Current Biology, Vol. 14, R629–R631, August 10, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.07.049

Homologous Recombination: Down to the Wire Dispatch

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Exchange of strands between homologous DNA molecules is catalyzed by evolutionarily conserved recombinases. These proteins can occur in different quaternary arrangements: rings or helical filaments. Recent results reveal that recombinase function follows from the filamentous form.

DNA recombination, the exchange of strands between homologous DNA molecules, is an essential biological process. Homologous recombination ensures accurate genome duplication, DNA damage repair and chromosome segregation [1]. The archetypal protein at the catalytic core of this process is the RecA recombinase, which can recognize DNA homology, pair homologous strands and mediate exchange [2]. RecA performs these reactions in the context of a helical protein filament that can hydrolyze ATP and is initially formed on one of the participating DNA molecules (Figure 1). Recent X-ray crystallographic studies [3,4] have found that a number of archaeal and eukaryotic homologs of RecA can exist in the form of rings, with either seven or eight protomers, rather than extended filaments. These findings raise a number of questions. Can homology recognition and strand exchange be performed with either architectural arrangement? Or does the ring form reflect intrinsic longitudinal flexibility of the filamentous form, with its pitch reduced to zero? The structure of the active filaments of several recombinases at nanometer scale, as well as recent biochemical experiments and a long awaited atomic level structure of an active recombinase nucleoprotein filament, argue strongly for the latter view.

RecA of *Escherichia coli* was the first homologous recombination protein identified, and is still the most extensively studied of the recombinases. Knowledge of the structure of this protein in complex with DNA is centrally important for understanding the mechanism of DNA strand exchange. RecA forms striking nucleoprotein filaments on both single-stranded and double-stranded DNA. It is believed that single-stranded donor DNA and double-stranded target DNA are simultaneously bound within this filament, where the poorly understood but necessary steps of identifying sequence homology and promoting strand exchange take place (Figure 1). The salient features of the recombination-competent nucleoprotein filament are the helical arrangement of RecA monomers and the extended and untwisted form of the bound DNA. Indeed, filament structure appears to be the most conserved feature of the recombinase proteins from different organisms; it is shared by the eukaryotic Rad51 and archaeal RadA recombinases, despite their limited overall amino-acid sequence similarity [5–7].

The observed correlation between helical nucleoprotein filament formation of the recombinases and DNA strand exchange activity is not absolute. Notably, the eukaryotic meiosis-specific recombinase Dmc1 [8], though similar to Rad51 and RecA, was not found to form filaments, though it does promote limited DNA strand exchange [9]. Curiously, Dmc1 forms rings, which bind both single-stranded and double-stranded DNA in stacks, not as helical filaments [10,11]. A recent X-ray crystallographic study [3] has provided an atomic level structural model of human Dmc1. The protein crystallizes in the absence of DNA and nucleotide cofactor as an octameric ring, as expected from lower resolution structural models from electron microscopy image reconstruction [11].

Because helical filaments of Dmc1 had not been reported, the ring was assumed to be the functional form. On the basis of this and the atomic-level structure, models of DNA interaction with Dmc1 in a ring configuration were devised to explain the protein's role in DNA recombination [3]. Though largely unsupported, it is not unreasonable to propose that Dmc1 functions mechanistically like a ring-type helicase or DNA pump to produce homologous DNA pairing intermediates.

But a more conventional picture of Dmc1-mediated recombination has also recently emerged. Dmc1 was found to form helical nucleoprotein filaments on single-stranded DNA in the presence of ATP, and to catalyze an active and extensive DNA strand exchange reaction [12]. ATP was found to be required for both helical filament formation and strand exchange activity, and it seems likely that the Dmc1–single-stranded DNA filament is also the catalytically relevant intermediate of this recombinase. However, a functional role for the ring form is not ruled out, and the requirement for hydrolysable ATP remains intriguing.

