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Need for Speed: Mechanical Regulation of a Replicative Helicase

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There is much debate about how helicases unwind DNA during DNA replication and how their activity is regulated. In this issue, Johnson et al. (2007) shed light on this conundrum using a single molecule approach to dissect the behavior of the T7 DNA helicase.

To duplicate genomic DNA before cell division, a helicase needs to separate double-stranded DNA into single-stranded templates for the replication machinery. Most replicative helicases are ring-shaped motor proteins, typically hexamers, which couple chemical energy stored in nucleoside triphosphates to move along and unwind the DNA. The demand on the replicative helicase is staggering. In *E. coli*, DNA replication occurs at ~1,000 base pairs per second, and the helicase would need to move at least as fast as this and over long distances. On the other hand, untimely generation of single-stranded DNA (ssDNA) is dangerous as ssDNA is prone to degradation and can induce potentially harmful processes for the cell such as illicit recombination. Therefore, the unwinding activity of helicases needs to be tightly regulated.

In this issue, Wang and colleagues (Johnson et al., 2007) present extraordinarily clear data revealing two important determinants of DNA unwinding speed: mechanical force and DNA sequence. They use a sophisticated physical technique called the optical trap to manipulate a single DNA molecule and to measure its unwinding by a bacteriophage T7 helicase.

Remarkably, when a force was applied experimentally in the direction of unzipping the DNA, the helicase unwound much longer stretches of DNA at a much higher speed (>7-fold) before falling off the DNA (Figures 1A and 1B). So the applied force was able to convert a relatively slow and finicky motor protein into one with speed and endurance. In addition, DNA unwinding slowed down in the DNA region rich in GC base pairs and sped up notably in the AT-rich region. Therefore, intrinsic stability of the DNA duplex regulates helicase activity (Galletto et al., 2004).

The force-induced outcome—i.e., a dramatic increase both in the enzyme speed and the distance it can travel—is highly reminiscent of the similar behavior of the T7 helicase when its activity is coupled to DNA polymerization (Lee et al., 2006; Stano et al., 2005). The T7 helicase moves on the lagging strand template, 5′ to 3′, whereas the leading strand DNA polymerase moves on the opposing strand, 3′ to 5′. As both the force (applied in the current study) and the polymerase are getting the unwound strand out of the way of the helicase (Figures 1B and 1C), it is conceivable that an interaction between the 3′ strand and the outer surface of the helicase (Figure 1A) negatively regulates the
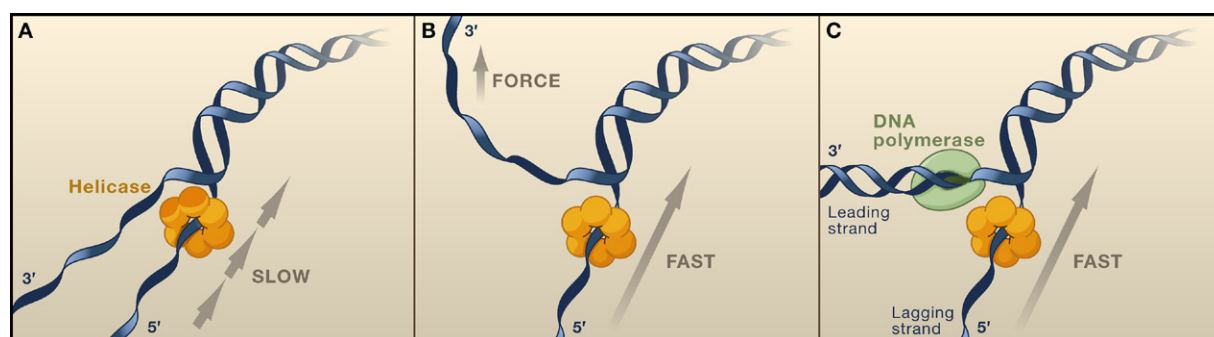
Figure 1. How Helicases Speed Up
(A) A hexameric helicase moves on the lagging strand in the 5′ to 3′ direction and unwinds the double-stranded DNA ahead. Its unwinding speed is much lower than its intrinsic translocation speed, possibly due to an inhibitory interaction between the helicase and the opposing strand. When a force is applied on the DNA strands (B) or when a DNA polymerase reaction is coupled with unwinding (C), the unwinding speed is dramatically enhanced. Therefore, regulation of helicase activities by other proteins may be mechanical in origin.

