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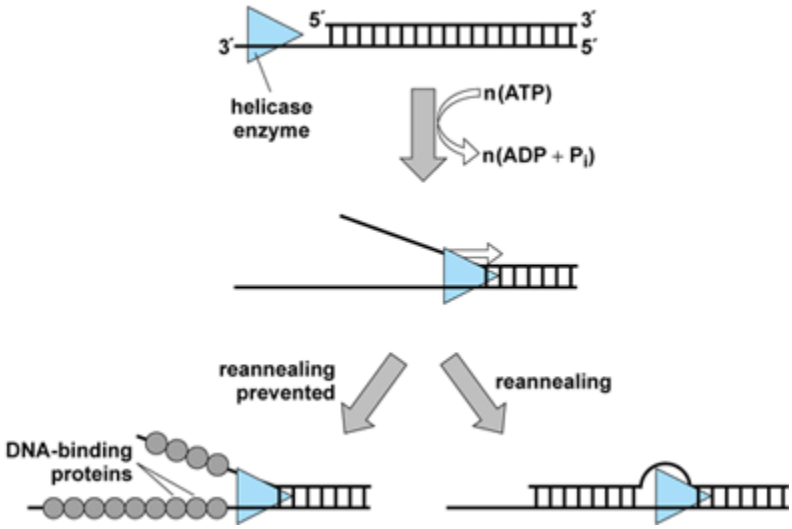
DNA helicases

In all cellular organisms from bacteria to humans, genetic information is locked within a double helix formed by the two antiparallel deoxyribonucleic acid (DNA) strands. Although double-stranded DNA (dsDNA) is the form most suitable for secure information storage, hydrogen bonds formed between complementary bases (Watson-Crick base pairing) impair readout of this information by the cellular machinery, which frequently requires a single-stranded DNA (ssDNA) intermediate as a template. The unwinding of dsDNA into ssDNA, a function critical for virtually every aspect of cellular DNA metabolism from DNA replication to homologous DNA recombination, is provided by a ubiquitous class of enzymes called DNA helicases. First identified in the 1970s, DNA helicases are motor proteins that convert chemical energy into mechanical work. Chemical energy is derived from the hydrolysis of adenosine triphosphate (ATP) or other nucleoside triphosphates, and is coupled with mechanical work during at least two important steps within the helicase reaction cycle (Fig. 1): (1) the unidirectional translocations along the substrate molecule and (2) the melting of the DNA duplex, which together result in the formation of the ssDNA intermediates essential for vital cellular processes.

Fig. 1 Schematic representation of the helicase reaction. The helicase enzyme translocates along the DNA molecule and separates the strands. Energy for this unfavorable reaction is provided by the hydrolysis of adenosine triphosphates (ATP) to adenosine diphosphates (ADP) and inorganic phosphate ions (P_i). In the presence of a single-stranded DNA binding protein, reannealing of the DNA duplex is prevented. The helicase depicted here displays a 3' →5' polarity, tracking unidirectionally along the lower of the two DNA strands in the duplex (the loading strand).



Classifications

Helicases are divided into five main superfamilies based on the presence and composition of conserved amino acid motifs (often referred to as the helicase signature motifs). (It is important to note, however, that only a small fraction of these putative helicases have been studied biochemically and, of those proteins, not all have been shown to possess nucleic acid strand separation activity.) Biochemical and structural data have suggested that helicases function as monomers, dimers, and multimers (predominantly hexamers) and that they can also be classified based on a substrate requirement for dsDNA, dsRNA, or DNA-RNA hybrids. To unwind dsDNA efficiently, many DNA helicases need to initiate from an ssDNA region adjacent to the duplex part of the substrate molecule. Based on the requirement for an ssDNA overhang of a certain polarity, helicases are divided into two functional groups: those that utilize a 3'-terminated ssDNA are designated as 3' →5' helicases, whereas enzymes that require a 5' overhang are designated as 5' →3' helicases.

Directional translocation

It is now generally believed that the observed polarity requirement of helicases is a consequence of a directional bias in translocation on ssDNA. For example, the enzyme depicted in Fig. 1 is a 3' →5' helicase. Upon binding to the ssDNA, it starts moving toward the 5' end of the loading strand, which brings the enzyme to the ssDNA-dsDNA junction and subsequently through the duplex portion of the substrate.

