

# A new look at the human Rad51 protein

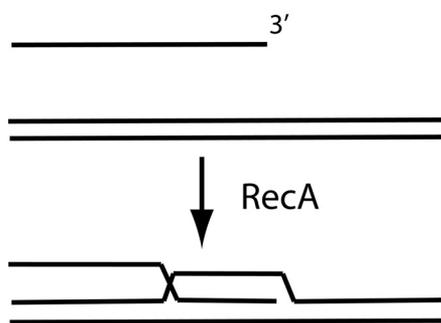
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The nearly ubiquitous RecA family of recombinases includes the bacterial RecA, archaeal RadA, and eukaryotic Rad51 and Dmc1 proteins (1–4). These proteins share sequence and structural homology and a close functional relationship. The proteins form extended filaments on DNA and promote the central DNA strand exchange steps of recombinational DNA repair. Despite extensive study, these proteins have proven to be tough subjects from which to extract mechanistic information. Until recently, molecular studies have been hampered by the limited availability of structural information, particularly with respect to the interaction of these proteins with DNA. The first 3D structures, provided by Steitz and coworkers (5, 6), represented a major step forward. However, they did not include DNA and proved to reflect an inactive conformation of the protein. Many similar structures have appeared in the intervening years, none featuring bound DNA. After a long period of failed efforts in many laboratories, the void has begun to be filled in the past year. First came the appearance of a RecA protein structure that included bound DNA (7). Now comes the publication in this issue of PNAS of a model for human Rad51 protein (hsRad51) bound to DNA (8).

The DNA transactions promoted by these recombinases are important, complex, and often amazing. The filamentous molecular form of RecA family recombinases is unusual and represents a substantial part of the challenge. The helical filaments formed on the DNA can involve thousands of tandem subunits. Most of these proteins form filaments initially on ssDNA. The bound DNA is then aligned with a homologous sequence in a duplex DNA. The initially bound strand is paired with its complement in the duplex, displacing the identical strand in the process. This basic reaction can lead to a variety of events associated with recombination and double-strand break repair, with a facilitated strand invasion by the 3' end of a DNA strand being possibly the most important (Fig. 1).

The new effort by Nordén and coworkers (8) takes advantage of multiple modern tools to forge an informative structural analysis. The model relies on published structures for two key domains of hsRad51, the DNA conformation provided by the RecA–DNA structure,



**Fig. 1.** The DNA strand invasion reaction promoted by RecA family recombinases. The recombinase filament forms on the ssDNA. When a 3' end serves as the invading end, it can be used as a replication primer terminus in subsequent steps of recombinational DNA repair.

the known structure of yeast Rad51 (albeit without DNA), a low-resolution structure of yeast Rad51 protein bound to DNA (reconstructed from electron micrographs and provided by Ed Egelman and colleagues), and linear dichroism data providing the angular orientation of 12 labeled tyrosine residues within hsRad51–ssDNA complex. The result is a highly constrained and compelling model that provides a solid platform on which to base further studies.

Structural and mechanistic questions abound in this system. The new structures address some of the most intriguing questions, providing a much-enhanced view of RecA/Rad51 interactions with both DNA and the ATP cofactor. A particularly rich harvest of information is provided about the DNA interactions. In RecA family recombinases, bound DNA is extended  $\approx 50\%$  relative to native B-form DNA, suggesting a general disruption of base stacking. The molecular basis of this extension was largely a mystery before the RecA–ssDNA structure provided by Pavletich and coworkers (7), and the answer was unanticipated. Instead of a uniform extension, triplets of adjacent nucleotides retain a B-like stacked conformation, with the backbone stretched between the triplets to explain the global extension (Fig. 2). In RecA protein, the triplets are separated to a large degree by the interpositioning of Ile-199. Not surprisingly, this residue is highly conserved in bacterial RecA proteins. The new model for hsRad51 reveals that this role is reprised by Tyr-232. In archaeal RadA and eukaryotic Rad51 proteins, this residue is

highly conserved as Tyr or Phe. In both new studies (7, 8), DNA binding loops in the protein interiors, inferred but largely unresolved in earlier structures, are found adjacent to the DNA, allowing the role of key amino acid residues to be assigned. The new picture of recombinase–DNA interactions, coupled to published kinetic studies (e.g., ref. 9), should allow a much more detailed analysis of the DNA strand exchange reaction pathway.

