DNA helicases: **One small step for PcrA, one giant leap for RecBC?**

Dale B. Wigley

One might imagine that the mechanism of helicases would relate to the number of base pairs that are unwound for each ATP that is hydrolysed. Recent studies, however, suggest the situation can be more complicated than this.

Address: Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.
E-mail: wigley@eric.path.ox.ac.uk

Current Biology 2000, 10:R444–R446

0960-9822/00/$ – see front matter

Helicases utilise the free energy of hydrolysis of nucleotide triphosphates (NTPs) to unwind nucleic acid duplexes. They are ubiquitous and are required for almost every process that involves nucleic acid. Until recently, the mechanism by which these enzymes could harness NTP hydrolysis to unwinding was a mystery. But a recent study [1] has begun to illuminate this dark world for one enzyme system, the bacterial helicase RecBCD.

RecBCD is a multisubunit enzyme involved in DNA repair [2]. The enzyme is a highly specific and processive DNA helicase which is able to unwind as many as 30,000 base pairs from an initial DNA binding event at a blunt double-strand end, created for example by a double-strand DNA break. The complex also contains a 3′–5′ nuclease activity that trims back the DNA on one strand. But upon reaching specific sites — referred to as recombination hotspots or 'χ' sites — the 3′–5′ nuclease activity is attenuated and a weak 5′–3′ nuclease activity is activated. The combined effect of these changes is the production of a 3′ single-stranded DNA tail that is recognised by RecA protein, thus instigating recombinational repair.

Separation of the two strands of a DNA duplex into its component strands is an energy requiring process, providing one reason for the NTPase activity of all helicases. One might, therefore, expect the number of base pairs that can be disrupted by each hydrolysis event to relate to the ratio of the free energy changes associated with each process. It turns out, however, that there are several other considerations. In order to separate the duplex strands, helicases translocate along one of the single-strand products with a defined polarity, either 3′–5′ or 5′–3′. This translocation has been shown to be unidirectional for several helicases [3,4], an energetically unfavourable process because of the entropic cost of this ordered, non-random behaviour. Furthermore, helicases are also able to utilise some of the free energy of hydrolysis of NTP to displace proteins that are bound to DNA ahead of them, as demonstrated by the displacement of streptavidin from biotin-labelled DNA substrates [4]. The stability of this latter interaction suggests that helicases must have a significant 'excess' of energy available to be able to prise streptavidin from its ligand. Untangling the relative contributions that each of the above processes make to the utilisation of the energy available from NTP hydrolysis remains an important question in the field.

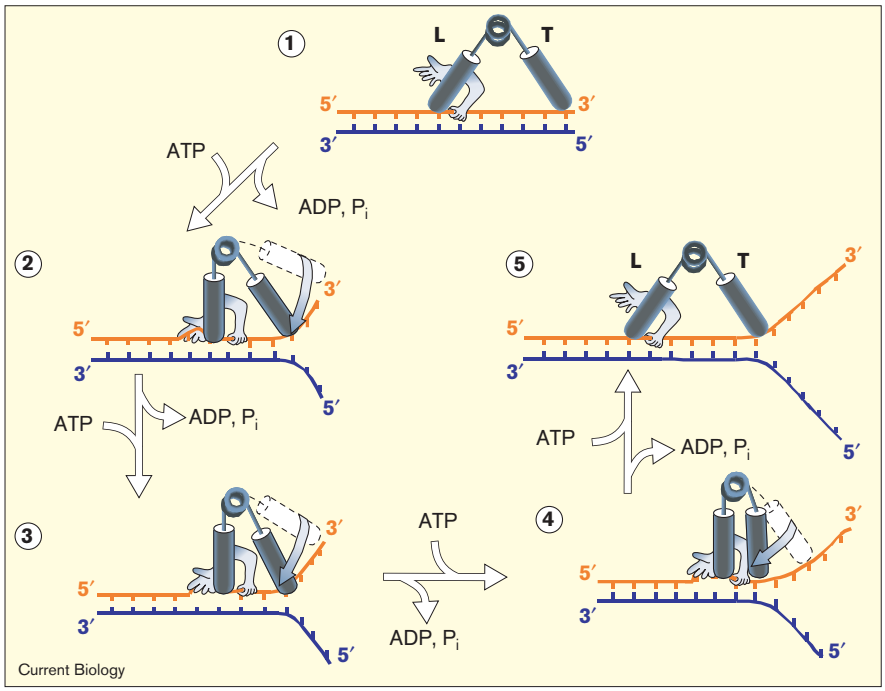
Attempts to make theoretical estimates for the number of base pairs that can be separated for each NTP molecule hydrolysed by an efficient helicase have varied from 2–4 [5] to as many as 9–12 [6]. The variation of these estimates is a consequence of the difficulties associated with estimating the free energy changes associated with base pair separation and NTP hydrolysis under the conditions that helicases experience in a cell rather than in a test tube. Attempts to determine the efficiency of helicases directly have also been problematic. Measurements of the amount of NTP that is hydrolysed by helicases during the unwinding of a nucleic acid duplex are dependent upon the assumption that all hydrolysis events are productive. As helicases are able to hydrolyse NTP in the presence of single-stranded DNA alone, this assumption clearly does not hold true. With this in mind, it is likely that experimental estimates of the efficiency are underestimated — more NTPs are hydrolysed in these experiments than are actually required to separate the duplex *per se*.

A further complication arises from the mechanism(s) of the enzymes: does each NTP hydrolysis event represent one catalytic turnover event, or are there other processes that occur with a lower frequency? In other words, how many bases are unwound each time an NTP molecule is hydrolysed? Is catalytic activity merely a repetition of this process, or are translocation and unwinding separate processes? These questions have led to the idea of a 'step size' for helicases, and estimates for this event are almost as numerous as the number of groups working in the field.

