Mechanisms of Helicases*

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Helicase protein was first discovered in Escherichia coli in 1976 (1, 2). Since then RNA and DNA helicases with diverse functions have been found in all organisms. Helicases couple the chemical energy of NTP binding and hydrolysis to separate the complementary strands of double-stranded nucleic acids, remove nucleic acid–associated proteins, or catalyze homologous DNA recombination. Although helicases display these activities in isolation, most work efficiently as part of a larger protein complex. Interests in understanding helicase mechanisms stem not only from their importance in various cellular processes. Helicases also serve as a model system to understand NTPase–coupled motors. The helicase function is required for efficient and accurate replication, repair, and recombination of the genome. Similarly, helicase functions facilitate RNA metabolic processes such as transcription, ribosome biogenesis, translation, RNA splicing, RNA editing, RNA transport, and RNA degradation. The structure, function, and mechanisms of helicases have been widely discussed in the literature (3–16). Here, we focus on the strategies that helicases use to translocate and catalyze strand separation coupled to NTP binding and hydrolysis.

Diseases

Mutations in helicases have been linked to a number of human diseases such as cancer and premature aging (17–25). Many of the implicated helicases such as the RecQ family of helicases are involved in DNA repair and in processes assuring genome stability. To link the defect in a particular helicase to the human disease will require defining the cellular roles of these helicases and determining the exact steps in which they are involved in the metabolic pathway. Many viruses use their own helicases that are essential for viral genome replication (26). Identifying and targeting the unique features of these viral helicases remains a feasible strategy for antiviral therapy (27–30).

Sequence and Structure

Based on conserved amino acid sequence motifs, helicases are classified into families and superfamilies (31). Helicases of different families share similarities in their three-dimensional folds (RecA-like fold) (32–34). SF1 and SF2 helicases are closely related and typically contain several domains and a single NTP binding site at the interface of two RecA-like domains. Ring-shaped SF3 and SF4 helicases contain an NTP binding site at the interface of adjacent subunits (34). The nucleic acid binding site in helicases is distinct but allosterically linked to the NTPase site. Examination of sequence, structure, and biochemical properties reveals that helicases of the same superfamily often show different substrate specificity (RNA versus DNA) and directionality of translocation (3’→5’ versus 5’→3’). This suggests that small changes in the primary structure of the helicase can be responsible for different substrate specificity and opposite directionality. The structural basis for the substrate specificity and directionality of translocation remains poorly understood.

Active State of Helicase

Even though helicases share a similar three-dimensional fold, they assemble into various oligomeric states that are often required to display full activity. The most established form of assembly is exemplified by the hexameric class of helicases (Fig. 1a) in which six subunits assemble to form a ring-shaped structure (T7 gp4, E. coli DnaB, RepA, Rho, MCM, SV40-LTag) (38). Monomers of ring-shaped helicases (T7 gp4 helicase) are not active in catalyzing NTPase or unwinding reaction, and hexamer formation is essential (35). The ring-shaped structure is stabilized by the binding of NTP, a metal ion, or both, and by the nucleic acid substrate (8). Most studies of ring-shaped helicases are consistent with the nucleic acid bound within the central channel. The enclosure of the nucleic acid by the protein subunits decreases the probability of helicase falling off, thus increasing the ability of the helicase to stay on track. Another advantage of this arrangement is the coupling of NTPase cycles between the hexameric subunits that can increase the efficiency of the NTPase cycles in promoting translocation.

Oligomerization is an important strategy for non-ring-shaped SF1 and SF2 helicases as well (Fig. 1, b–e). Even though many helicases function as monomers (Fig. 1a, T4 Dda, HCV NS3h, E. coli RecQ) (36–38), most non-ring-shaped helicases studied either require their activity is greatly enhanced by the formation of dimers or higher oligomers (38–42). Some helicases utilize a structural interaction whereas others rely on a functional interaction. Structural interactions result in the formation of a homodimer (Fig. 1c, UvrD) or heterodimer (Fig. 1d, RecBCD) that converts the helicase into a more effective enzyme (39, 40, 43). RecBCD helicase consists of two interacting helicases, RecB and RecD, that together are more active than individual helicases (44, 45). Many helicases show functional cooperativity and enhanced processivity when multiple molecules of helicases are loaded on the tracking strand (Fig. 1e). These helicases (T4 Dda and HCV NS3h) are functional as monomers, do not form stable oligomers, and do not show cooperativity in NTPase or nucleic acid binding. Yet their activity is enhanced when multiple helicases are loaded on the tracking strand, which is attributed either to prevention of backward helicase slips or simply the availability of additional helicase molecules when one falls off the track (38, 41, 42, 46).