The recombinases share a highly conserved ATPase motif and are characterized by DNA-stimulated ATPase activity. Both ATP binding and interaction with DNA likely influence the conformation of these proteins in a mechanistically important manner. Atomic level structural models of RecA, RadA, Dmc1 and the ATPase domain of Rad51 show almost identical structure at their nucleotide binding cores [3,4,13,14]. These structures were determined from crystals grown in the absence of DNA and also lacked bound nucleotide cofactors (though the RadA crystals were formed in the presence of ATPγS). Dmc1 and RadA both crystallized as rings. RecA crystallized as a filament but with characteristics different from the filaments formed on DNA (see below). Indeed, all of the recombinases can form rings, most often in the absence of DNA and usually also in the absence of nucleotide cofactors [2]. To understand function we

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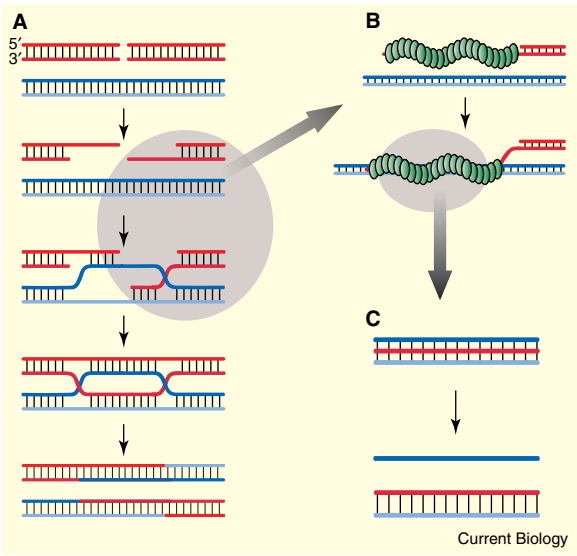

Figure 1. Schematic representation of homologous DNA recombination and recombinase function.

(A) A model for one of the functions of homologous recombination, DNA double-strand break repair, illustrated at the level of DNA. Duplex DNA molecules are indicated by the ladders with the rungs representing base pairs. Processing of double-stranded DNA ends result in 3′ single-stranded tails onto which the recombinase proteins form helical filaments. The grey oval represents the part of the process that is shown in more detail in B. (B) A helical nucleoprotein filament formed on single-stranded DNA can recognize homologous sequence in intact double-stranded DNA resulting in pairing of the two DNA molecules. (C) Within the context of the filament, DNA strand exchange takes place.

should take a careful look at the structural information that allows comparison of protein or filament conformation in the presence and absence of DNA and nucleotide cofactors.

Given that architectural arrangements as filaments and rings are both possible, there must be important differences in subunit interactions that influence the quaternary structure of these recombinase complexes. The subunit interface interactions have been extensively analyzed from the available atomic level structural models of RecA, RadA and Dmc1. This information may be most useful for understanding potential control of conformational changes that require disruption of this interface [3,4]. Indeed, the different arrangement of subunits in ring and filament forms of the recombinases, as well as some helicase proteins with related ATPase cores, indicates that the interface between subunits in rings and filaments have to be very different [2].

With the new information on improved DNA strand exchange activity and filament formation by Dmc1, helical nucleoprotein filaments are absolutely correlated with strand exchange activity. Though the DNA gymnastics of strand exchange are cloaked within this structure, they are likely to be accompanied by rearrangements of the proteins with respect to each other and or the DNA in the filament. Changes in nucleoprotein filament structure should provide clues to mechanism of strand exchange. For instance, ATP binding is required for DNA strand exchange by RecA, Rad51 and Dmc1, while hydrolysis of ATP is apparently required only by Dmc1.

Even in the absence of atomic level structural information on active filaments, there is a wealth of information suggesting a variety of quaternary structures for the RecA and Rad51 nucleoprotein filaments. Helical filaments formed on DNA by RecA, RadA and Rad51 all display striking variability, sometimes in different regions of the same filament, in the extension of DNA, the helical pitch of the filament, and the degree of regularity and relative orientation of protein domains, depending on the cofactor bound [5,15–17]. All of this variability indicates conformational flexibility of the recombinase-DNA filaments. This flexibility might be due to changes at the protein subunit interfaces, unstructured or flexible regions of the proteins that allow changes in the relative orientation of domains, or changes in the continuity of the filament by disassociation/re-association of subunits.