Recent work [3] on the PcrA helicase has used a novel assay for single-strand DNA translocation to try to answer this question. This work has shown that a single ATP molecule is hydrolysed every time that the enzyme moves along single-strand DNA by one base — a step size of one, the smallest to date. This proposal is also consistent with a helicase mechanism for PcrA that was proposed based on crystal structures of substrate and product complexes [7]. But this may not be the same for other enzyme systems. Work on the RNA helicase encoded by

Figure 1

Mechanism for RecBC helicase proposed by Bianco and Kowalczykowski [1]. Step 1: the leading (L) domain of RecBC is bound to the DNA duplex ahead of the trailing helicase (T) domain. Step 2: ATP binding and hydrolysis drive the helicase domain forward and DNA unwinding takes place but with an undefined 'step' size (probably between one and five base pairs). Steps 3–4: step 1 is repeated until the trailing domain catches up with the leading domain. Step 5: possibly as a consequence of contacting the trailing domain, the leading domain steps forward by approximately 23 base pairs (the 'step size' measured from the gapped substrates). The figure is not to scale. (Adapted from [1].)



the hepatitis C virus genome has suggested a step size of two bases per ATP molecule hydrolysed [8]. Work on UvrD helicase has suggested a rate-limiting step occurs every 4–5 base pairs [9], which also appears to be true for RecBCD [10], although it is not clear how many NTP molecules are hydrolysed in these systems at each step, which could be the cumulation of more than one hydrolysis cycle [9]. To complicate the issue further, recent work on RecBC helicase has revealed an even higher level of complexity that was not appreciated previously.

Using a series of 'gapped substrates', Bianco and Kowalczykowski [1] have now shown that RecBC — RecBCD lacking the D subunit — is able to traverse single-stranded gaps in DNA duplexes during processive unwinding, provided that these gaps are not on the translocating strand (they also proved that RecBC translocates along one strand in a 3′–5′ direction). Furthermore, by varying the size of the oligonucleotides on either side of the gap, a periodicity was detected which is indicative of a step size of about 23 base pairs. This observation has major implications for how this enzyme is interacting with its DNA substrate, because the contour length of 23 base pairs of B-form duplex DNA — over two complete turns of the helix — would span approximately 75 Å. If this does indeed represent the step size for the protein, the contact region must be even larger.

Evidently, the step size of 23 base pairs is too great to result from a single NTP hydrolysis event, so Bianco and Kowalczykowski [1] infer that several NTPase-dependent steps must occur for each leap of 23 bases (Figure 1). These smaller steps might relate to the 4–5 base pair 'steps' reported for RecBCD [10], or could even be as small as the one base pair step reported for PcrA [3]. Whatever the answer finally turns out to be, we are left with the conclusion that the way that helicases utilise the free energy of NTP hydrolysis is probably not straightforward, and that any or all of the various 'step sizes' that are observed for different helicases might be coupled to NTP hydrolysis in different ways. It is of course possible, however, that NTP-dependent base separation could be similar in all enzymes, but with an overlying 'step size' that relates to other specific requirements of the proteins, such as improved processivity or recognition of specific structures within the DNA. The wide range of activities that are carried out by helicases might fit with this proposal.

Enzymes that manipulate DNA frequently undergo extra-ordinary molecular gymnastics in order to allow them to deal with a substrate that is larger than the enzyme. It will be fascinating to see the crystal structure of RecBCD so that we can understand the details of how this enzyme performs what appears to be a remarkable molecular mechanism.

References

1. Bianco PR, Kowalczykowski SC: **Translocation step size and mechanism of the RecBC DNA helicase.** *Nature* 2000, **405:**368-372.
2. Kowalczykowski SC: **Initiation of genetic recombination and recombination-dependent replication.** *Trends Biochem Sci* 2000, **25:**156-165.

3. Dillingham MS, Wigley DB, Webb MR: **Demonstration of unidirectional single stranded DNA translocation by PcrA helicase: measurement of step size and translocation speed.** *Biochemistry* 2000, **39:**205-212.
4. Morris PD, Raney KD: **DNA helicases displace streptavidin from biotin-labelled oligonucleotides.** *Biochemistry* 1999, **38:**5164-5171.
5. Roman LJ, Kowalczykowski SC: **Characterisation of the adenosine-triphosphatase activity of the *Escherichia coli* RecBCD enzyme: relationship of ATP hydrolysis to the unwinding of duplex DNA.** *Biochemistry* 1989, **28:**2873-2881.
6. Lohman TM, Bjornson KP: **Mechanisms of helicase-catalyzed DNA unwinding.** *Annu Rev Biochem* 1996, **65:**169-214.
7. Velankar SS, Soultanas P, Dillingham MS, Subramanya HS, Wigley DB: **Crystal structures of complexes of PcrA helicase with a DNA substrate indicate an inchworm mechanism.** *Cell* 1999, **97:**75-84.
8. Porter DJT, Short SA, Hanlon MH, Preugschat F, Wilson JE, Willard DH, Consler TG: **Product release is the major contributor to kcat for the hepatitis virus helicase-catalysed strand separation reaction of short duplex DNA.** *J Biol Chem* 1998, **273:**18906-18914.
9. Ali JA, Lohman TM: **Kinetic measurement of the step size of DNA unwinding by *Escherichia coli* UvrD helicase.** *Science* 1997, **275:**377-380.
10. Vindigni A, Gregorian R, Lucius AL, Ali JA, Taylor AF, Smith GR, Lohman TM: **DNA unwinding step-size of *E. coli* RecBCD helicase.** *Biophys J* 2000, **1:**1786.