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A General Model for Nucleic Acid Helicases and Their "Coupling" within Macromolecular Machines

Review

Peter H. von Hippel* and Emmanuelle Delagoutte
Institute of Molecular Biology and
Department of Chemistry
University of Oregon
Eugene, Oregon 97403

Introduction

In this article we present a general framework that can be used to describe the molecular mechanisms whereby ATP-driven helicases separate and rearrange the complementary strands of double helical nucleic acids. This framework also permits us to consider how these helicases might be functionally coupled to other components within the macromolecular machines that carry out physiological processes. We then proceed to define parameters that can be used to quantify helicase function and derive simple thermodynamic equations that describe helicase reactions in isolation and in coupled systems.

Aspects of the molecular mechanisms of helicase function are then developed using known systems of increasing complexity. We begin by considering simple DNA "melting proteins" that can—under some conditions—open DNA without binding or hydrolyzing ATP. We then discuss the cargo-carrying molecular motors that, like helicases, use the chemical free energy of ATP hydrolysis to translocate directionally along specific cytoplasmic "tracks" but do not, of course, "open" the lattices on which they travel. This leads us to a discussion of the helicase properties of transcription complexes, functioning in both a coupled and an uncoupled mode, and these overall approaches are integrated in terms of the well-studied helicase properties of *E. coli* transcription termination factor Rho. Finally, we apply the general model that results to the helicases of DNA replication, and outline how the mechanisms described here might be further developed and tested for a variety of systems displaying helicase activity.

Helicases: Definitions, Properties, and Parameters

Nucleic acid helicases are defined as enzymes that translocate directionally through double-stranded nucleic acid (dsNA) substrates to catalyze the separation of the complementary NA strands. Furthermore, in conjunction with other components of the macromolecular machines within which they function to facilitate various biological processes (such as DNA replication, RNA transcription, DNA recombination, DNA repair, and RNA editing and splicing), nucleic acid helicases also catalyze the transfer of single-stranded nucleic acid (ssNA) products of the dsNA opening reaction to other proteins or to different complementary ssNA partners, or release the ssNA products directly into solution. Properties of some of the well-studied helicases of *E. coli* are listed in Table 1; most of these helicase functions are clearly required in other organisms as well. Helicase reactions tend to be driven by the hydrolysis of ATP or other nucleoside triphosphates (NTPs). This process is generally catalyzed by a cryptic ATPase activity carried by the helicase protein and activated by the binding of a segment of the relevant ssNA lattice. Finally helicases, when functioning within a macromolecular machine that drives one of the above cellular processes, tend to be highly processive, efficient, specific for the type (DNA or RNA) of ssNA substrate (lattice) to which they bind, and directional (5′→3′ or 3′→5′) in their movements along the target ssNA lattice (for a general review of helicase mechanisms see Lohman and Bjornson, 1996).

The *processivity* of a helicase at a given lattice (e.g., template) position is defined as the probability that the helicase at that position will continue to translocate forward by one step along the NA substrate, divided by the probability that the helicase will dissociate from the substrate lattice at that position. The processivity of a helicase is often regulated by additional protein components or "coupling" factors, which may interact with the helicase either directly, or indirectly via the nucleic acid components of the system (see Table 1 and below). Such processivity coupling factors can be operationally defined as components that interact functionally with the helicase to "trap" intermediate ssNA reaction products of the dsNA opening reaction and facilitate their subsequent use (e.g., as an ssNA template) by the macromolecular machine within which the helicase operates (see Table 1). In the absence of such factors, the processivity of an isolated helicase may be low.