Evidence for directional translocation on ssDNA was provided by two different approaches. The first examined the dependence of helicase ATPase activity on the length of the ssDNA substrate. The second, based on the ability of many helicases to create sufficient force during ssDNA translocation to disrupt the tight interaction between streptavidin and biotin (K_d = 10-15 M), measured the ability of the helicase to increase the rate of streptavidin dissociation from DNA substrates biotinylated at either the 3' or 5' end. This second method was used successfully to determine the directionality of movement of several helicases on ssDNA.

High-resolution structural data suggest that the helicase signature motifs are not essential for the duplex DNA separation per se, but for the ATP-dependent unidirectional motion of the helicases on either single- or double-stranded DNA lattices. Consequently, it was proposed that the helicase signature motifs define a modular structure that functions as the DNA motor, while additional domains, which may vary from one protein to another, may be responsible for the DNA unwinding.

Accessory factors

Once dsDNA unwinding is achieved, spontaneous reannealing of the duplex may be avoided if the nascent ssDNA strands are trapped by single-stranded DNA binding proteins or other "coupling factors" that hand off the intermediates to the next step in a reaction pathway (Fig. 1). Although ssDNA binding proteins have frequently been shown to stimulate helicase activity in vitro, helicase activity can also be stimulated by other accessory factors that increase the rate or processivity of unwinding. The primary replicative helicase of *Escherchia coli*, DnaB, is a good example of a helicase that acts poorly in isolation from the accessory factors with which the enzyme is intended to operate. As part of the replisome (the DNA synthesis machinery of the cell), the role of DnaB is to separate the DNA strands at the replication fork. It was shown recently that the rate of movement of the replication machinery at the fork is coordinated by an interaction between DnaB and DNA polymerase (enzyme that synthesizes a daughter strand of DNA residues) that is mediated by the τsubunit of the DNA polymerase. The τsubunit bridges the polymerase dimer and the hexameric helicase, inducing a conformational change in DnaB that enhances its translocation rate by almost 30-fold to 1000 base pairs per second. In the absence of τ, the replication machinery is uncoupled, and the polymerase simply follows DnaB as it unwinds DNA at approximately 35 bp/s.