Interactions with Nucleic Acid Substrate

Most helicases need a single-stranded nucleic acid region to bind and to initiate their action of strand separation (Fig. 2, a and b). Once loaded on the strand, they show a directional bias and translocate either 5’→3’ or 3’→5’. Helicases like RecBCD contain two helicases of opposite polarity and can initiate from blunt ended DNA (47). Ring-shaped helicases require Y-shaped nucleic acid structures with a loading strand and a non-complementary strand of an optimum length to initiate unwinding (Fig. 2, c–e) (48–50). A bulky adduct can replace the non-complementary strand to facilitate the unwinding process. In the absence of the non-complementary strand or a bulky adduct, ring-shaped helicases (DnaB, T7 gp4, MCM) encircle the duplex DNA and pass over it without unwinding the strands (51, 52). During unwinding, helicases may assume different nucleic acid binding modes depending on their NTP ligation state (53). Determining the mode of helicase interactions with the nucleic acid substrate during unwinding (Fig. 2) is an important step toward understanding the helicase mechanism.

Helicases show different degrees of tolerance to changes in the chemical nature of the loading strand while translocating. Some are sensitive to breaks (discontinuities in sugar phosphate backbone; substituted with ethylene glycol, etc.), to abasic sites, or to electrostatic disruptions (54). While unwinding, certain helicases (NHPP-II, Dda) show no sensitivity to the nature of the displaced strand (55–57). On the other hand, the nature of the displaced strand appears to influence the activity of HCV NS3 helicase (58).

Helicase Translocation Mechanisms

During genome replication, repair, or recombination, helicases need to unwind nucleic acids much longer than their binding sites. In such a case, helicase–catalyzed unwinding occurs in a stepwise manner, where the helicase stays on track and catalyzes repeated cycles of base pair separation steps coupled to unidirectional translocation. Many helicases have the ability to translocate unidirectionally along nucleic acids uncoupled from base pair separation. Distinction is therefore made here between translocation mechanisms and base pair separation mechanisms in discussing the proposed models of helicases. Many different mechanisms have been proposed for translocation and base pair separation. All of the mechanisms involve NTPase-coupled nucleic acid affinity changes and a conformational change (power stroke or ratchet) to explain biased movement that results in base pair separation or translocation. The differences in the proposed mechanisms reflect the diverse biochemical properties including the oligomeric state of the helicase, its mode of binding the nucleic acid at the unwinding junction, and the effect of the NTP ligation state on nucleic acid binding properties.

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Stepping Mechanisms—The stepping mechanisms involve two nucleic acid binding sites that independently bind and release nucleic acid in response to the signals received from the NTPase site (53, 59, 60). In the stepping models, the helicase is always bound to the nucleic acid via one nucleic acid binding site. In an inchworm type stepping model for a monomeric helicase, a cycle of nucleic acid binding, release, and translocation events begins with one helicase site bound tightly to the nucleic acid and the second helicase site bound weakly to the nucleic acid (Fig. 3A). The weak site dissociates from the nucleic acid and in a power stroke motion moves away from the tight site to bind at a position ahead. After the weak site has moved and made tight interactions ahead, the original tight site becomes weak, and as it dissociates from the nucleic acid, in a power stroke motion it moves forward to get close in distance to the site ahead. One cycle in an inchworm stepping mechanism is completed in six conformational changes.

The inchworm stepping mechanism is applicable to dimeric helicases as well. In a monomeric helicase, the two nucleic acid binding sites would present on the same polypeptide and under the control of a single NTP binding site. In dimeric helicase, each subunit would contain a nucleic acid binding site that can be controlled by the NTP binding site of that subunit. In dimeric helicases, therefore, coordinated NTPase activity can lead to coordinated binding and release of nucleic acid. An alternative stepping mechanism (rolling model) for a dimeric helicase has been proposed for DNA unwinding (53). In this model, each of the two subunits of the helicase alternate their binding to single-stranded and duplex DNA as they translocate one strand through its nucleic acid binding site while excluding the complementary strand (Fig. 4A, C). Many ring-shaped helicases also translocate along duplex DNA to bring about strand separation in an efficient manner.

