

A CONSERVED MECHANISM FOR IDENTIFYING BREAKS IN DNA

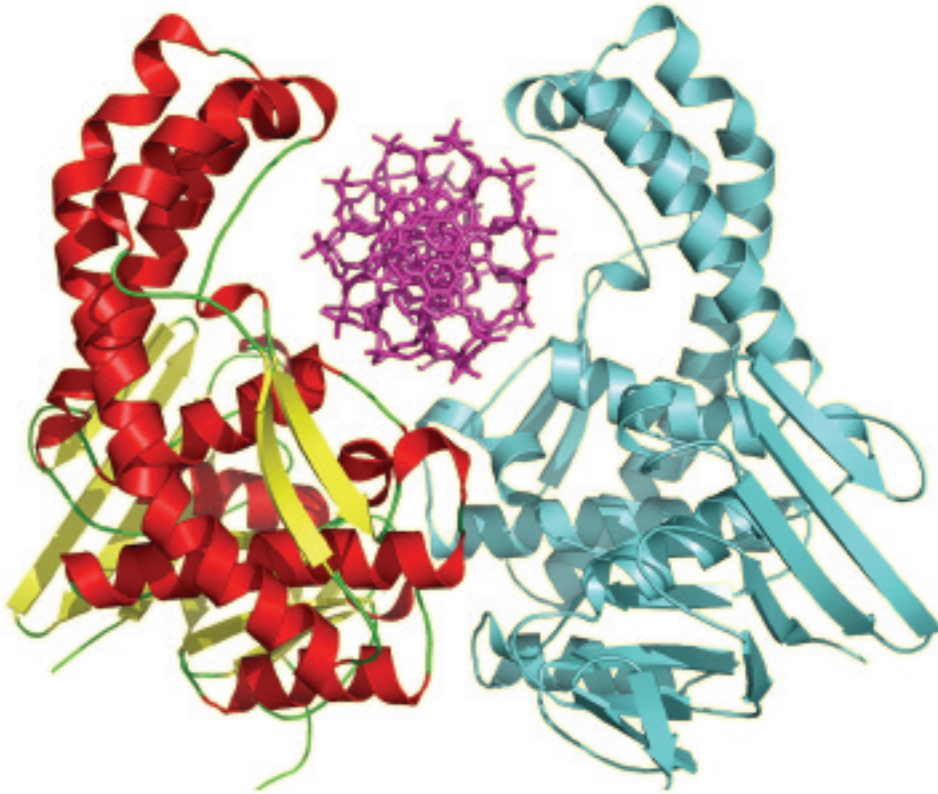


Fig. 1. Representation of the structure of the RecF dimer bound to DNA. Two RecF monomers are shown in the dimer predicted from the structural data and from modeling based on all known ABC ATPases known, including Rad50. One RecF monomer is shown in cyan only and one is shown with structural elements represented; α -helices are shown in red, β -strands in yellow, and loops are shown in green. DNA is shown in pink as it is predicted to lie in the "pocket" created by the RecF dimer (looking down the helix).

One of the most fundamental steps in the progression of any species is the replication of DNA that creates a copy of the genome for the new daughter cell. If DNA replication fails, the whole process can go awry. For this reason, the mechanisms that repair mistakes and breaks in DNA that are integral to the replication process have been evolutionarily conserved from bacteria to higher eukaryotic organisms. This conservation is reflected in genes that are conserved among species and can sometimes be seen in conserved protein structural elements. An elegant example of a conserved structure involved in DNA repair was solved by researchers using data collected at the SBC-CAT 19-ID-D beamline at the APS, who elucidated the structure of a bacterial DNA repair enzyme, RecF, at a resolution of 1.62 Å. The work revealed important structural conservation between the bacterial repair enzyme, RecF, and one from eukaryotic organisms called Rad50. Structural conservation of this type clearly indicates evolutionary preservation of RecF function. In addition, their study makes important findings regarding ATP-dependent dimerization of RecF and its mechanism of binding to DNA at the site of repair.

RecF belongs to a family of proteins that serve as recombination mediators (RMs), which are important for repair of single-stranded DNA (ssDNA) gaps. These proteins recognize the gap and recruit other repair enzymes to the site to fix the problem. Analysis of the amino acid sequence of RecF has revealed the presence of conserved motifs that identify a possible region responsible for DNA-dependent ATP binding. The first step in this study was to test the function of these regions. By using techniques of molecular biology to mutate these conserved amino acids, investigators from the St. Louis University School of Medicine and Portland State University were able to show that ATP binding is essential to RecF dimerization and DNA binding, providing important clues about the mechanism of action of this protein.

The next step was to solve the protein's three-dimensional structure, which revealed that the bacterial RecF protein contains two domains, both of which look like domains in the eukaryotic Rad50 protein. The first section of RecF is structurally similar to the ATPase domain of Rad50, and the second section is similar to the subdomain that is important for DNA recognition. This suggests that even though Rad50 binds double-stranded DNA (dsDNA) ends and RecF binds

ssDNA breaks, they probably recognize and bind DNA in the same way.

Once they had solved the structure of RecF, the researchers were able to put their mutational analysis together with information from the structure to form a hypothesis about how DNA repair gets started. Here's how they think it might work. First, a DNA break occurs and is recognized by RecF. RecF bound to ATP can form a dimer with another ATP-bound RecF molecule, and the dimer binds to the DNA break (Fig. 1). This creates pockets for the two sides of the damaged DNA replication fork. Interestingly, the structure revealed that the inside of the RecF dimer pocket is positively charged, favoring binding of the negatively charged DNA molecule. The model predicts that one side of the dimer binds dsDNA, and the other side binds ssDNA. In Rad50, which recognizes blunt-ended dsDNA breaks, one side binds dsDNA, and the other side is free. Once the break is recognized, the RecF dimer recruits another protein, RecR, which binds RecF or DNA only when they are presented together. This suggests that RecF binding to the DNA break may change its conformation to create a binding site that can be recognized by RecR. In fact, it is predicted that four copies of RecR can bind to the RecF-DNA break complex to make what is called a tetrameric clamp. The resulting complex is a sort of molecular vice grip that holds the DNA break in place for enzymes to make the fix. — *Sandy Field*

See: Olga Koroleva¹, Nodar Makharashvili¹, Charmain T. Courcelle², Justin Courcelle², and Sergey Korolev^{1*}, "Structural conservation of RecF and Rad50: implications for DNA recognition and RecF function," *EMBO J.* **26**, 867 (2007). DOI: 10.1038/sj.emboj.7601537

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These works were supported by the E.A. Doisy Trust Fund and SLU Medical School Start Up Fund and by NIH grant GM073837. J.C. and C.T.C. are supported by National Science Foundation Career Award MCB0551798. Use of the Argonne National Laboratory Structural Biology Center beamlines at the APS was supported by the US Department of Energy, Office of Biological and Environmental Research, under Contract No. W-31-109-ENG-38. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

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