Single-molecule translocation visualization

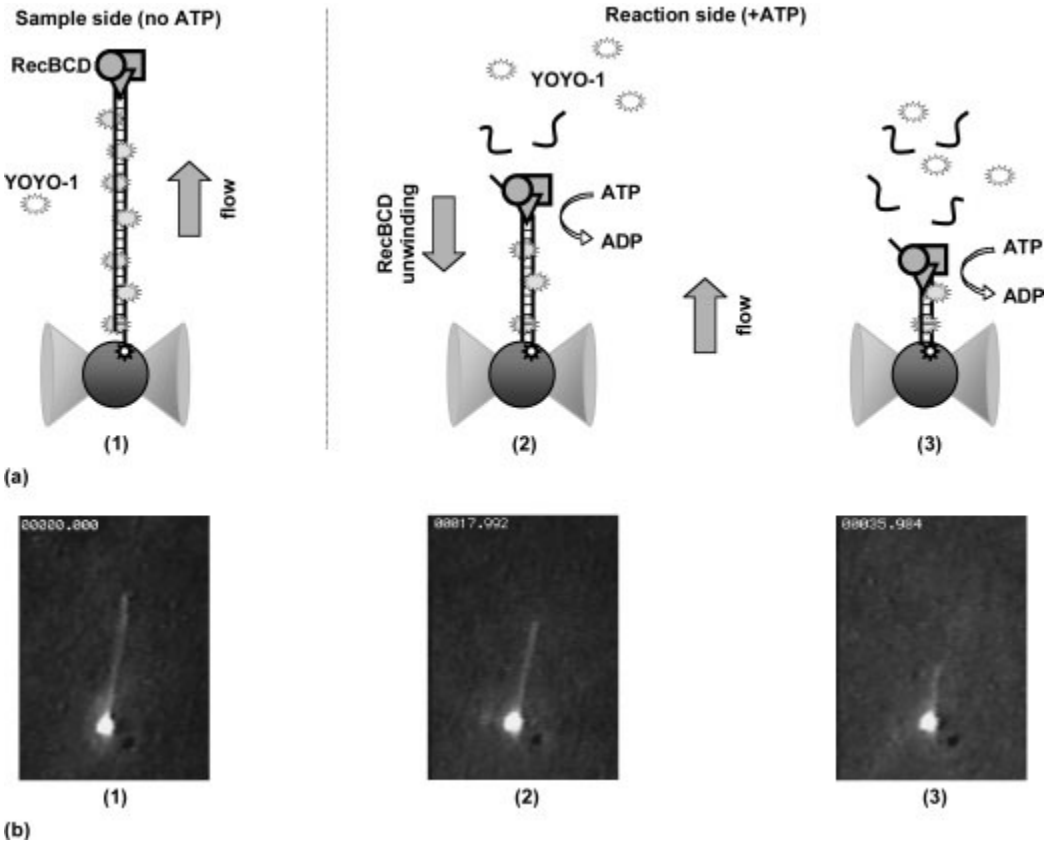
Until recently, all biochemical data on helicases were derived from conventional bulk-phase techniques, which observe the population-averaged properties of large molecular ensembles. In 2001 two new approaches to visualize translocation by a single molecule of a helicase were reported. These new techniques successfully visualized translocation of a single molecule of RecBCD, a multifunctional heterotrimeric enzyme employed by *E. coli* to initiate homologous recombination at dsDNA breaks. RecBCD is an exceptionally fast helicase that is furnished with all of the processivity and accessory factors it requires. The enzyme has a high affinity for blunt or nearly blunt dsDNA ends, and it can unwind, on average, 30,000 bp of dsDNA per binding event at a rate of 1000 bp/s, while simultaneously degrading the ssDNA products of its helicase activity.

Optical trap visualization

In one approach, a device called an optical trap was used to manipulate individual, fluorescently labeled DNA molecules and to visualize their unwinding and degradation by the RecBCD enzyme (Fig. 2*a*). A dsDNA molecule, biotinylated at one end, was attached to streptavidin-coated polystyrene beads. The RecBCD enzyme was then prebound to the free DNA end in the absence of ATP. The bead was caught and held by lasers (the optical trap); buffer flowing through the optical cell caused the DNA to

stretch out behind the trapped bead. The dsDNA was visualized by staining with a fluorescent intercalating dye (YOYO-1) and appeared as a bright 15-micrometer rod. Upon addition of ATP, the RecBCD enzyme mediated the unwinding of dsDNA, which was observed as a progressive shortening of the fluorescently labeled DNA molecule (Fig. 2*b*).

Fig. 2 Optical trapping and visualization. (*a*) Optical trapping method for studying RecBCD helicase/nuclease at the single- molecule level. (1) A polystyrene bead is held in the optical trap with dsDNA (stained with the fluorescent dye YOYO-1) stretched out in the flow behind it. (2) Upon addition of ATP, the helicase begins to unwind and degrade the DNA. (3) Unwinding continues until the helicase reaches the bead or falls off of its DNA track. (*b*) Frames from a movie of DNA unwinding and degradation in the optical trap apparatus. The frames are equivalent to the representation in *a*. (*The original movie of the helicase in action may be viewed in its entirety at http://microbiology.ucdavis.edu/sklab/kowalczykowskilab.htm*)