Brownian Motor Mechanism—A two-state Brownian motor model (Fig. 3B) has been proposed as an alternative to the stepping mechanisms (61, 62). This model invokes Brownian motion and power stroke, and it is based on two conformational states of the helicase. Structural and biochemical studies have identified two distinct conformational states of helicases with weak and tight nucleic acid binding modes resulting from the different NTP ligation states. In the tight state, the helicase-nucleic acid energy profile is deep and sawtooth-shaped, and helicase movement along the nucleic acid is not possible. To translocate the helicase needs to loosen its interactions with the nucleic acid, and this happens when the helicase changes its NTP ligation state (NTP, hydrolysis, or product release). In the weak state, the helicase-nucleic acid energy profile is shallow and symmetric, and the helicase can move in either direction (Brownian motion) or completely dissociate from the nucleic acid (accounting for the observed low processivity of helicases). The short lifetime of the weak state (because of a rapid change in the NTP ligation state) keeps the helicase close to the starting position. When the helicase resumes the tight state, it makes a step forward (power stroke). Those molecules that have fluctuated in the opposite direction return to the original position. Repetition of these steps leads to net forward movement of the helicase along the nucleic acid.

Future challenges lie in distinguishing between the various models of helicase translocation. Accurate measurement of the step size can provide insights into a Brownian motor type versus a deterministic stepping type mechanism of helicase translocation.

Base Pair Separation Mechanisms

Base pair separation occurs at the junction of single-stranded and duplex regions. Helicases unwind long stretches of duplex nucleic acids by coupling base pair separation to translocation. Translocation along nucleic acid can occur by any of the above mentioned mechanisms. Depending on how the base pairs are separated, the base pair separation mechanisms are classified as active or passive (9, 62, 63). In a passive mechanism, the helicase waits for the base pairs to open spontaneously by thermal fluctuations before it moves and binds the newly opened bases. Because the terminal base pair at the junction opens and closes at a very fast rate (64), this type of a mechanism is attractive for helicases that can move and occupy one base at a time. The probability of several base pairs opening at the same time near the junction is very low. Therefore, if the helicase needs to move and bind more than one base at a time, it would employ some type of an active mechanism to bring about strand separation in an efficient manner.

One simple way for helicases to separate the base pairs at the junction is to translocate one strand through its nucleic acid binding site while excluding the complementary strand (Fig. 4A, wedge, wire stripper, or strand exclusion type mechanism). Many ring-shaped (T7 gp4, DnaB, MCM) and non-ring-shaped helicases (NPH II, Dda) have been proposed to unwind nucleic acid by this mechanism (48, 51, 52, 55, 57, 65–67). This mechanism and the mode of nucleic acid binding provide a way to prevent immediate reannealing of the unwound strands.

Helicases may employ additional ways to destabilize the duplex DNA. Helicases may interact directly with the duplex region near the unwinding junction and distort it prior to fully separating the strands by directional translocation (Fig. 4B, helicase-nucleic acid affinity changes (tight to weak transitions). Brownian motor mechanism. On the right, the helicase is shown to undergo nucleic acid affinity changes (tight to weak). In the weak state (2), the helicase fluctuates in either direction. Upon resuming the tight state (3), some helicase molecules move forward (3) and some return to their original position (1). On the left, the free energy of the helicase-nucleic acid complex is shown along the nucleic acid length. In the tight state (1), the helicase is trapped in a deep energy well unable to move. In the weak state (2), thermal fluctuations allow the helicase to fluctuate in either direction or to completely dissociate from the nucleic acid (4). Upon resuming the tight state, the deep energy profile is restored and some helicases move forward (3).

Many ring-shaped helicases have been proposed to unwind DNA by the strand exclusion model (Fig. 4C), which is essentially the wedge mechanism in Fig. 4A. Many ring-shaped helicases also translocate along duplex DNA to catalyze homologous DNA recombination at a Holliday junction. Ring-shaped helicases are also involved in the initiation of DNA replication, and they have the ability to bind specific sequences at the origin. Although the exact mechanism of unwinding DNA at the origin is not well understood, several models...
Helicases are required for the efficient catalysis of most DNA and RNA metabolic processes where they perform diverse functions. The basic activity of helicases in bulk experiments is to bind, unwind DNA efficiently with a high processivity under single-molecule conditions where the DNA was attached to a magnetic tweezer setup (78). The processivity of HCV NS3 increased with higher forces applied to the optical beads that were attached to the ends of the unwound strands (76). The single-molecule studies also identified distinct pauses as components of helicase unwinding (NS3), helicase switching DNA strands and moving on the opposite strand (UvrD), and repetitive backward movements when the helicase encountered a block such as the duplex DNA (Rep).

