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Staying on Track: Common Features of DNA Helicases and Microtubule Motors

Minireview

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Linear motor proteins are enzymes that couple energy from nucleoside triphosphate hydrolysis to translocation along a polymer lattice. DNA helicases translocate along a DNA lattice as they unwind duplex DNA to form the single-stranded DNA intermediates required for DNA replication, recombination, and repair. Microtubule motors, such as kinesin, move along linear microtubule polymers in order to transport cargo, such as membrane organelles, within the cell. Although their functions are quite different, recent studies indicate that both classes of motor proteins share a number of mechanistic features.

One important property shared by DNA helicases and conventional kinesin, the founding member of the kinesin superfamily, is the ability to move along their polymer lattice for long distances without dissociating (termed processive movement). A high degree of processivity is crucial for helicases involved in DNA replication, where millions of base pairs (bp) must be replicated quickly. Processivity also enables small numbers of kinesin motors to transport vesicles efficiently over long distances in nerve axons. In contrast, muscle myosin and axonemal dynein operate in arrays where large numbers of motor proteins generate motion and thus neither require nor display processivity.

To translocate processively, a linear motor must maintain at least partial contact with the polymer lattice at all times, since Brownian motion will rapidly separate a detached motor from its track. One strategy is for the motor to create a physical barrier to prevent its dissociation from the polymer. For example, DNA polymerases often associate with accessory proteins that encircle the DNA, thereby creating a "sliding clamp" that prevents dissociation but allows one-dimensional motion along the polymer. A second strategy, used by oligomeric motor proteins that possess multiple lattice-binding sites involves a "hand-over-hand" interaction with the polymer (also called "subunit switching" or "rolling"). This mechanism requires that individual subunits of the motor alternate binding to the lattice in a coordinated manner, so that at least one subunit is tightly bound to the lattice at any given time. *E. coli* Rep helicase and conventional kinesin both function as homodimeric enzymes and are examples of motor proteins that appear to translocate by this mechanism. This review compares the mechanisms used by these two processive enzymes. Aspects of the mechanisms discussed here also are likely to be relevant to other motors with multiple polymer-binding sites, such as the hexameric helicases (Geiselmann et al., 1993; Lohman and Bjornson, 1996). However, these helicases differ from Rep in that they form ring-like structures that encircle at least one strand of the DNA (Yu et al., 1996), which may act as a fail-safe to ensure high processivity.

Definition of Processivity

Translocation processivity, P, reflects the kinetic partitioning of the motor between forward motion and dissociation and can be defined quantitatively as $P = k_f/(k_d + k_f)$, where k_f and k_d are the net rate constants for forward movement (per step) and complete dissociation of the enzyme from the linear lattice, respectively. As defined, P ranges between 0 and 1; $P = 0$ for nonprocessive enzymes and $P = 1$ for infinitely processive enzymes. *E. coli* Rec BCD, a highly processive recombinational helicase ($P = 0.99997$), can unwind $(1 - P)^{-1} = \sim$33,000 base pairs (bp) of duplex DNA before dissociating (Roman et al., 1992). Although a quantitative estimate of Rep's processivity has not been made, *E. coli* UvrD, a related dimeric helicase with 40% amino acid similarity to Rep, has an ~5 bp step size and unwinds an average of 50 bp in vitro before dissociating ($P = 0.9$) (Ali and Lohman, 1997). It is important to note that estimates in vitro often depend strongly upon salt and ATP concentrations as well as temperature, since these factors influence the rate of dissociation from the DNA. Processivity of DNA helicases can also be enhanced by accessory proteins; the ϕX174 gene A protein, for example, enables Rep to unwind at least 7,000 bp processively. Therefore, a low processivity measured under one set of conditions in vitro does not preclude higher processivity in vivo.

Using single molecule motility assays, conventional kinesin has been shown to take ~100 steps along microtubules before dissociating ($P = 0.99$) (Block et al., 1990). The high degree of processivity of conventional kinesin may be an unusual feature of this particular kinesin, since other members of the kinesin superfamily, such as Ncd, do not display detectable processivity.