The *efficiency* of a helicase in ssNA translocation or dsNA unwinding can be defined, in terms of the helicase step-size, either as the number of nucleotide residues (nt) traversed by the helicase on an ssNA lattice or as the number of base pairs (bp) separated per ATP molecule hydrolyzed. An ATP hydrolysis event that results in helicase advance by less than a full translocation or unwinding step size may reflect weak binding of the helicase to (or poor "articulation" of the helicase with) the NA lattice, resulting in helicase "slippage." An apparent decrease in efficiency in these terms could also reflect the nonproductive binding of the helicase to the product ssNA, resulting in "abortive" ATP hydrolysis. Coupling factors may serve to reduce such slippage and nonproductive binding, and thereby improve the apparent efficiency of the helicase. We note that the efficiency of a given helicase may (but need not, see below) correspond to the size (in nt) of the single ssNA binding site of the helicase subunit.

The *specificity* of a given helicase for its dsNA substrate is indicated by designating these enzymes as DNA-DNA, RNA-DNA, or RNA-RNA helicases to define the dsNA species that is opened, with the ssNA strand to which the helicase specifically binds and along which it translocates being underlined for helicases that unwind heterologous dsNA (e.g., the transcription termination RNA-DNA helicase Rho of *E. coli*.)

Most well-studied proteins that interact with ssNA

*To whom correspondence should be addressed (e-mail: petevh@molbio.uoregon.edu).

Table 1. *E. coli* Helicases Involved in Various Biological Processes

Helicase	Function	Specificity	Directionality	Association State	"Coupling" Factors[b]
DnaB	Replication	DNA-DNA	5′→3′	Hexamer (6 Identical subunits)[a]	DNA polymerase III ssDNA binding protein (primase)
RNA polymerase (core)	Transcription (elongation)	DNA-DNA	5′→3′	Tetramer (4 different subunits)	Next required NTP (see Section 5)
Rho	Transcription (termination)	RNA-DNA	5′→3′	Hexamer (6 identical subunits)[a]	(NusG)
RecBCD	Recombination	DNA-DNA	3′→5′	Trimer (3 different subunits)	(RecA) (ssDNA binding protein)
UvrD	DNA Repair	DNA-DNA DNA-RNA	3′→5′	Not fully defined	DNA polymerase I ssDNA binding protein

[a] Subunits identical in sequence, though not necessarily in conformation.
[b] Here we include both factors that alter the kinetics of the helicase reaction by direct "trapping" mechanisms and (in parentheses) factors that facilitate helicase function (e.g., loading) in other ways (see text).

bind with a defined orientation relative to the polar ssNA backbone (McGhee and von Hippel, 1974). Thus, the *directionality* of a given helicase is generally defined in terms of the polarity of the ssNA segment or "tail" that is required to "load" most helicases at the single-stranded-double-stranded NA (ss-dsNA) junction at which the unwinding process that is catalyzed by the helicase is initiated. For example, an RNA-DNA helicase such as Rho, which functions optimally from an ssRNA tail located at the 5′ end of the RNA component of a ds(RNA-DNA) hybrid, is defined as a 5′→3′ helicase to describe the direction in which the helicase moves along its specific ssRNA lattice from the helicase loading site.

In terms of structural and biochemical properties, many (though not all) of the better characterized DNA-DNA replication helicases operate as hexamers of identical subunits, often arranged in six-membered rings with pseudo-C3 structural (and functional) symmetry (reviewed by Patel and Picha, 2000). This symmetry is defined, in part, by enzymatic and physical biochemical studies that show that many of these hexameric helicases actually work as trimers of functionally asymmetric dimers (Dong et al., 1995; Hingorani and Patel, 1996; Yu et al., 1996b). This structural arrangement also applies to the RNA-DNA helicase Rho (Geiselmann et al., 1992).

Helicase Assays and Coupling

A helicase operating in isolation may be difficult to assay, since the ssNA intermediates of the reaction are transient and the final dsNA product of the overall reaction is unaltered from the initial substrate (Figure 1A). However, any protein or enzyme that traps or processes these ssNA intermediates and thus prevents the reannealing of the unwound strands can, in principle, work as a helicase "coupling" factor and its binding or activity can be used to monitor helicase action. For example, the ability of the Rep helicase of *E. coli* to sensitize dsDNA to single-strand specific nucleases in the presence of ATP allowed early workers to attribute a dsNA unwinding activity to this protein (Takahashi et al., 1979), and changes in intrinsic protein fluorescence that accompany the binding of an ssDNA binding protein to ssNA intermediates were monitored to track the unwinding activity of RecBCD in real time (Roman and Kowalczykowski, 1989).