In both new studies (7, 8), the ATP binding site is found not in the interior of individual subunits (as suggested by earlier structural studies of the relatively inactive form of RecA) but at the subunit–subunit interface. This arrangement in active, DNA-bound recombinase filaments had been predicted by the EM image reconstructions of Egelman and colleagues (10). In the RecA–DNA structure, the positioning of key residues around the nucleotide cofactor is evident, providing a structural explanation for many studies of mutant RecA proteins. A network of amino acid residues, surrounding the ATP binding site and contributed by both adjacent subunits, brings about an observed cooperativity in ATP hydrolysis (11–16). Significantly, the new hsRad51 model is likely to inspire a more detailed investigation of ATP binding site residues and ATPase function.

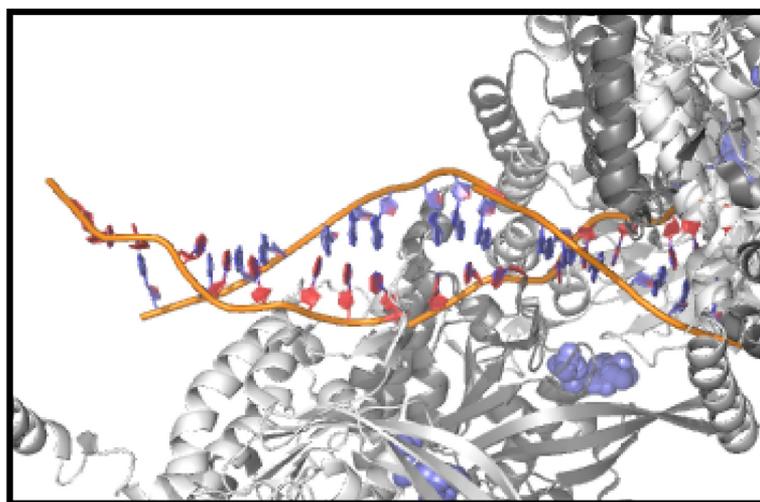
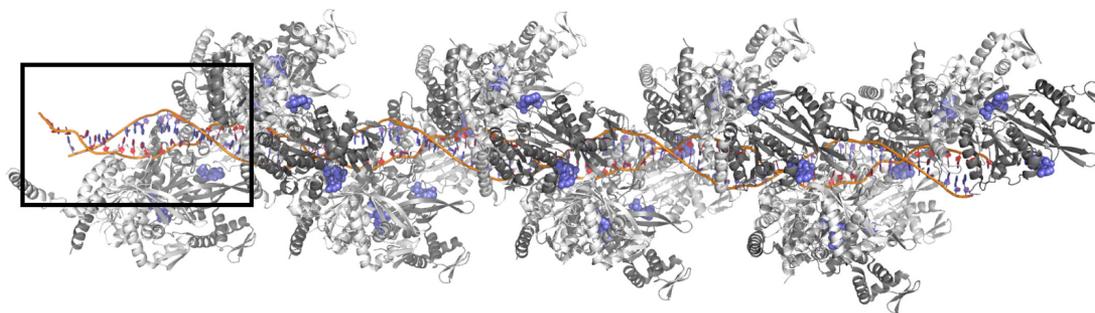
In important ways, these new structural insights serve to highlight the many questions that remain. In RecA, ATP hydrolysis is coordinated between filament subunits and coupled to extensive DNA strand exchange. The coupling mechanism remains to be elucidated. The filament assembly and disassembly pathways should eventually yield to the modern combination of kinetic, single-molecule (17), electron microscopy, and structural methods. For both RecA and Rad51, the recent structural advances reveal only one of several protein conformations that must exist during the ATP hydrolytic cycle. A more realistic structure, caught “in the act” might well have multiple conformations, with different conformations bound to ATP, ADP, or no cofactor in adjacent sub-

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**Fig. 2.** The RecA protein of *E. coli* bound to duplex DNA. (*Upper*) The structure shown is based on the structure reported by Pavletich and coworkers (7) and includes 24 RecA subunits. Alternating subunits are colored in lighter and darker shades of gray. The ATP cofactors are shown in blue. The DNA backbone is shown in orange. (*Lower*) A close-up of a segment of the bound DNA (boxed in *Upper*), showing the repeating structure of spaced triplets.

units, much like the cooperative ATPase cycles elucidated for some helicases. Although it is clear that ATP hydrolysis often results in subunit dissociation at one RecA filament end, the function of ATP hydrolytic events in subunits away from filament ends remains controversial. The bacterial RecA (18) and its archaeal and eukaryotic homologues

also interact with a myriad of regulatory proteins, interactions that are critical to proper cellular function. In humans, the relevant interactions with hRad51 can mean life or death. Very little is known about the details of many of these interactions, and it is likely that additional regulatory proteins remain to be discovered.

The recent advances in RecA and Rad51 structural analysis nevertheless are dramatic and hard-won. They provide a much firmer foundation for continued work on these fascinating proteins.

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