Subunit Switching during Processive Movement

The binding properties of Rep and kinesin to their respective polymer lattices, DNA and microtubules, are well suited to enable these motor proteins to "walk" along a linear lattice. The two subunits (also termed "heads") of these dimers display negative cooperativity for lattice binding, so that one head is tightly bound to the polymer while the second head is detached or weakly bound. Such negative cooperativity is important to prevent both subunits from being bound simultaneously to the lattice for extended periods of time, although transient species with both heads bound are proposed to be important intermediates for translocation. To obtain such behavior, an asymmetry must exist in the enzymatic (ATPase) and lattice-binding properties of each head. This asymmetric behavior must alternate between heads for the dimer to translocate, and therefore the two subunits of the dimer need to communicate when bound to the lattice. Both Rep and kinesin display

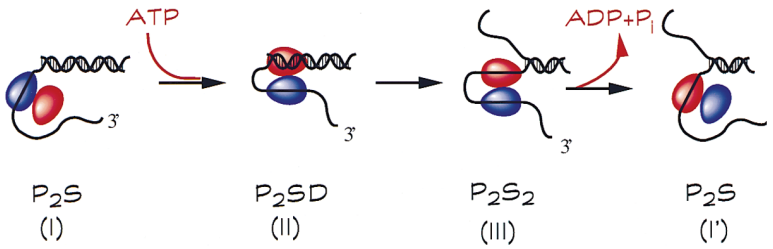

Figure 1. Active, Rolling Model for the Translocation/DNA Unwinding by the Homodimeric *E. coli* Rep Helicase

In this model (Wong and Lohman, 1992), the two subunits of the homodimeric helicase alternate binding to the duplex DNA. An alternative inch-worm model would have the same subunit remain as the leading subunit.

such "subunit switching" behavior, in which the two subunits alternate between high and low affinity binding to the lattice in a process that is regulated by ATP binding/hydrolysis.

E. coli Rep Helicase. Although *E. coli* Rep protein is monomeric (76 kDa) in the absence of DNA, binding of either single-stranded (ss) or duplex (ds) DNA induces dimerization, and the dimer is the active species in unwinding DNA (Wong and Lohman, 1992). Each subunit of the Rep dimer can bind either ss- or dsDNA competitively and ssDNA binds with a preferred orientation. However, the affinity of DNA for the second subunit is at least four orders of magnitude lower than for the first subunit. Importantly, this negative cooperativity for DNA binding is regulated allosterically by nucleotide binding (see Figure 1). In the absence of nucleotide, a Rep dimer is favored in which only one subunit is bound to ssDNA (a P_2S dimer); however, ADP binding promotes formation of a Rep dimer in which both subunits are bound to ssDNA (P_2S_2 dimer). Finally, binding of the nonhydrolyzable ATP analog, AMPP(NH)P, favors simultaneous binding of both ss- and dsDNA to the dimer, one to each subunit (a P_2SD dimer).

ATP turnover by Rep is stimulated ~10,000-fold by DNA binding and dimerization. Recent studies also provide evidence for communication between the two ATPase sites within the functional dimer. First, heterodimer formation between a wild-type Rep monomer and a mutant Rep monomer (Rep K28I), which can bind but not hydrolyze ATP, prevents ATP hydrolysis by the wild-type Rep subunit (Wong and Lohman, 1997). Second, although ATP hydrolysis does not increase the rate of ssDNA dissociation from the P_2S Rep dimer, it does stimulate (by >400-fold) the rate of dissociation of ssDNA from the P_2S_2 Rep dimer in a process that requires ATP interaction with both subunits of the dimer (Bjornson et al., 1996). Third, selective inhibition of either the DNA-bound or the detached subunit of the P_2S Rep dimer, through binding of the transition state analog, ADP-AlF_4^-, demonstrates that each subunit of the P_2S Rep dimer can hydrolyze ATP, but with quite different mechanisms (Wong and Lohman, 1997). Finally, kinetic evidence suggests that the two Rep subunits are asymmetric even when both are bound to ssDNA and that ATP hydrolysis stimulates a concerted conformational change in which the properties of the two subunits are switched, the tight site for ssDNA becoming the weak site and vice versa (Bjornson et al., 1996).