Gel assays involving the release of one of a pair of complementary ssNA oligomers that differ in size, annealed to the ends of a longer linear ssNA oligonucleotide that also contains a central ssNA region that can serve as a helicase loading site, have been used to define the polarity and specificity (and sometimes to follow the kinetics) of various helicases (e.g., see Venkatesan et al., 1982; Matson and Richardson, 1983; Bianco and Kowalczykowski, 2000). In addition, helicases (e.g., gp41) have been assayed indirectly by monitoring the rate of consumption of ATP as a function of lattice length during translocation of the helicase along ssDNA (Liu and Alberts, 1981; Young et al., 1994), although this approach only permits indirect inferences about what is happening to the protein and nucleic acid components in the course of the reaction because the efficiency of the isolated helicase may be low or variable. However, when combined with kinetic unwinding assays, experiments of this type can be used to measure helicase efficiency (Dillingham et al., 2000).

As indicated above, most helicases function poorly (inefficiently and with reduced processivity) when separated from the macromolecular machinery and coupling factors with which they are intended to operate. This is because the function of most helicases within such assemblies is not merely to catalyze the opening of a dsNA segment, but also to drive rearrangements in which one or both of the ssNA products end up bound to another macromolecular component. An example might be the ultimate transfer of both initial template strands to newly synthesized strands in semiconservative DNA replication. Intermediates in such rearrangement processes are often protein-ssNA complexes—e.g., DNA polymerases or single-stranded DNA binding proteins bound to transiently single-stranded DNA sequences at the replication fork.

Often the inclusion of loading (Barry and Alberts, 1994; Morrical et al., 1994) or trapping factors within the reaction assay mixture can improve helicase activity. A loading factor facilitates *initiation* of the helicase reaction, while a trapping component (e.g., ssNA binding protein; see Figure 1B) facilitates *elongation* by stabilizing ssNA intermediates in the reaction as they are formed. However, such components may also alter the molecular mechanisms of the helicase from those displayed

