DNA RECOMBINATION

Two ways to unwind

The *Escherichia coli* RecBCD enzyme uses its helicase and nuclease activities to process double-stranded (ds)DNA breaks for repair by homologous recombination. From electron-microscopic studies it has been known for years that RecBCD unwinds dsDNA in an unusual manner — the 5′-ended strand forms a long ssDNA tail, and the 3′-ended strand forms a single-stranded (ss)DNA loop and a short ssDNA tail. This led researchers to believe that another helicase might be at work, besides the RecB subunit, which has 3′→5′ helicase activity. Now, reporting in complementary papers in *Nature*, Taylor and Smith, and Dillingham, Spies and Kowalczykowski show that RecD is also a helicase, but of opposite polarity to that of RecB. These findings have led to a revised model of how RecBCD unwinds DNA.

Using 3′- or 5′-end-labelled DNA substrates to identify the termini of unwound DNA (anti-biotin antibody-conjugated gold beads bound to the DNA ends are visible as electron-dense particles in electron micrographs), Taylor and Smith determined the strand polarity of the unwinding structures produced by a RecB-helicase-defective mutant, $RecB^{K29Q}CD$. The electron micrographs showed a ssDNA loop and only one, 5′-ended, ssDNA tail with equivalent lengths. So, the authors concluded that the inactive RecB subunit remained at the 3′ end where it initially bound, producing a loop but no second tail. The rate of unwinding was nearly identical to that of wild-type RecBCD, indicating that RecD helicase, which binds to the 5′ end, is a faster helicase than RecB and of opposite polarity.

To test the contribution of the RecB helicase, Taylor and Smith used RecBC enzyme (lacking RecD) and found that it produced Y-shaped molecules with two equal-length ssDNA tails but without loop formation, as expected for a single-helicase enzyme. In addition, a RecD-defective mutant, $RecBCD^{K177Q}$, produced 'loop two-tail' unwinding structures, but with a 3′-ended long ssDNA tail and a very short 5' tail, implying a strand polarity opposite to that of the wild-type enzyme. The inactive RecD subunit remained bound at or near the 5′-ended strand where it formed a loop and a nearly equal-length ssDNA tail on the opposite strand. Either removal of the RecD subunit or inactivation of its helicase activity, by using the $RecBCD^{K177Q}$ mutant, resulted in an unwinding rate ~20% of that of the wild-type enzyme — confirming that RecB is the slower helicase that functions on the 3′-ended strand.

In a companion paper, Kowalczykowski and colleagues purified a histidine (his)-tagged RecD (^{his}RecD) protein and verified that it has ssDNA-dependent ATPase activity. They showed that it also has a DNA helicase activity that has the requisite ATP dependence. However, the RecD protein translocated in a 5′→3′ direction to unwind DNA, implying that it could travel on the complementary DNA strand opposite RecB but still travel in the same direction as RecB.

Next, Kowalczykowski and co-workers verified that the purified ^{his}RecD protein could be reconstituted with RecBC enzyme to produce a functional wild-type RecBCD enzyme, which showed rapid processive unwinding and correct recognition of the DNA sequence, Chi, a recombination hotspot. They also showed that both of the mutant single-motor RecBCD enzymes, $RecB^{K29Q}CD$ or $RecBCD^{K177Q}$, retained almost wild-type levels of helicase activity, indicating that the RecB and RecD helicases have independent, complementary activities.

These studies led both groups to propose a 'dual-helicase' model. Although seemingly complex, RecBCD's bipolar helicase activities provide a simple mechanism by which the two helicases can bind to opposite, anti-parallel strands at the dsDNA break, but translocate in the same direction relative to the duplex. The two motors might also explain the exceptionally high processivity of RecBCD, as well as the unusual 'loop two-tail' unwinding intermediates seen in electron micrographs — if two linked motors travel on opposite strands at unequal speeds, the faster helicase activity produces a long ssDNA tail, and a ssDNA loop accumulates on the strand with the slower helicase. It will be interesting to see whether bipolar, non-identical helicase motors are used by other DNA-processing enzymes.

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References and links

ORIGINAL RESEARCH PAPERS Taylor, A. F. & Smith, G. R. RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature* **423**, 889–893 (2003) | Dillingham, M. S. *et al*. RecBCD enzyme is a bipolar DNA helicase. *Nature* **423**, 893–897 (2003)

WEB WATCH

Desperately seeking…

- The EF-Hand Calcium-Binding Proteins Data Library
http://structbio.vanderbilt.edu/cabp_database/index.html
- Calmodulin Target Database
http://calcium.uhnres.utoronto.ca/ctdb/ctdb/home.html

"Small, ubiquitous, divalent ion with high versatility seeks partner for transient or long-term relationship". Visiting the web sites above might help you find Ca^{2+}'s ideal partner.

On the Vanderbilt data library homepage is an EF-hand-protein structure; a more comprehensive list of EF-hand proteins, along with their functions, is just a click away ('Summary of proteins included'). The 'Structural information' option provides information on aspects such as interhelical angles and distance difference matrices. There's also sequence information (alignments, two-dimensional sequence maps) and — rather hidden — what might be called 'a beginner's guide to EF hands' ('Home pages for residues'). Well worth a click-through is the 'Other web resources' option, which details reseachers and societies.

The Calmodulin Target Database, pioneered by the Ikura laboratory at the Ontario Cancer Institute, offers an introduction to calmodulin (CaM). On binding Ca^{2+}, this EF-hand-containing Ca^{2+} receptor changes shape, enabling it to bind to various targets. The 'Guided tour' facility leads you through the available options, providing information on some of the known CaM-binding proteins and motifs, and lets you search for putative CaM-binding sites in your protein. If it does bind, you can do a binding-site analysis … and find out more, through the 'Resources' option, in the up-to-date list of CaM-target reviews.

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