# Dual engines moving on antiparallel tracks

Steven W Matson

**Two recent papers provide evidence that the *Escherichia coli* RecBCD complex, which unwinds double-stranded DNA to supply a substrate for recombination, has two helicase subunits that bind opposite DNA strands and move with opposite unwinding 'polarities'.**



The unwinding or 'unzipping' of duplex DNA to produce single-stranded DNA (ssDNA) is essential for all processes that involve a ssDNA template or reaction intermediate, including DNA replication, repair, recombination and transcription[1]. Enzymes called DNA helicases catalyze this unwinding of duplex DNA in an energy-requiring reaction, using the energy provided by the NTPase activity intrinsic to all helicases. As we currently understand this conceptually simple but mechanistically complex reaction, a helicase enzyme binds and translocates along one DNA strand, cycling through multiple conformational states as NTP is bound, hydrolyzed and the hydrolysis products released, while simultaneously unwinding the DNA duplex[2]. These enzymes are therefore viewed as molecular 'motors' required for DNA strand separation.

Given the antiparallel orientation of the two strands that make up duplex DNA, it is reasonable to expect that some helicases unwind DNA in a 5′→3′ direction, while others unwind DNA in a 3′→5′ direction, depending on which strand the protein utilizes as a lattice for translocation. This is found to be the case, as most helicases are unidirectional and prefer to initiate unwinding on ssDNA with a specific polarity (and are classified as 3′→5′ or 5′→3′ enzymes, accordingly). This has important implications for understanding the molecular roles played by helicases in the cell. But one enzyme, the *E. coli* RecBCD helicase/nuclease, which processes duplex DNA to produce the ssDNA required for homologous recombination, unwinds DNA from a blunt (or nearly blunt) end and so has been difficult to assign a specific unwinding polarity. Two recent papers published in *Nature*[3,4] now reconcile the idea of unidirectional translocation/unwinding and initiation at a blunt end, while adding a new mechanism to the growing list of models describing the helicase-catalyzed unwinding reaction (**Fig. 1**). These results also provide an unambiguous and logical explanation for the unusual unwinding reaction intermediates previously reported for RecBCD[5], as well as a plausible explanation for the high processivity observed for RecBCD-catalyzed unwinding reactions.

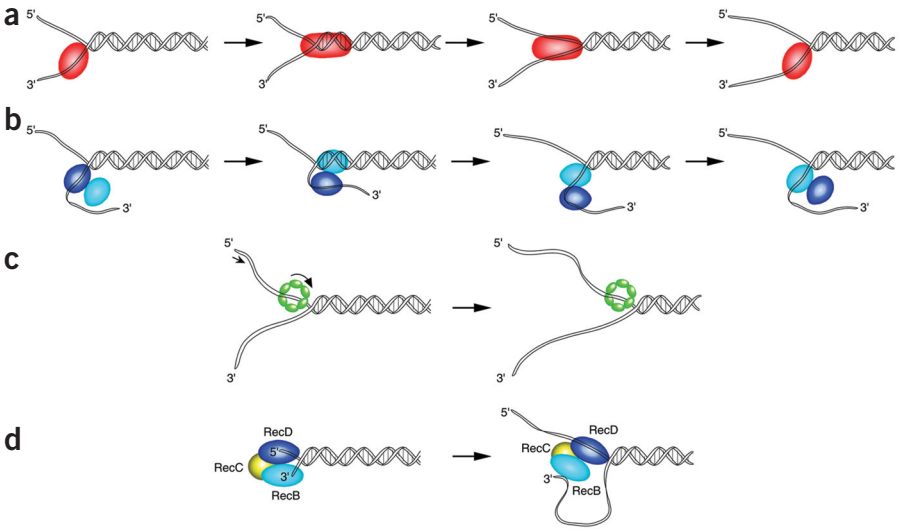


**Figure 1** Models depicting four unwinding reaction mechanisms for DNA helicases. (**a**) The inchworm mechanism for a monomeric helicase. The enzyme cycles through energetic and conformational states as it hydrolyzes NTP to couple unidirectional DNA translocation with duplex destabilization. (**b**) The functional dimer model utilizes a dimeric helicase composed of two identical protomers. The protomers alternate in binding to ssDNA and duplex DNA as the protein translocates along and unwinds duplex DNA. (**c**) A hexameric helicase, with ssDNA moving through the central channel of the hexamer, utilizes NTP hydrolysis to fuel unidirectional translocation along one strand while displacing the other strand to the outside of the enzyme. (**d**) The RecBCD complex utilizes two active helicases, one moving along one strand in the 5′→3′ direction and the other moving along the other strand in a 3′→5′ direction. The two helicase motors have opposite unwinding polarities but move in the same overall direction as they translocate along the antiparallel strands of duplex DNA. The RecD helicase unwinds DNA at a faster rate than the RecB helicase resulting in the formation of the 'loop-tails' intermediate shown.