These results are consistent with an "active, rolling" or "subunit switching" mechanism for DNA translocation/unwinding proposed for the dimeric Rep helicase (see Figure 1) in which ATP hydrolysis cycles the two heads of the Rep dimer through a coordinated series of conformational states that change the relative affinities of the two subunits for ss- versus dsDNA (Wong and Lohman, 1992). In this model, duplex DNA is actively unwound upon binding one subunit of the Rep dimer to the duplex region ahead of the ss-/dsDNA junction. The description of this as a rolling model was based on the assumption that the Rep dimer would have a conserved 2-fold axis of symmetry relating its two potential DNA binding sites. However, based on recent structural information (see below) (Korolev et al., 1997), it is more likely that the symmetry of the dimer varies throughout the unwinding cycle. These data are also consistent with inch-worm models, in which each subunit alternates between tight and weak binding to the DNA, but the same (leading) subunit of the dimer interacts transiently with both duplex and ssDNA while the trailing subunit interacts only with ssDNA.

Conventional Kinesin. For conventional kinesin, motility data indicate that processive motion requires a two-headed molecule. Kinesin dimerizes through a coiled-coil domain C-terminal to the motor domain, but a monomer can be produced by truncation prior to the coiled-coil. While monomeric kinesin is capable of directed motion in assays involving many motors interacting with a single microtubule, it does not move processively as a single molecule (Berliner et al., 1995; Vale et al., 1996). Since only the dimer is processive, head–head interactions are essential for processivity.

The kinesin chemomechanical cycle involves allosteric communication between the nucleotide and microtubule binding sites. The affinity of the motor for the microtubule depends upon the nucleotide in the active site; ATP and ADP states correspond to tight and weak microtubule binding states, respectively. Moreover, binding of microtubules to kinesin accelerates the steady state ATPase rate, primarily by accelerating ADP release from the active site 1000-fold. For kinesin dimers in the absence of microtubules, the enzymatic properties of the two heads appear to be identical. However, upon binding microtubules, ATP binding and hydrolysis become asymmetric with one head possessing high affinity for nucleotide and the other possessing low affinity (Ma and Taylor, 1997). Hackney (1994) first demonstrated this enzymatic asymmetry by showing that dimeric kinesin rapidly releases ADP from only one of its two heads upon binding to microtubules. This result suggests formation of an intermediate akin to the P_2S form of the Rep dimer, in which one kinesin head is tightly bound to the microtubule and weakly bound to ADP while the second head is in the opposite state. In support of this idea, cryo-electron microscopy studies show kinesin dimers with only one head bound to the microtubule (Amos and Hirose, 1997). Subsequent release of the tightly bound ADP from the detached head is driven by ATP binding/hydrolysis by the microtubule-bound

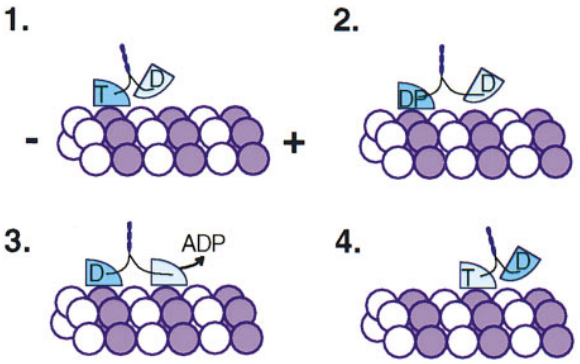

Figure 2. Hand-Over-Hand Model of Kinesin Motility

T, DP, and D represent the ATP, ADP·Pi, and ADP states of the kinesin motor domain, respectively. Binding of dimeric ADP-kinesin to microtubules leads to kinesin bound tightly to microtubules via one head. The orientation of the two heads is that observed in the dimer structure (Kozielski et al., 1997). ATP hydrolysis causes the two heads to separate and biases the binding of the detached head to the next available β-tubulin subunit (purple) toward the plus end of the microtubule (steps two and three). Completion of the hydrolysis cycle in step 3 allows binding of the forward head, which in turn triggers ADP release. This loss of ADP allows the rear head to detach and ATP to bind, returning kinesin to its original state in step 4.

head, which is coupled to strong binding of the detached head to the next available tubulin-binding site on the microtubule lattice.

