] Simultaneous relocations of these ssDNA- and dsDNA-binding sites play important roles in the catalytic functions of *Sso*RadA proteins. For example, in the 3_1 extended right-handed filament, L1 and NTD together form an outwardly open palm structure at the outer surface of the helical filament. Inside this palm structure, five conserved basic amino acid residues (K27 and K60 of the NTD and R117, R223, and R229 of the L1 motif) surround a 25-Å pocket that is wide enough to simultaneously accommodate an ssDNA and its dsDNA target (Fig. 5, modified from Fig. 4 in Ref. 19). Such an interesting structural arrangement does not exist in other crystal structures of RecA family proteins, such as the RecA or RadA-AMP-PNP 6_1 right-handed filament,[15,35] the Rad51 ring,[12] or the RadA 4_3 left-handed helical filament.[18] Subsequent biochemical analyses revealed that these 5 positively charged residues are essential for RadA function in DNA binding and in promotion of D-loop formation (see Figs. 5–7 in Ref. 18).

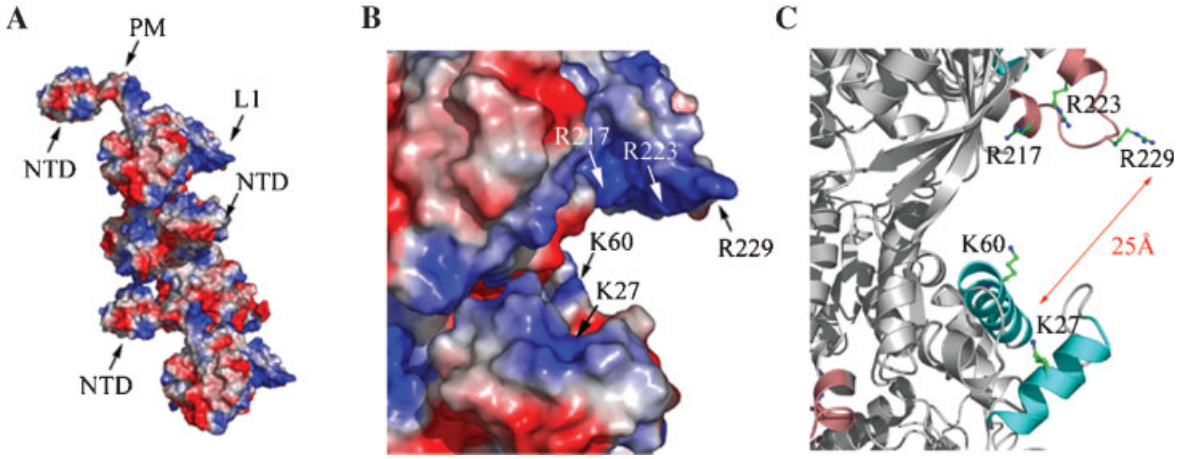

Figure 5. Spatial arrangement of the L1 motif and the N-terminal domain (NTD) along the 3_1 extended right-handed *Sso*RadA filament. **A:** Quaternary structure. The putative dsDNA-binding regions in the NTD are shown in blue. The L1 and L2 ssDNA-binding motifs are shown in pink and green, respectively. ATP binding sites are shown in yellow. The polymerization motif (PM) is indicated by an arrow. **B:** Local surface charge potential of the L1 motif and the NTD region. Positive and negative charges are indicated by blue and red, respectively. **C**: A ribbon diagram of two neighboring protomers (grey) showing the L1 motif (pink) and the NTD (cyan). The side chains of key basic residues K27, K60, R217, R223 and R229 are depicted in ball-and-stick representations. This figure is reproduced from Fig. 4 in Chen LT, Ko TP, Chang YW, Lin KA et al. 2007 PLoS One 2:e858.

