HHS Public Access

Author manuscript

J Mol Biol. Author manuscript; available in PMC 2018 March 21.

Published in final edited form as:

J Mol Biol. 2012 July 13; 420(3): 139–140. doi:10.1016/j.jmb.2012.04.030.

Helicase Unwinding: Active or Merely Perfect?

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DNA helicases were first isolated as enzymes that utilize the energy of ATP hydrolysis to unwind double-stranded DNA (dsDNA) into two single strands.[1] All DNA helicases share several common biochemical properties. These include binding to single-stranded DNA (ssDNA) and dsDNA and nucleoside 5′-triphosphate (generally ATP) hydrolysis, coupled to polar unwinding of duplex DNA into its component single strands.[2,3] Although the outcome of the action of DNA helicases is the same (i.e., dsDNA unwinding), the manner in which they achieve this goal is quite diverse. This is dictated by the *in vivo* role of the enzyme, its oligomeric structure, and the partner proteins with which it interacts.[4,5] Several methods have been proposed to classify this diversity of helicases, both for RNA and DNA, including groupings based on structure and sequence[6] or also taking into account physical properties, such as directionality of movement.[7]

Furthermore, as many helicases translocate along ssDNA and unwind the duplex into its component strands, the mechanism used to separate strands can also be used to classify these enzymes.[8] This grouping classifies helicases into passive and active DNA unwinding nanomachines. A passive enzyme is an opportunistic one that binds to a single-strand tail and then waits for the adjacent duplex to open, primarily by thermal fluctuations.[9] Once the duplex opens, the helicase captures the flayed end, as it now moves one or a few bases along the nascent ssDNA strand, driven by ATP hydrolysis. Then, it has to wait for a subsequent base-pair opening event before further movement can occur. An enzyme using this mechanism would be expected to translocate rapidly on ssDNA but move much more slowly through dsDNA, and furthermore, its unwinding would be inhibited by increasing GC content of the duplex. In addition, ATP hydrolysis would not be directly coupled to unwinding but instead would be used to drive ssDNA translocation forward through a series of conformation changes during the ATP hydrolysis cycle. Finally, DNA unwinding by passive enzymes would be enhanced by an assisting force that destabilizes the DNA duplex: ~15 pN of force is required to separate the strands of DNA mechanically.[10]

In contrast, an active DNA helicase is one that binds to the duplex and induces strand separation, an event directly coupled to its movement along the DNA. Enzymes using this type of mechanism would translocate on ssDNA and unwind the DNA duplex at a rate comparable or identical to that of translocation on ssDNA. Furthermore, DNA unwinding would be largely insensitive to GC content and be directly coupled to nucleoside 5′-triphosphate hydrolysis. This follows because the free energy of hydrolysis of ATP is

approximately −10 kcal/mol whereas only +1.6 kcal/mol is required to open a base pair of average composition:[11] many helicases move only one base pair per ATP.[12] Consequently, for an active enzyme, the DNA unwinding rate is expected to be unaffected by an assisting force below 15 pN.

DNA helicases can in principle be fully active, can be completely passive, or can use a mechanism that is intermediate between the two. Consequently, a method was developed to distinguish the type of mechanism, based on relative rates of helicase movement.[8] The rates of translocation on ssDNA (V_{trans}) and unwinding (V_{un}), both in terms of bases per second, are measured, and the V_{trans}/V_{un} ratio is calculated. Those enzymes whose ratio approaches 1 are considered to be highly active while those with ratios <0.25 are considered to be passive. This idea of active or passive unwinding is relevant not only to the idea of efficiency of ATP usage but also to the relationship of movement to force. In the case of helicases, single-molecule techniques, based on laser trapping beads or the magnetic bead technique,[13] are enabling the interdependence of force and movement of heli-cases to be elucidated in ways not easily possible with solution assays.

The bacteriophage T4 DNA helicase, known as Dda, functions in DNA replication and recombination.[14] It is active as a monomer, although monomers have been shown to line up on DNA in a “train” fashion resulting in enhanced processivity: if the leading monomer dissociates, the next is ready to take its place.[15,16] In this issue of the *Journal of Molecular Biology*, Byrd *et al.* used a combination of pre-steady-state kinetics and single-molecule biophysics to discern the mechanism of DNA unwinding for Dda. They measured the translocation rate on ssDNA, the unwinding rate of duplex DNA, and the effects of GC content on DNA unwinding for Dda monomers. Remarkably, they have determined that V_{un} is almost identical with that of V_{trans}, resulting in a V_{trans}/V_{un} ratio of 0.96. This suggests that Dda is an active enzyme. To further investigate this, they examined the effects of increasing GC content on the DNA unwinding rate. The results show that the DNA unwinding is insensitive to the duplex GC content, producing a $V_{un}{}^{GC}/V_{un}{}^{AT}$ ratio of ~1. These findings support an active helicase mechanism for Dda. Finally, this study showed that the rate of unwinding by single molecules of Dda is independent of an assisting force in the 5- to 13-pN range, producing V_{trans}/V_{un} ratio of 0.96 for the whole range of forces studied. Taken together, this combination of single-molecule and pre-steady-state kinetics demonstrates that Dda is essentially a nearly perfect active DNA helicase.

This leads to several new questions. “What is perfection?” and why would any helicase be less than perfect? Of course, each helicase has specific roles and may need to modulate its activity depending on interactions with other proteins. Speed *versus* efficiency is a familiar question in the realm of cytoskeletal molecular motors:[17,18] the need for high speed or force may preclude high efficiency. For a helicase such as Dda, presumably the helicase activity must be coordinated with that of other proteins in the functional complex. Such coordination might include the appropriate processivity and speed that optimizes all aspects of the function(s) as the complex acts on the DNA. A passive helicase could be highly energy efficient, if the ATP hydrolysis does not occur without forward movement, but could be more prone to premature dissociation at a blockage on the DNA, such as bound protein, and thus reduce processivity. A passive helicase may also require other additional components to

separate dsDNA optimally and possibly, thereby, convert it to active unwinding. Currently, there is considerable effort to determine the properties of helicases within their full, active complexes, and from these studies, we will be able to understand how the various properties such as processivity, speed, and degree of active unwinding come together to produce coordinated processing of DNA.

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