Although translocation is an important activity to understand, direct methods to measure translocation along single-stranded and duplex nucleic acids in the absence of unwinding are lacking. Transient state kinetic methods have been used to measure the rate and processivity of helicase translocation along nucleic acids without strand separation (88, 92, 93). Such assays indicate that helicases translocate unidirectionally and efficiently along single-stranded nucleic acid, and therefore directional translocation is an intrinsic property of these helicases. Interestingly, helicases (UvrD, T7 gp4) translocate along single-stranded DNA at a much faster rate than during DNA unwinding implying that the duplex DNA poses a barrier to the movement of the helicases (87, 94).

**Coupling of NTPase to Translocation and Unwinding**

Translocation and base pair separation activities of helicases are driven by NTP binding and hydrolysis. During each NTPase cycle, the helicase goes through defined NTP ligation states including empty, NTP, NDP, and NDP. One or more of the NTP ligation states causes changes in the affinity of the helicase for the nucleic acid and brings about a power stroke that leads to translocation and/or strand separation. Power stroke and nucleic acid affinity modulation steps are energetic events; therefore, only those changes in the NTPase cycle that are associated with an observable energy change are likely to drive these events at the nucleic acid binding site. Identifying the high energy steps requires dissection of the kinetics and thermodynamics of the NTPase reaction as well as determining the relative nucleic acid binding affinities of the different NTP ligation states.

One common feature of helicases found through structural studies is NTP binding at an interface between two separately folded domains. These domains are reminiscent of the structure of pre-recombinational single-stranded coating protein RecA. The binding of NTP at the interface serves several purposes. The changes in the NTP binding pocket as NTP assumes the different ligation states (empty, NTP, NDP, NDP) can be amplified as domain (or subunit) movements if the predominant contacts between them are through the NTP. A conserved arginine residue from the neighboring domain (or subunit) that is essential for NTP hydrolysis has been implicated to sense the change in the NTP ligation state and transmit the change to cause domain (or subunit) movement (34, 95). This conformational change can ultimately result in nucleic acid affinity changes or a power stroke (by change in distance between domains or subunits).

In monomeric helicases, a single NTPase site controls the conformational changes whereas there is potential for coordination between NTPase sites in an oligomeric helicase. Ring-shaped helicases potentially can bind and hydrolyze six NTPs, and many show cooperativity in NTP binding and hydrolysis. Several mechanisms can be envisioned for the coordination of the NTPase activity between the subunits of the hexameric helicase. A random or stochastic mechanism of NTP hydrolysis, where the subunits independently hydrolyze NTP, was ruled out for T7 helicase from the steep inhibition of the NTPase in a mixed hexamer containing wild-type and an inactive mutant helicase (96, 97). In a concerted or simultaneous mechanism, all six subunits bind and hydrolyze NTP and move DNA at the same time. Such a mechanism has been proposed for SV40 L-Tag (98). A three-site sequential mechanism of NTP hydrolysis and passage of nucleic acid between those subunits was proposed (34, 99, 100). Recently, however, a six-site sequential mechanism of NTP hydrolysis and passage of nucleic acid from one subunit to the neighboring one were proposed (Rho and T7 gp4) based on the kinetic studies of NTPase in the presence of nucleic acid and kinetic modeling (96). Such studies combined with high resolution structures of ring-shaped helicases with nucleic acid in different NTP ligation states are necessary for a complete understanding of the mechanochemical process.

**Perspectives**

Helicases are required for the efficient catalysis of most DNA and RNA metabolic processes where they perform diverse functions. The basic activity of helicases is to couple NTP binding and hydrolysis to conformational changes that bring about separation of base pairs or translocation along nucleic acid. Presently we lack a unified mechanism that explains this motor activity of helicases. The differences in the proposed mechanisms arise from the diverse nucleic acid bind-
ing modes of helicases and the various oligomeric states that are required to observe activity. It is recognized that even though helicases can work in isolation, the associated proteins regulate helicase activity, and therefore it will be important to understand the functions of helicases in the context of the cellular machinery. Advances in understanding helicase mechanisms will come from characterizing helicase structures in different NTP ligation states and by accurate measurements of helicase parameters such as the physical and chemical step sizes. Many questions remain unsettled. The identity of the elementary step appearing as the rate-limiting one in bulk unwinding assays or as pausing in single-molecule assays remains to be resolved. High resolution structures have yet to provide the basis for substrate specificity and helicase directionality. Deficiencies in helicase functions result in complex human diseases with varying phenotypes. Elucidating the underlying cause for these diseases at a mechanistic level is necessary to develop targeted therapeutics.

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