Collectively, these results demonstrate that the two kinesin heads behave differently in the dimer, and that ATP binding/hydrolysis drives subunit switching between distinct microtubule binding states. These results have suggested a hand-over-hand mechanism for kinesin in which the two heads alternate in binding to the polymer (Figure 2) (Hackney, 1994; Ma and Taylor, 1997; Gilbert et al., 1998), analogous to the one proposed independently for the Rep helicase (Figure 1). At any given time, one head will have high microtubule affinity and low nucleotide affinity, and the other head will have opposite affinities. After a forward step is completed, the identities of the heads and their corresponding affinities interchange, and the cycle begins over again. Thus, the motor is tightly regulated such that the same set of sequential transitions is carried out repeatedly, and the two heads of kinesin are always in different nucleotide- and microtubule-binding states. This "exclusion principle" is important for ensuring unidirectional translocation, for if the two heads were in identical states, the asymmetry distinguishing the forward head from the back head would be lost.

Translocation (Unwinding) Mechanisms

A molecular understanding of how a motor protein translocates along its linear polymer lattice must consider the basis for directionality of movement. DNA translocation/unwinding and microtubule translocation present distinct problems in this regard. Microtubule motors display directionality in their movement along microtubules, with conventional kinesin moving toward the plus end, whereas other members of the kinesin superfamily (e.g., Ncd) move toward the minus end. Although microtubules have a polar structure with intrinsic directionality, identical binding sites exist on either side of the microtubule-bound subunit. Therefore, the kinesin dimer must bias the direction of rebinding of the forward head to achieve directional motion. A DNA helicase operating on ssDNA faces the same problem. However, DNA helicases do not generally function on ssDNA alone, but rather at a junction between ss- and dsDNA. Therefore, an intrinsic asymmetry in the DNA lattice exists on either side of the helicase (duplex DNA is ahead, whereas ssDNA is behind the helicase). Some helicases, such as Rep and UvrD, require a 3′ ssDNA flanking the duplex (3′ to 5′ helicases), whereas others require a 5′ ssDNA flanking region (5′ to 3′ helicases). Hexameric helicases appear to require both a 3′ and 5′ flanking ssDNA tail (Hacker and Johnson, 1997). An unsettled question is the extent to which the observed polarity of unwinding reflects an intrinsic property of the helicase and/or the asymmetry within the DNA lattice itself.

A molecular description of a "walking" mechanism for a motor protein also requires information about its "step size" and the coupling ratio between steps and ATP hydrolysis. Kinesin moves in 8 nm increments, consistent with the spacing between its binding sites (tubulin heterodimers) along a single protofilament. Recent studies indicate that one ATP is hydrolyzed per 8 nm step at near-zero load, making this a tightly coupled and highly efficient motor in that regime (see review in this issue by S. Block).