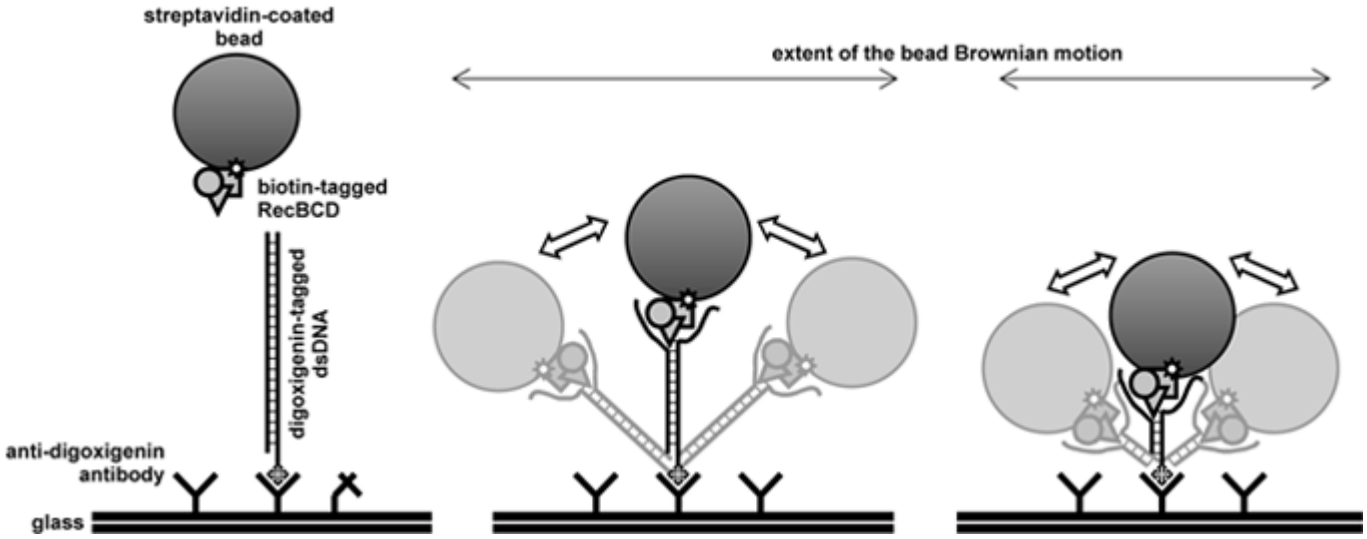


Tethered particle motion visualization

An alternative single-molecule approach used light microscopy to follow translocation of a biotin-tagged RecBCD enzyme bound to a streptavidin-coated polystyrene bead. In the tethered particle motion experiment (Fig. 3), dsDNA molecules, modified with digoxigenin at one end, were attached to a glass surface coated with antidigoxigenin

antibodies. Bead-labeled RecBCD molecules were bound to the free (unmodified) dsDNA ends. Because the DNA acts as a flexible tether, RecBCD translocation was observed as a decrease in the Brownian motion (the irregular motion of small particles caused by the random bombardment by molecules in the surrounding medium) of the bead as it was pulled toward the glass surface.

Fig. 3 Tethered particle motion experiment to study DNA translocation by single RecBCD helicase/nuclease molecules. A dsDNA molecule is attached to a glass surface, and RecBCD molecules are attached to polystyrene beads. As RecBCD tracks along the DNA molecule in an ATP-dependent manner, it gradually draws the bead closer to the glass surface. This translocation results in a decrease in the Brownian motion of the bead that can be measured by light microscopy. (*Adapted from http://www.bio.brandeis.edu/~gelles/movies.html*)



Combined observations

The two single-molecule experiments are different yet complementary: the tethered particle motion experiment directly measures translocation, whereas the optical trap method (and conventional bulk assays) measures dsDNA unwinding. Therefore, together, the studies provide additional powerful evidence for the coupling of DNA strand separation with movement of the helicase protein on its substrate lattice. Both single-molecule visualization methods show that RecBCD translocates unidirectionally and processively on dsDNA, with each molecule moving at a constant rate (within the limit of experimental detection). Although the average translocation rate is similar to that derived from bulk measurements, considerable variation is observed in the translocation rate of individual RecBCD enzymes. This surprising observation is an example of the kind of information that is accessible only by single-molecule studies.

Conclusion

In the last 10 years, considerable progress has been made in the understanding of the molecular mechanisms of DNA helicases. Although many questions remain, perhaps the next challenge in this field is to understand how these DNA motors are incorporated into and used by large multiprotein complexes, such as the replisome, to orchestrate complex DNA processing events.

See also: Adenosine triphosphate (ATP); Deoxyribonucleic acid (DNA); Enzyme; Molecular biology; Nucleoprotein

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- View of Brownian motion of DNA-tethered beads

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