McGRAW-HILL YEARBOOK OF Science & Technology

2003

example, immune responses are always amplified in vivo). Pressure-mediated DNA delivery, including the hydrodynamic force–based approach (a rapid injection of a large volume of DNA solution into the bloodstream), has also been explored. DNA can also be delivered with a "gene gun," a particle bombardment device that accelerates DNA-coated gold microparticles to an extremely high speed to penetrate cell membranes, thus introducing large amounts of DNA into cells. Electrical force represents a different class of DNA delivery, called electroporation. In this approach, high-voltage electrical pulses (in the millisecond range) are applied to puncture cell membranes transiently, allowing DNA to diffuse into a cell. Although electroporation is one of the most efficient ways of DNA delivery, it is also one of the most toxic methods. Magnetofection represents a relatively new approach. DNA vectors are first associated with superparamagnetic nanoparticles; localized delivery is then achieved by application of a magnetic field. Other interesting methods of DNA delivery include sonoporation, in which DNA delivery is accomplished by ultrasonic cavitation, and photochemical transfection, in which light and photosensitizing compounds are used to direct DNA delivery.

Challenges and the future. It is clear now that there will be no single, universal DNA delivery system that suits all needs; each clinical setting needs a particular delivery method tailored toward the treatment or prevention of that particular disease. The greatest challenge is to develop a nonviral DNA delivery system that has high efficiency and specificity but low toxicity and cost. Thus future DNA delivery may rely on a hybrid system that combines the advantages of both viral and nonviral components, or a modular system that incorporates many currently developed methods to meet the challenge.

For background information *see* DEOXYRIBONUCLEIC ACID (DNA); GENE; LIPOSOME; PLASMID; VACCINATION in the McGraw-Hill Encyclopedia of Science & Technology.

Dan Luo

Bibliography. M. E. Davis, Non-viral gene delivery systems, *Curr. Opin. Biotechnol.*, 13:128–131, 2002; M. A. Kay, J. C. Glorioso, and L. Naldini, Viral vectors for gene therapy: The art of turning infectious agents into vehicles of therapeutics, *Nat. Med.*, 7: 33–40, 2001; F. Liu and L. Huang, Development of non-viral vectors for systemic gene delivery, *J. Control Release*, 78:259–266, 2002; D. Luo and W. M. Saltzman, Synthetic DNA delivery systems, *Nat. Biotechnol.*, 18:33–37, 2000; W. M. Saltzman, *Drug Delivery: Engineering Principles for Drug Therapy*, Oxford University Press, New York, 2001; W. M. Saltzman and D. Luo, *Synthetic DNA Delivery Systems*, Landes Bioscience, in press.

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.

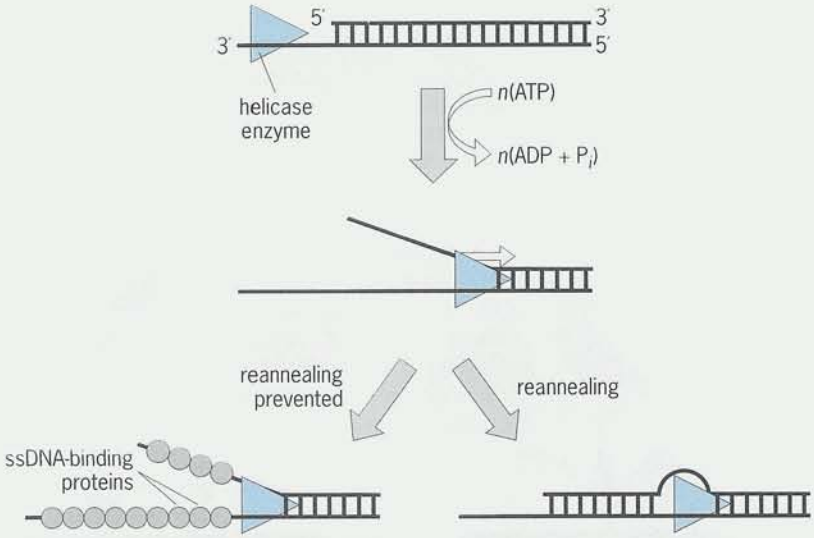

Fig. 1. Schematic representation of the helicase reaction. The helicase enzyme translocates along the DNA molecule and separates the strands. Energy for this unfavorable reaction is provided by the hydrolysis of adenosine triphosphates (ATP) to adenosine diphosphates (ADP) and inorganic phosphate ions (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 $3' \rightarrow 5'$ helicases, whereas enzymes that require a 5′ overhang are designated as $5' \rightarrow 3'$ 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 $3' \rightarrow 5'$ 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