Using different and complementary techniques, the new results[3,4] demonstrate that RecBCD, composed of one subunit each of RecB, RecC and RecD, contains *two* active helicase motors with opposite unwinding polarities and different unwinding rates. The RecB helicase unwinds DNA in a 3′→5′ direction and is relatively slow, while the speedier RecD helicase unwinds DNA in the 5′→3′ direction. Thus, the RecBCD helicase/nuclease binds to the blunt end of a duplex DNA molecule with the RecB helicase bound to the 3′ terminus and the RecD helicase bound to the 5′ terminus. Then, using two motors with opposite unwinding polarities moving along antiparallel tracks—but in the same physical direction—the complex unwinds duplex DNA (**Fig. 1d**).

The evidence for both RecB and RecD having intrinsic helicase activity is compelling. Earlier studies had shown RecB to have helicase activity[6], and the newly published biochemical experiments[4] directly demonstrate the helicase activity and the unwinding polarity of the RecD protein. Moreover, using point mutants containing a mutation at a key amino acid residue that abrogates helicase activity in one motor or the other, the authors have shown that both motors function in the wild-type RecBCD complex.

*The author is in the Department of Biology and the Curriculum in Genetics & Molecular Biology, University of North Carolina, Chapel Hill, North Carolina 27599, USA.*
*e-mail: smatson@bio.unc.edu*

Electron microscopic studies[3], again using specific point mutants that inactivate one motor or the other, explain the 'loop-tails' unwinding intermediates observed previously in RecBCD-catalyzed unwinding reactions[5]. When the slow helicase (RecB) is inactivated, a long ssDNA tail and a ssDNA loop of equal length are observed. This can be explained by RecD-catalyzed unwinding along one strand while the inactive RecB remains bound to the end of the other strand. As expected, based on the polarity of RecD-catalyzed unwinding, the long ssDNA tail terminates at the 5′ end. When the fast helicase (RecD) is inactivated, unwinding proceeds at a much slower pace and the intermediates observed contain a long 3′ ssDNA tail, a loop and a very short 5′ ssDNA tail. These results are consistent with an active 3′→5′ helicase and an inactive 5′→3′ helicase. When the RecD helicase is absent, the overall unwinding reaction is markedly slowed and the unwinding intermediate contains two ssDNA tails of equal length. Together, these results make a convincing case for the model presented by the two groups of authors—two motors moving along antiparallel strands with opposite unwinding polarities and at differing speeds. The loop-tail unwinding intermediates can be attributed to the fact that (i) the two helicases are tethered in a complex; (ii) each motor is bound to one of the two DNA strands; and (iii) both helicases are active with the fast helicase far ahead of the slow helicase.

This discovery merits our attention for several reasons. First, it extends our understanding of how helicases unwind duplex DNA, a subject of considerable interest. There are three current models (**Fig. 1a–c**) for DNA unwinding by different helicase proteins, each of which enjoys significant experimental support. The inchworm model, originally proposed more than two decades ago[7], envisions a monomeric enzyme with two DNA-binding sites that 'inches' along one strand of DNA while displacing the other strand (**Fig. 1a**). X-ray crystallographic evidence obtained using PcrA, an essential monomeric helicase from *Bacillus stearothermophilus*, provides experimental support for this model[8]. The functional dimer model posits the existence of an active dimeric helicase with alternating subunits engaged in unwinding the duplex or tethering the enzyme to product ssDNA as the enzyme cycles through energetic states (**Fig. 1b**)[9]. The third mechanism, utilized by several helicases involved in DNA replication, involves a hexameric, doughnut-shaped helicase that encircles and translocates along one DNA strand while the other strand is displaced to the outside of the enzyme (**Fig. 1c**)[10]. We can now add a model utilizing two active helicases in a single complex that move along antiparallel tracks (**Fig. 1d**). This concept was, in fact, first advanced over 20 years ago by Hoffmann-Berling[11] to explain unwinding ahead of the advancing replication fork using the Rep and UvrD helicases. Although we now know that the hexameric DnaB catalyzes unwinding ahead of the replication fork, and that Rep and UvrD unwind DNA in the same direction, the basic notion of using two helicase motors with opposite polarities to unwind DNA clearly explains the behavior of RecBCD.

