Implications of the RecA structure

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Abstract

The RecA protein has been the most intensively studied protein involved in homologous genetic recombination, but until recently very little has been known about the molecular details of how RecA can bring two DNA molecules into juxtaposition and switch strands between them. A recent RecA-DNA crystal structure provides some striking new insights.

Introduction and context

The *RecA* gene was first identified in *Escherichia coli* more than 40 years ago [1], and hundreds (if not thousands) of papers have shown this gene to be centrally important to both the recombination and repair of DNA [22,3,4]. While genetic recombination helps create genetic diversity and therefore increases the fitness of a population, pathways for genetic recombination appear to have arisen as a mechanism for the repair of DNA, using an identical or homologous stretch of one DNA molecule to repair a second DNA molecule that has been damaged. The RecA protein is produced quite copiously in bacteria after massive DNA damage and actually can be the single most abundant protein in the cell. In addition, pure RecA protein *in vitro* can catalyze a remarkable reaction in which two DNA molecules exchange a strand. These reactions have served as models for the action of Rad51, the eukaryotic homolog of RecA. The active forms of both RecA and Rad51 are helical protein polymers formed on DNA [5], and this has made many high-resolution structural studies difficult or impossible.

Electron microscopic and topological studies had shown that RecA has a profound effect on DNA structure when it binds: the DNA is stretched by ~50% [6], and the DNA is untwisted by almost a factor of two, from ~10 to ~19 base pairs per turn [7]. Since the human Rad51 protein has been shown to induce similar changes in DNA [8], it is reasonable to believe that this unusual DNA structure is important for the mechanism of genetic

recombination. A crystal structure of RecA protein had been solved many years ago [9], but based upon electron microscope observations [10], the filament within the crystal appeared to be in a compressed inactive conformation. Furthermore, the crystal did not contain DNA, so it provided few clues about how RecA induced this unusual DNA conformation. Modeling studies for stretched DNA have been attempted [11], but these are relatively unconstrained by detailed experimental observations. A very challenging study using nuclear magnetic resonance (NMR) was undertaken to look at oligonucleotides complexed with RecA [12], but the interpretation of those results appears to have depended upon the assumption that the structure is actually uniform, with every base in the same conformation.

Major recent advances

A new crystal structure of a complex between RecA and DNA has been solved [13], and one of the most surprising observations is that there is no uniform stretched DNA structure. Three bases adopt a fairly B-like conformation (Figure 1), with stacking of the bases to exclude solvent within these triplets, and then there is a very large stretch and a left-handed twist to reach the next set of triplets. The left-handed twists interspersed between the triplets in right-handed B-like conformations account for the global untwisting that has been measured, whereas the stretching of ~7.8 Å between triplets accounts for the global average of ~5.1 Å rise per base or base pair (from ~3.4 Å per base pair in B-DNA).

Figure 1. The single-stranded DNA from the complex [13] of RecA and DNA (PDB 3CMW).

Three bases are stacked and have a B-like twist but are separated from adjacent triplets by regions of left-handed twist and extreme stretching. This conformation appears to be the basis of a search for homology with a second DNA molecule that will locally have a B-like conformation.

In addition to giving us the first high-resolution picture of this unusual DNA conformation (and one that is likely to be conserved from bacteria to humans), the crystal structure is a technical *tour de force*. The problem with many helical polymers is that, unless there are exactly two, three, four, or six units per turn, the polymer cannot be crystallized so that every subunit is in an identical environment. Polymerization can also be competing with crystallization, so that under the conditions of high protein concentrations needed for crystallization many polymeric proteins will polymerize. Pavletich and colleagues [13] solved this problem for RecA by engineering a RecA polyprotein containing four, five, or six copies of RecA linked together, with the ends modified to prevent polymerization. They could thus crystallize the polyprotein as an asymmetric object.

The new crystal structure [13] also shows that RecA subunits have the ability to rotate in the filament from the position seen in the first crystal filament [9], consistent with predictions made using both electron microscopy [14] and spectroscopy [15]. The subunits of the active filament are bound to ATP, whose hydrolysis releases the DNA from the filaments [4]. This rotation brings ATP molecules bound to one subunit into a cleft between adjacent subunits where they can be hydrolyzed.

Future directions

Although the new crystal structure provides a major advance in understanding how RecA-like proteins function, many questions still remain. However, these questions now can be examined in a framework that is very different from the one that has existed previously. It has been known that a second DNA molecule can be untwisted by the complex of RecA bound to a first DNA molecule [16], but the new crystal structures do not provide direct information about how DNA is untwisted at this second binding site. Models can be built, and the new framework provides many constraints that will aid in designing experimental tests. All of this will advance our understanding of how the fundamental biological process of homologous genetic recombination takes place, and will undoubtedly have an impact on human health as well. Diseases like cancer can be caused by defects in the repair of damaged DNA, so a molecular understanding of how proteins such as human Rad51 act in the repair of DNA will be crucial for many advances.

Abbreviations

NMR, nuclear magnetic resonance.

Competing interests

The author declares that he has no competing interests.

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