Specifically, the 3 arginine residues (R217, R223, R229) in the L1 motif constitute a positively charged linear patch that is responsible for formation of the RadA-ssDNA nucleoprotein filament (see Fig. 2 in Ref. 19). Lys27 and Lys60 are specifically required for dsDNA binding, and they localize at each end of a 92° arched basic patch along one face of the NTD (see Fig. 3 in Ref. 19). This 92° arched basic patch likely binds dsDNA along its border, leading to dsDNA bending or even distortion and flipping of base pairs. These unique structural configurations not only allow the L1 motif to extensively stretch ssDNA but also provide a framework for the formation of new base pairs between a RadA-ssDNA nucleoprotein filament and the target dsDNA (see Fig. 8 in Ref. 19). Therefore, we suggest that the 3_1 extended right-handed filament structure likely represents or is similar to a structural intermediate during the homologous search and pairing process.[19]

It is noteworthy that the DNA-binding pocket formed by these five positively charged residues offers no obvious structural clue to differentiate 5′ to 3′ or 3′ to 5′ polarity during homology pairing and strand-exchange reaction. Since these residues are evolutionarily conserved among archaeal and eukaryotic RecA family proteins, it may explain why eukaryotic Rad51 proteins can promote ATP-dependent strand exchange in either a 5′ to 3′ or 3′ to 5′ direction during the strand-exchange reaction.[23–25] On the contrary, Arg217, Arg223 and Arg229 are not conserved in *E. coli* RecA.[19] Such structural variation not only explains why human Rad51 and *E. coli* RecA exhibit profound mechanistic differences in ssDNA binding,[41] but also reveals an intriguing clue for the 5′ to 3′ polarity of the RecA-mediated strand-exchange reaction.[21,22,25,27]

Fourth, the clockwise or right-to-left axial rotation of RecA family protein helical filaments can couple ATP binding and hydrolysis to homology pairing and strand-exchange reactions. We identified an arginine (Arg83) in the SRM of the *Sso*RadA protein that is evolutionarily conserved in all RecA protein family members. We refer to this arginine as R_0. In the RadA-AMP-PNP 6_1 right-handed filament, the guanidinium group of R_0 forms salt bridges with the carboxyl groups of two glutamate residues: Glu96 (denoted E_1) of the same protomer and Glu157 (denoted E_2) of the neighboring protomer. These two salt bridges are likely to directly control opening and closure of the ATP-binding pocket between two neighboring protomers. In the closed ring, R_0 interacts with E_2 but not with E_1. In the RadA–AMP–PNP $P6_1$–61 right-handed filament, R_0–E_1 and R_0–E_2 interactions (the E_1–R_0–E_2 triad) function as a clip to fasten the AMP-PNP binding between the two protomers (see Fig. 6 in Ref. 18). This model is supported by our finding that point mutations of R_0 to glutamate in *Sso*RadA,[18] *E. coli* RecA and *Sc*Rad51 (Kuei-Ann Li and Chien-Der Lee, unpublished results) all result in significant decreases in ATP binding affinity. As the 6_1 RadA helical filament undergoes clockwise axial rotation in 2 discrete approximately 120° steps to the 3_1 extended right-handed filament and then to the 4_3 left-handed filament, E_1 and E_2 break their ionic interactions with R_0 and gradually move away from R_0. This clockwise axial rotation progressively opens up

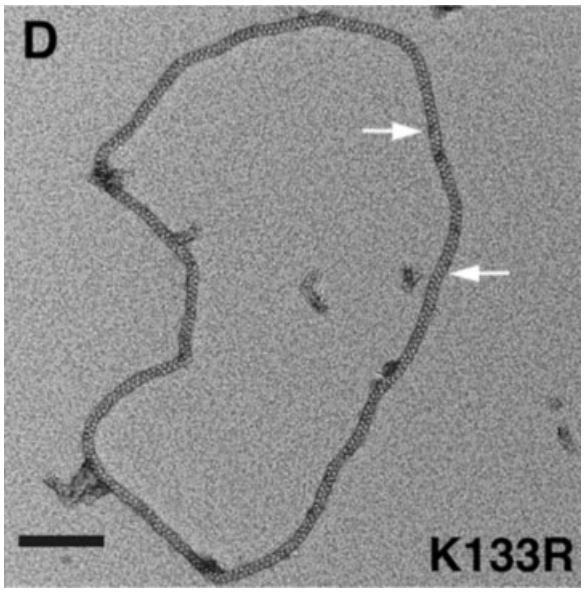

Figure 6. Electron micrograph of a human Rad51 K133R mutant nucleoprotein filament formed on a circular ssDNA in the presence of ATP. The segments with right- or left-handed helical pitches are marked with white arrows, respectively. This figure is reproduced with permission from Patrick Sung, Peter Chi (Yale University, USA), and *DNA Repair* (adapted from Fig. 9D in Chi P, Van Komen S, Sehorn MG, Sigurdsson S, Sung P 2006 DNA Repair 5:381–391).