Although a step size has not been measured for the Rep helicase, recent measurements for the related *E. coli* UvrD helicase indicate that it unwinds duplex DNA in increments of 4–5 base pairs; i.e., a rate-limiting step is repeated during the unwinding cycle and 4–5 bp are unwound between successive rate-limiting steps. This result is independent of temperature and ATP concentration; thus, the 4–5 bp unwinding increment reflects an intrinsic property of UvrD helicase and is not consistent with a mechanism requiring thermal fraying of the duplex DNA. However, since a monomer of UvrD covers ~10 nucleotides when bound to ssDNA, consistent with the structure of a Rep monomer bound to ssDNA (Korolev et al., 1997), it is not clear whether this "step size" relates directly to the distance moved by a UvrD subunit, or possibly some sub-step size that is repeated for each physical step. While the type of movement executed is uncertain, it does not appear that translocation occurs by a sliding mechanism, whereby only one subunit maintains continuous contact with the DNA. This is demonstrated by the fact that the Rep and UvrD dimers can unwind duplex DNA in which the 3′ ssDNA flanking region contains either a stretch of non-DNA or ssDNA where the phosphodiester backbone polarity has been reversed (reviewed by Lohman and Bjornson, 1996). The fact that a covalent linkage formed between a subunit of the Rep dimer and ssDNA does not inhibit its steady state ATPase activity also rules out a tightly coupled directed sliding mechanism (Wong and Lohman, 1996).

Structural Data: Bridging the Gap between Binding Sites

Structural information on the functional dimeric motor proteins is needed to address the details of the proposed hand-over-hand mechanism. Is there structural asymmetry in the dimers that mirrors the enzymatic asymmetry of the two heads? What conformational changes accompanying ATP binding/hydrolysis and polymer lattice binding promote directional movement?

Recent x-ray crystallographic studies of the *E. coli* Rep protein in complex with ssDNA ($dT(pT)_{15}$) and ADP show that it is a two-domain protein with each domain having two subdomains (Korolev et al., 1997). ADP is bound at the base of a cleft between the two domains, and ssDNA binds across the cleft above the nucleotide-binding site. The asymmetric unit of the crystal contains two Rep monomers bound to $(dT)_{16}$; however, biochemical evidence suggests that this is not the functional dimer. Two conformations of the Rep monomer are observed within the asymmetric unit of the crystal, which differ by an $\sim$130° rotation about a hinge region connecting the one subdomain to the other three subdomains. Other studies indicate that movement about this hinge region is coupled to ssDNA binding and thus may be functionally significant. Such large movements could potentially function in translocation and indicate that part of the required flexibility for movement may be built into the Rep monomer. Furthermore, the large conformational changes observed for the monomer suggest that the structure and symmetry of the Rep dimer may change dramatically during the course of DNA unwinding and translocation. This latter point should be kept in mind when attempts are made to use static structures to understand motility.

Crystal structures have been obtained for the kinesin monomer and dimer. The quandary presented by the kinesin dimer structure (Kozielski et al., 1997) is that the distance between the microtubule-binding sites on the two heads is 55 Å, which is less than the spacing between binding sites along the microtubule protofilament ($\sim$80 Å). Thus, it seems difficult for both heads to bridge this gap without some element unfolding, since the kinesin head (unlike Rep) is a single domain and contains no obvious extensible elements. To allow the heads to separate, the coiled-coil dimerization domain known as the neck has been proposed to unfold, since it contains destabilizing amino acids in the core of the coiled-coil. However, the kinesin neck can be deleted or replaced with a more stable artificial coiled-coil (free energy of unfolding $>$20 kcal/mol) without abolishing processivity, which makes it likely that other structural changes occur to allow the kinesin dimer to span the distance between two tubulin subunits (Romberg et al., 1998).

Summary

Both kinesin and Rep face a similar problem—how to stay tightly associated with a linear polymer while still translocating rapidly and unidirectionally along it. While they have quite different structures and operate on different polymers, these enzymes appear to have evolved similar solutions to this problem. Both appear to use mechanisms in which one of the subunits of the dimer is tightly bound to the lattice while the second subunit moves forward to the next binding site. The two subunits then exchange identities and the process is repeated for further steps. The physical and biochemical evidence supporting this mechanism is convincing, but many important details remain to be deciphered. Importantly, the physical mechanism by which ATP hydrolysis is converted into forward motion and the nature of the intermediate in which the two subunits bind to the polymer and exchange identities are both poorly understood. It is likely that many other oligomeric motors operate using similar mechanisms, so understanding kinesin and Rep will hopefully provide molecular insight into many forms of intracellular motility.

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