Secondly, a complex with two active helicases provides a credible explanation for the processivity of RecBCD helicase. Previous experiments have shown that RecBCD can processively unwind duplex regions in excess of 40 kilobase pairs[12]. To remain stably bound on the DNA while cycling through the various conformational states associated with DNA unwinding, the enzyme must have at least two DNA binding sites. If contact between the helicase and one binding site is lost, contact with the other binding site will keep the enzyme bound to the substrate and prevent dissociation of the helicase from the DNA. Hexameric helicases accomplish this task by encircling the DNA strand upon which they translocate; dimeric helicases have at least two binding sites and monomeric helicases, which are not usually highly processive, are postulated to contain two DNA binding sites. With two active and tethered motors, if one helicase dissociates the other will remain bound and the processivity will be high as observed with RecBCD.

Finally, as indicated above, tethered helicase motors moving at different rates explain the loop-tail intermediates observed in RecBCD-catalyzed unwinding[5]. These intermediates are critical to the role played by RecBCD in recombination, as the coordinated helicase and nuclease activities of RecBCD provide the 3′ ssDNA required to initiate strand invasion. As RecBCD unwinds duplex DNA, the 3′-terminated DNA strand is preferentially degraded by the intrinsic nuclease until a specific DNA sequence called a Chi site is encountered. The asymmetric Chi sequence is recognized by the enzyme approaching from its 3′ side and this encounter attenuates the 3′→5′ nuclease while stimulating the 5′→3′ nuclease. This results in the formation of a 3′-terminated ssDNA, and RecBCD directs the loading of RecA onto this ssDNA[13]. The slow 3′→ 5′ movement along one strand may enhance the ability of the enzyme to recognize a Chi site and alter the polarity of the nuclease. The RecA–DNA filament produced is the mediator of the search for homology that is crucial in homologous recombination.

Will this mechanism be observed for other DNA unwinding complexes? It seems likely this will be the case. The eukaryotic TFIIH transcription/repair complex contains two helicases of opposite polarity[14], although whether each helicase is actively engaged and moves along antiparallel DNA strands simultaneously is not yet known. It is also likely that new dual motor helicase complexes will be discovered as we continue to understand how other proteins modulate the activity of a helicase. Thus far, the possibility of one helicase modulating the activity of another helicase has not been carefully considered. This will likely change and we may discover further examples of dual, tethered helicase motors moving along antiparallel tracks to catalyze the unwinding of duplex DNA.


1. Matson, S.W., George, J.W. & Bean, D.W. *BioEssays* **16**, 13–22 (1994).
2. Lohman, T.M. & Bjornson, K.P. (1996) *Annu. Rev. Biochem.* **65**,169–214 (1996).
3. Taylor, A.F. & Smith, G.R. *Nature* **423**, 889–893 (2003).
4. Dillingham, M.S., Spies, M. & Kowalczykowski, S.C. *Nature* **423**, 893–897 (2003).
5. Taylor, A.F. & Amith. G.F. *Cell* **22**, 447–457 (1980).
6. Phillips, R.J., Hickleton, C.D., Boehmer, P.E. & Emmerson, P.T. *Mol. Gen. Genet.* **254**, 319–329 (1997).
7. Yarronton, G.T. & Gefter, M.L. *Proc. Natl. Acad. Sci. USA* **76**, 1658–1662 (1979).
8. Velankar, S.S., Soultanos, P., Dillingham, M.S. Subramanya, H.S. & Wigley, D.B. *Cell* **97**:75–84 (1999).
9. Wong, I. & Lohman, T.M. *Science* **256**, 350–355 (1992).
10. Patel, S.S. & Picha, K.M. *Annu. Rev. Biochem.* **69**, 651–697 (2000).
11. Geider, K. & Hoffmann-Berling, H. *Annu. Rev. Biochem.* **50**, 233–260 (1981).
12. Bianco, P.R. *et al. Nature* **409**, 374–378 (2001).
13. Anderson, D.G. & Kowalczykowski, S.C. *Cell* **90**:77–86 (1997).
14. van Brabant, A.J., Stan, R. & Ellis, N.A. *Annu. Rev. Genomics Hum. Genet.* **1**, 409–459 (2000).