the ATP-binding pocket. We propose that the 6_1 right-handed, 3_1 extended right-handed, and 4_3 left-handed filaments represent the TP (ATP-bound), DP (ADP + P_i- or ADP-bound), and E (empty) states of the RadA proteins, respectively (see Fig. 8 in Ref. 18). In this scenario, the RadA helical filament is functionally similar to the F1-ATPase. The F1-ATPase is a rotary motor in which a central gamma subunit rotates against a surrounding cylinder made up of $\alpha_3\beta_3$-subunits. Driven by the three beta subunits that sequentially hydrolyze ATP, the motor produces clockwise rotation in three discrete 120° steps. These steps are also denoted TP, DP and E.[42] Since the ATPase domains of F1-ATP and the RecA family proteins are structurally and functionally conserved,[9] we favor the possibility that the neighboring dimers or monomers along a RadA helical filament, like those of the F-ATPase motor, proceed through a "sequential" TP-DP-E structural transition during their catalytic cycles (see below for further discussion).

Fifth, axial rotation of RadA helical filaments provides a mechanism for ssDNA exclusion at the end of the strand-exchange reaction. In the 4_3 left-handed helical filament, the carboxylic acid groups of E_1 and E_2 are exposed on the exterior surface of the RadA protein and are located in the virtual "right-handed" spiraling path connecting all L1 and L2 motifs (see right panel of Fig. 6E in Ref. 18). These carboxyl groups can interfere with ssDNA binding by electrostatically repelling the phosphate groups in DNA. On the other hand, in the 6_1 right-handed RadA-AMP-PNP filament, these two carboxylic acid groups are both electrically neutralized and buried in the protein interior by the guanidinium group of R_0. Moreover, E_1 and E_2 are excluded from the filament axis in which the L1, L2 and HhH motifs are located (see left panel of Fig. 6E in Ref. 18). Therefore, establishing the E_1–R_0–E_2 triad in the 6_1 right-handed RadA-AMP-PNP filament allows better binding to ssDNA or dsDNA. In contrast, a clockwise axial rotation of the RadA helical filament (from right-handed to left-handed helical pitch) may result in ssDNA exclusion.

Finally, we reported that *Sso*RadA mutant proteins carrying a point mutation (R83A or N85P) in the SRM motif exhibit hyperactive ATP binding and hydrolysis in the presence of ssDNA substrate; however, these two mutants were defective in promoting the D-loop formation reaction (see Fig. 8 in Ref. 18). These mutants are analogous to the F1-ATPase mutations that uncouple ATPase activities from transport functions.[42] These results further support the notion that SRM plays important roles in coupling the axial rotation of the RadA filament to RadA functions (i.e. ATP hydrolysis and D-loop formation).

Potential pitfalls and considerations

There are at least two potential problems for the clockwise axial rotation model. The first arises from the stoichiometry of the RadA–DNA interaction. If we assume that continuous transformation requires conservation of stoichiometry (three nucleotides or three base pairs per RadA), the 3_1 extended right-handed filament (three monomers per turn with 98 Å pitch) cannot be obtained by continuous transformation from a right-handed filament with six monomers per turn with a 95- or 107-Å pitch. The axial spacing between consecutive bases would need to increase to at least 10.9 Å in the 3_1 overwound right-handed filament. In fact, the actual spacing would also need to be significantly larger than 10.9 Å, since the DNA-binding sites are located on the exterior of the filament, and the DNA would have to wrap around the filament. Such interbase distances require that the DNA breaks into mono-nucleotide pieces, and this certainly does not happen during DNA pairing and strand exchange. This dilemma may be addressed in one of two ways. First, there is no experimental evidence to support the conservation of stoichiometry (three nucleotides or three base pairs per protein monomer) throughout the catalytic cycles of RadA or RecA family proteins. Therefore, conservation of stoichiometry may not be a prerequisite for continuous transformation. Second, the dilemma rests on the assumption that all RadA monomers in a helical filament produce axial rotation at the same time. As described above, we speculate that RadA proteins undergo sequential structural transformation of dimeric (or monomeric)