unwinding activity. Lifting such an inhibition via force or the action of a polymerase may allow the helicase to unwind DNA at its intrinsic speed. Such a mechanism would have strong regulatory consequences, whereby a robust unwinding activity is switched on precisely when it is needed. It would also prevent the helicase from running far ahead of the polymerase (for example, when the polymerase is stalled due to a lesion in the leading strand). In previous studies, crystal structures of *E. coli* Rho helicase (Skordalakes and Berger, 2003) and single-molecule analysis of *E. coli* Rep helicase (Myong et al., 2005) showed evidence for secondary binding sites on the helicase surfaces with regulatory implications.

The sequence dependence observed in this study raises an interesting question. Would the helicase also speed up in AT-rich regions of the genome when it is working with the replication machinery? If so, can the lagging-strand polymerases keep pace with the helicase? Alternatively, direct or indirect interactions between the helicase and the polymerases may keep their movement at a constant speed regardless of sequence.

This study also addresses the fundamental question of how a ring-shaped helicase unwinds DNA. Although there are several proposals involving transportation of double-stranded DNA through the ring, the most popular view envisions the helicase encircling the ssDNA that it moves along (Figure 1). Like many other helicases, the T7 helicase rapidly translocates on ssDNA in a particular direction (i.e., 5′ to 3′; Jeong et al., 2004). So one can imagine a purely passive unwinding mechanism where a helicase awaits thermal fluctuations to melt base pairs at the unwinding fork before taking a forward step to trap the transient intermediates. Because an assist-ing force would bias the spontaneous dynamics of the replication fork toward melting and a high GC content would hinder base pair melting, the force- and sequence-dependent data of this study seem to support the passive mechanism.

Not quite so, according to Wang and colleagues. To test the passive model rigorously, they also measured the ssDNA elasticity, sequence-dependent mechanical stability of duplex DNA, and ssDNA translocation speed of the T7 helicase. It turns out that less than half of the force-induced increase in unwinding speed is attributable to a purely passive mechanism. Rather, the authors argue, the helicase must actively destabilize the duplex ahead; indeed, an active-passive hybrid model with an ssDNA translocation step size of 2–4 nucleotides would fit the data.

The mathematical models used here are simplistic by necessity. For instance, potentially inhibitory interactions between the helicase and the opposing strand, which would be reduced by force, were not considered. As in any good study, their specific model is testable. For example, if future higher resolution studies show that the step size of translocation is one nucleotide, as suggested by a DNA-bound crystal structure of another hexameric helicase E1 of papillomavirus (Enemark and Joshua-Tor, 2006), or if there exists an additional layer of much larger steps as shown for a nonhexameric helicase HCV NS3 (Dumont et al., 2006), the current model involving uniform DNA translocation steps of a larger size will have to be amended.

This study is a beautiful exhibition of the power of new in singulo approaches for revealing fundamental details of elementary biological processes and in particular for demonstrating how mechanical parameters influence biochemi-cal reactions. The measurements were done at relatively high forces because spatial resolution of force-based methods deteriorates precipitously at low forces. Ideally, one wishes to understand the mechanical regulation of reactions also at low forces, which would be more prevalent in vivo than the strong and persistent forces found in typical single-molecule analyses. Further technical developments are in order, for example, by combining mechanical manipulation with the single-molecule fluorescence methods which are capable of measuring DNA unwinding and helicase conformational changes with high resolution at arbitrarily low forces (Ha et al., 2002; Myong et al., 2005). Given the fascination of biophysicists with motor proteins and the rapid pace of technical developments, more detailed and often surprising views of helicases in action are eagerly anticipated.

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