Intriguingly, when the atomic level monomer structures were docked into the nanometer resolution filament structures, the predicted subunit interfaces were very different from those observed in the crystallized ring forms [15]. Specifically, the nucleotide binding site is positioned at an interface between protomers in the models of active filaments on DNA, and it is not at such an interface in the protein-only crystal structures. This suggests an obvious role for nucleotide cofactor binding and hydrolysis in dynamic subunit interactions and subsequent filament flexibility.

Models can be tantalizing but never as satisfying as actual data. Those with a keen interest in recombinases and recombination need no longer be frustrated, as an atomic level structure of an active recombinase is at long last available [18]. Rad51 from *Saccharomyces cerevisiae* was crystallized with all the components necessary for a functional filament, single-stranded DNA and ATPγS as a nucleotide cofactor. So what does it look like? For starters, the interface between Rad51 protomers is not the same as that observed in crystals of the protein alone. Notably, the nucleotide binding site is now located at the interface between protomers, as had been modeled into active RecA filaments. This arrangement can explain some data obtained with mutant RecA, where an amino acid substitution far from the ATPase site in the RecA structure nevertheless influenced this activity. The equivalent amino acid in Rad51 is in contact with the ATPase site of its neighbor in the filament. In addition, there is asymmetry among the protomers in the active filament structure. The amino- and carboxy-terminal domains of adjacent monomers alternate in their relative orientation. This results in an alternation in the interface ATPase domains, placing an amino acid from the adjacent protomer either within or removed from the nucleotide binding pocket of its neighbor. The asymmetry that is obvious in this filament structure agrees nicely with asymmetry previously proposed for RecA filaments based on enzymology experiments [19].

Is the mechanism of DNA strand exchange also revealed in the structure of the Rad51 filament? The absence of electron density for DNA in the structure

means that some secrets remain concealed [18]. But consistent themes that must reflect function do emerge from the synthesis of this and other structural information. Most importantly, all evidence indicates that the nucleoprotein filaments are flexible structures. This flexibility is evident in the variation in pitch — reflected in the number of protomers per turn of the helix and rise per protomer, the ring forms being one extreme with a pitch of zero — of the filaments observed by electron microscopy, even between segments of the same filament [5,15]. The Rad51 crystal has a rather high pitch of 130 Å per turn, though this is within the range seen in electron microscopy for filaments formed in active conditions (with DNA and nucleotide cofactor).

In contrast, the RecA crystal structure, though a helical filament, has a pitch of 83 Å, which is in the range observed by electron microscopy of filaments formed in inactive conditions. Conformational dynamics within the filament could even be explained based on altering the asymmetric state of the ATPase sites between protomers in the Rad51 filament crystal. The recombinase filaments may form springs that can expand and contract along their long axis as the result of conformational changes at protomer interfaces. There is at least some experimental support for this idea from single molecule dynamic studies of RecA–DNA filaments. The stretch modulus of RecA–DNA filaments was found to differ depending on the nucleotide cofactor present, indicating that a springier filament is formed with hydrolysable ATP than with ATPγS [20].

If the protein part of the filament is a springy helix, what about the DNA? The arrangement of DNA in the nucleoprotein filaments is at least partly determined by the number of nucleotides bound per recombinase protomer. Though a figure of three nucleotides per RecA protomer is often quoted in the literature, it seems there is room for interpretation, especially when considering single-stranded DNA. The high pitch of the Rad51 filament crystal is inconsistent with some estimates of the Rad51-to-DNA stoichiometry [18]. Even for RecA it seems there is evidence for different stoichiometries [19]. In this respect, the absence of DNA density in the Rad51 crystal may be useful information. One explanation for the unresolved DNA is that it adopts a variety of conformations in the crystal filaments or is actually mobile. Rice and colleagues [18] suggest that different protomers within the filament may bind DNA at different stoichiometries. Interestingly, single molecule dynamic measurements showed that the increase in stiffness of RecA–DNA filaments on double-stranded DNA versus single-stranded DNA was less than expected from adding a second DNA strand [20]. This was interpreted to mean that only one strand of DNA was bound tightly in the protein helix, and that the other one could slide with respect to the protein structure.

There is now a clear correlation between recombinase filament formation and recombination function, as well as new information about the variable protein interface in an active filament that can be used to focus our attention on the activities within, homology recognition and DNA strand exchange. Tantalizing models abound, suggesting new tests and a hard look at old data.

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