conformations from that in a 6_1 right-handed filament (for ssDNA binding) to that in a 3_1 overwound right-handed filament (for homology pairing), and finally to that in a 4_3 left-handed filament (for strand exchange, ssDNA exclusion, or protein dissociation).[18] According to this scenario, DNA–protein stoichiometry can be conserved, and DNA substrates will remain intact. Moreover, because the monomeric structure of RadA protein in the 3_1 overwound right-handed filament is more extended along the axis of the helical filament than are those in the 6_1 or 4_3 filaments, it allows a transient extension or stretch of ssDNA to promote homologous pairing to a dsDNA target (Fig. 8 in Ref. 19).

Our model seems at least partly incompatible with the previous reports that *E. coli* RecA[21,22,26,27] and eukaroytic Rad51[28–30] proteins can catalyze homology pairing and strand-exchange reactions without (or with very little) ATP hydrolysis. This problem can be addressed in the following way. Our structural and functional studies of archaeal RadA proteins reveal that there is tight coupling between the local structures of the ATP-binding pocket and the overall quaternary structures. Therefore, structural changes in the ATP-binding pocket will eventually affect axial rotation (or quaternary structures) of the RecA family protein filaments. Accordingly, point mutations of amino acid residues involved in ATP binding/hydrolysis and inclusions of ATPγS or the ADP–aluminum fluoride complex into the wild-type RecA family proteins may lead to alterations of the overall filament structures and subsequently promote homology pairing and strand-exchange reactions. This new hypothesis is in contrast to a previous conclusion that presynaptic filaments become greatly stabilized (presumably as a 6_1 helical filament) when ATP hydrolysis is prevented, enhancing their stability.

It is noteworthy that our hypothesis is more compatible with two experimental results previously reported by other investigators. First, as described above, Yu and Egelman showed that *E. coli* RecA filaments formed on linear dsDNA in the presence of ATP and aluminum fluoride showed both right- and left-handed helical pitches (Fig. 3).[38] Second, a recent EM imaging analysis indicates that a nucleoprotein filament of human Rad51 K113R mutant proteins and a circular ssDNA also display both left- and right-handed helical pitches (Fig. 6).[30] This Rad51 K113R mutant is defective in ATP hydrolysis but retains the ability to bind ATP or ssDNA. Because there is a huge controversy regarding interpretation of EM results, it is important to perform high-resolution AFM imaging analysis of the nucleoprotein filaments of ATPase-defective mutant variants or wild-type proteins using nonhydrolyzable ATP analogs.

Conclusions

RecA family proteins couple ATP binding and hydrolysis to the DNA strand-exchange reaction in a manner that promotes axial rotation of RecA protein filaments from a right-handed to a left-handed helical pitch. As a result, the L1, L2 and HhH DNA-binding motifs move concurrently to mediate DNA binding, homology pairing and strand exchange, respectively. Therefore, the energy of ATP is used to rotate not only DNA substrates but also RecA family protein filaments. This new model is fully compatible with the facilitated DNA rotation model,[34] except that the latter model, like all current models of RecA family proteins, overlooks the fact that RecA family proteins are flexible enough to form both right-handed and left-handed helical filaments. From this perspective, our new model opens a new avenue for understanding the molecular mechanisms of RecA family proteins. Ultimately, the validity of our model must be tested by determining the structures of RadA–DNA nucleoprotein filaments or by visualizing ATP-fueled axial rotation of RecA family protein filaments during homology pairing and strand-exchange reactions through single-molecule studies.

HR is not only an important topic in biology and medicine; it is also the foundation of modern genetics. In the past two decades, many studies of RecA family proteins were performed using a variety of experimental techniques. However, most of these results were interpreted on the basis of there being right-handed helical filaments with six protomers per helical turn. It would be interesting and probably important to re-examine such published results from a different perspective.

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