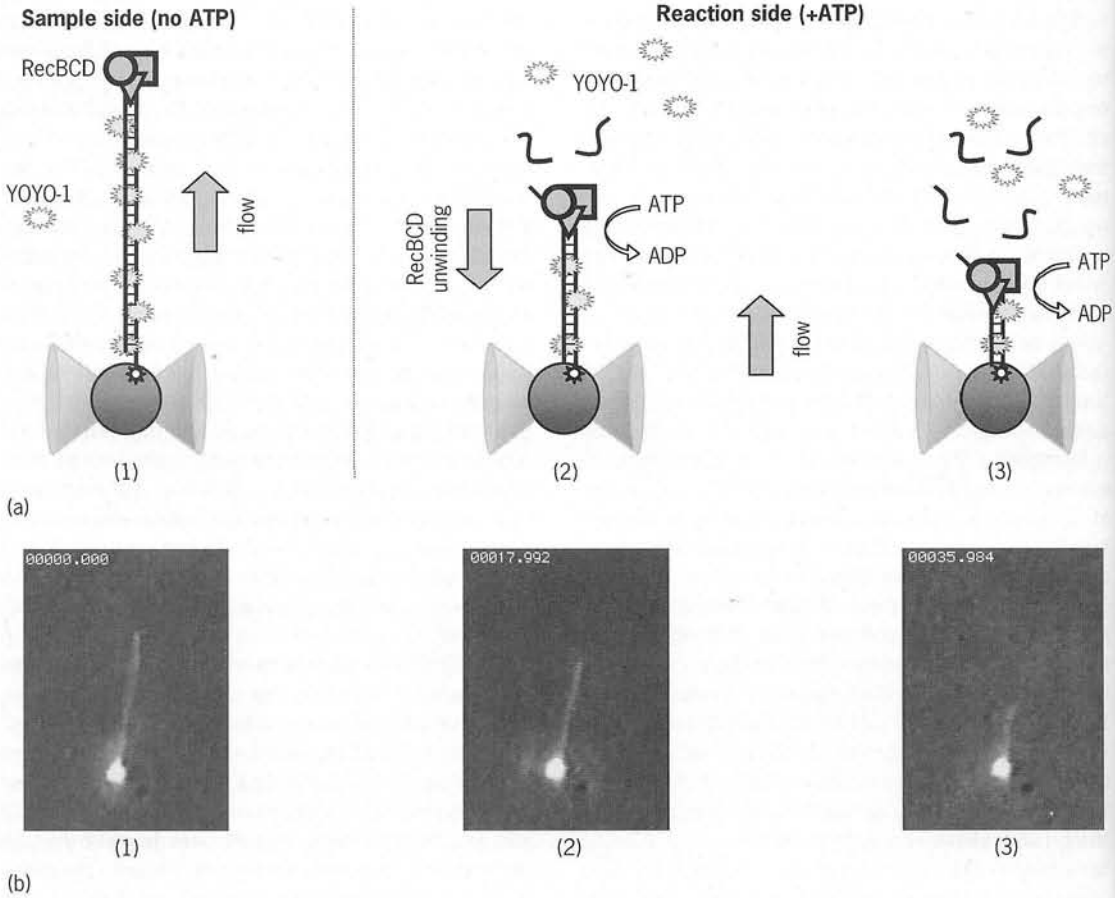

Fig. 2. Optical trapping and visualization. (*a*) Optical trapping method for studying RecBCD helicase/nuclease at the single-molecule level. (1) A polystyrene bead is held in the optical trap with dsDNA (stained with the fluorescent dye YOYO-1) stretched out in the flow behind it. (2) Upon addition 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*)

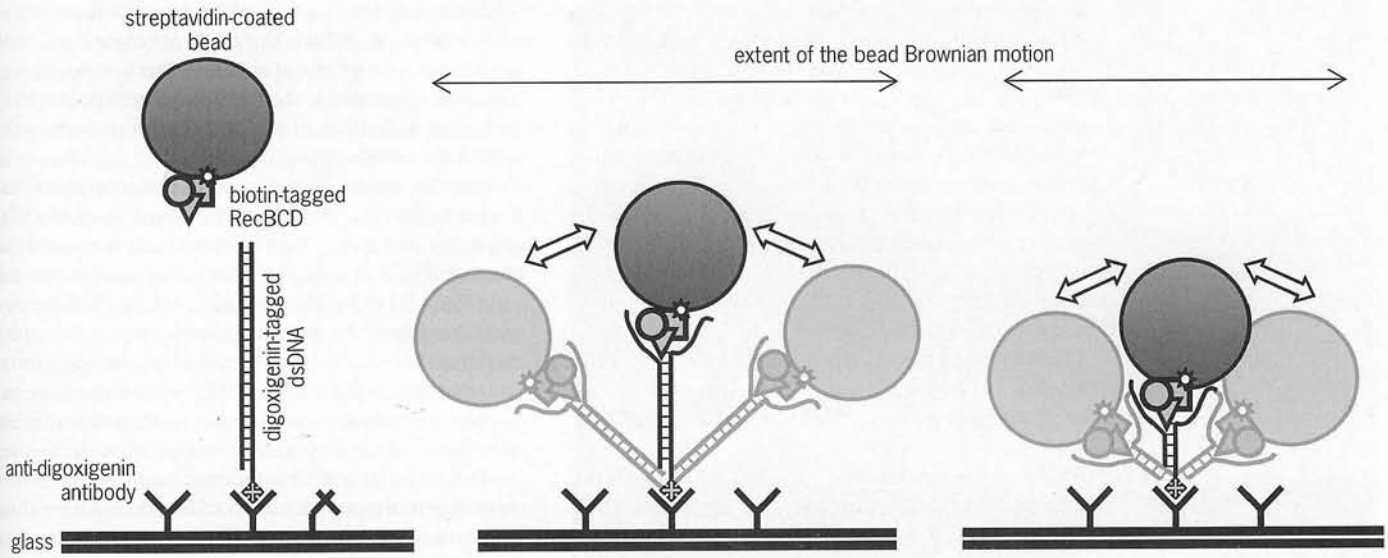

Fig. 3. Tethered particle motion experiment to study DNA translocation by single RecBCD helicase/nuclease molecules. A dsDNA molecule is attached to a glass surface, and RecBCD molecules are attached to polystyrene beads. As RecBCD tracks along the DNA molecule in an ATP-dependent manner, it gradually 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*)

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 τ subunit of the DNA polymerase. The τ subunit 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*).

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.

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.

For background information *see* ADENOSINE TRIPHOSPHATE (ATP); DEOXYRIBONUCLEIC ACID (DNA); ENZYME; MOLECULAR BIOLOGY; NUCLEOPROTEIN in the McGraw-Hill Encyclopedia of Science & Technology.

Maria Spies; Mark S. Dillingham; Stephen C. Kowalczykowski

Bibliography. B. Alberts et al., *Molecular Biology of the Cell*, 3d ed. (II, Chap. 6), Garland Publishing, New York, 1994; P. R. Bianco et al., Processive translocation and DNA unwinding by individual RecBCD enzyme molecules, *Nature*, 409(18):374–378, 2001; K. M. Dohoney and J. Gelles, χ-Sequence recognition and DNA translocation by single RecBCD helicase/nuclease molecules, *Nature*, 409(18):370–374, 2001; H. Lodish et al., *Molecular Cell Biology*, 4th ed. (Chap. 12), W H Freeman, New York, 2000; P. Soultanas and D. B. Wigley, Unwinding the "Gordian Knot" of helicase action, *TIBS*, 26(1):47–54, 2001.

Dynamic brain atlas

Magnetic resonance imaging (MRI) has become the imaging technique of choice for the diagnosis of many brain disorders. It is also increasingly used to assist diagnosis of psychiatric disorders and dementia, which are characterized by subtle abnormalities in the size and shape of brain structures, instead of the distinct lesions visible in patients with, for example, brain tumors. Unfortunately, these subtle abnormalities are difficult to describe precisely, making diagnosis of individual brain MRI scans difficult. However, the dynamic brain atlas, a new technique for analyzing brain MRI scans, may assist physicians to overcome these diagnostic obstacles.

Brain atlases. In neuroimaging research, brain atlases are widely used to assist in data analysis. Typically, brain atlases are based on brain MRI scans and are generated either by fusing images from multiple subjects or fusing multiple images of a single representative subject. A brain atlas is considered a reference brain with which the brain MRI scan of a specific subject or group of subjects can be compared. Atlases from multiple subjects have the advantage of giving an indication of natural variability in the size and shape of structures.

Static. In today's practice, brain atlases are built statically (that is, all images are fused at once after acquiring the data). Brain atlases can be found in neurological textbooks or, more and more often, in electronic form on the Internet. Although these atlases have been very successful in research applications, they are not so helpful for the diagnosis of individual patient brain MRI scans. For patient diagnosis, it is necessary to have information relevant to the specific patient (that is, relevant to the patient's age, gender, background, and medical history), but static precalculated atlases do not have this property.

Dynamic. The ideal atlas would be dynamic, that is, customized to the current patient by being made up of images from individuals of the same age, gender, background, and medical history as the study subject. This would make the process of identifying subtle or diffuse brain disease (for example, psychiatric disorders and dementia) more straightforward, as slight differences between the current patient and similar normal individuals could be spotted at a glance. Dynamic atlases will be increasingly valuable as hospitals computerize their storing and handling of medical images.

Computational grids. A dynamic brain atlas requires large collections of images from which a subgroup that matches the current patient can be chosen. As digital-image archive systems are becoming more common in hospitals and image data are being incorporated into multimedia patient records, widespread computational analysis of clinical images is becoming possible for the first time. However, the time-consuming fusion of large numbers of images cannot be performed on one standard computer in practical time, and few hospitals are likely to want to invest in expensive supercomputer facilities. The capabilities provided by computational grids, however, could make it possible to use the huge quantity of distributed on-line patient images for decision support in diagnosis. Similar to the document-sharing concept of the World Wide Web, the grid concept is based on the coordinated sharing of computational resources via the Internet. Grid users could have at their disposal distributed high-performance computers that are able to access and process large amounts of data stored in global databases, as well as the appropriate tools to control these resources. By using this grid infrastructure, the task of fusing images is distributed to grid computers that process the data simultaneously, instead of being performed on the local machine. Hence, a task that could last for days on one computer can be processed in a matter of minutes on the grid. Additionally, no local high-performance computer is necessary anymore, because the local machine serves just as a "gateway"