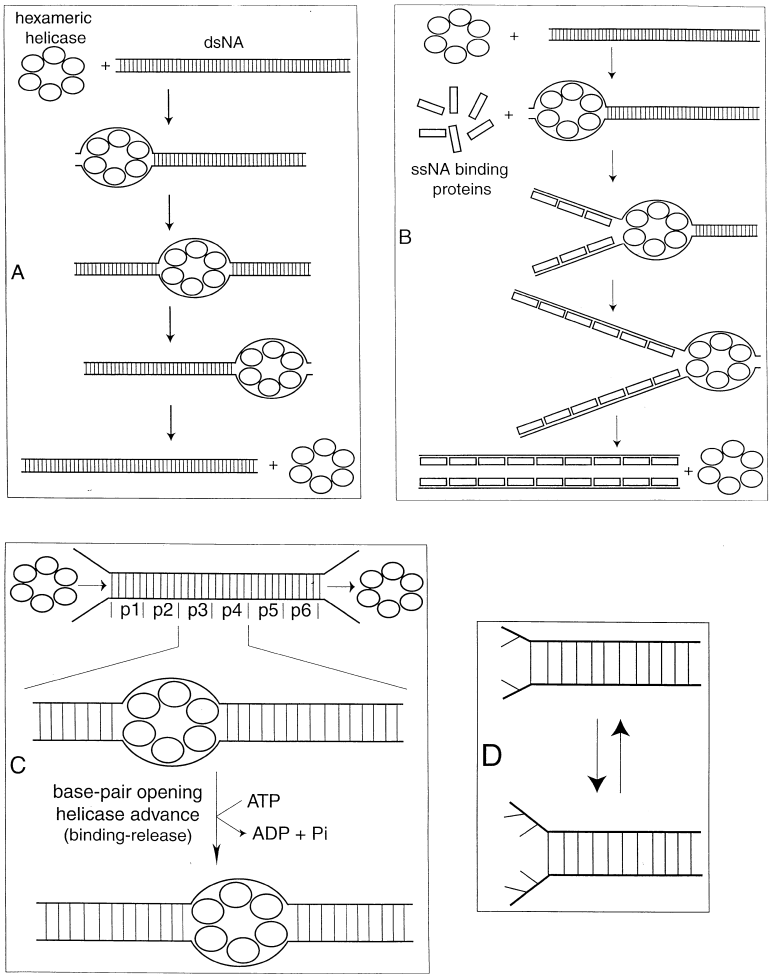

Figure 1. Uncoupled and Coupled Overall Helicase Reactions, the Single-Step Helicase Reaction Cycle, and the Base Pair Opening Process

(A) The advance of a typical hexameric helicase through a dsNA segment in an uncoupled reaction.

(B) The advance of a hexameric helicase through a dsNA segment in a reaction coupled to the binding of single-stranded NA binding protein.

(C) Depiction of the "single-step" helicase reaction cycle resulting from the binding and hydrolysis of a single molecule of ATP. In this example the helicase unwinds this 30 bp duplex in six single-step cycles, therefore with an efficiency equal to 5 nt. The positions reached at the end of each cycle are numbered p1 to p6.

(D) The thermally driven sequential (one bp at a time) NA unpairing ("fraying") process that is required for helicase advance.

in isolation, or when the helicase is properly coupled within the relevant macromolecular machine. One of the challenges of studying helicases within the context of a macromolecular machine is to understand not only the molecular mechanism(s) of the helicase itself, but also how these mechanisms are altered (coupled) when the helicase functions within the complete biological process.

The Helicase Reaction Cycle

To examine the basic principles of helicase function in the uncoupled and in various coupled states, we reduce the overall helicase reaction cycles of Figures 1A and 1B to the smaller intermediate "single-step" reactions that make up the overall process. We call such intermediate reactions single-step cycles (even though they do, in fact, consist of multiple substeps; see below), because these intermediate cycles can be defined in terms of the efficiency of the helicase—i.e., the distance (*k* bp) advanced by the helicase into the dsNA substrate as a consequence of consuming a single ATP molecule. This process, which defines the ATPase cycle of the helicase, represents a single-step (Figure 1C) in the "elongation phase" of the helicase reaction. We note that this efficiency parameter (which can also be defined in terms of an apparent helicase step-size) may vary, depending on experimental conditions and on whether the helicase is operating in isolation (Figure 1A), is coupled to a trapping component (Figure 1B), or is functioning within a complete macromolecular machine. A systematic study as a function of reaction and coupling conditions is often required to define the dependence of the apparent efficiency on these factors.

The single-step reaction cycle that we define in Figure 1C includes not only the ATP binding and hydrolysis cycle, but also two more elementary processes. These are: (i) the sequential opening of the individual base pairs located at the moving ss-dsNA junction (Figure 1D); and (ii) the advance of the helicase along the template (Figure 1C), which must involve the (at least partial) release of the helicase from the ssNA lattice, followed by rebinding to a newly exposed downstream ssNA segment.

The Thermodynamics of the Single-Step Helicase Cycle

The Uncoupled Helicase Reaction

The intermediate helicase reaction cycle schematized in Figure 1C represents the single-step helicase-catalyzed opening of *k* bp as a consequence of the binding and hydrolysis of one ATP molecule. For a helicase moving

through a dsNA segment in isolation (i.e., in an uncoupled reaction—Figure 1A) this single-step reaction can be written:

$$\{(\text{dsNA})_{k\ \text{bp}}\}_1 + \{(\text{ssNA})_{2k\ \text{nt}}\}_1 + \text{ATP} \Leftrightarrow \{(\text{dsNA})_{k\ \text{bp}}\}_2 + \{(\text{ssNA})_{2k\ \text{nt}}\}_2 + \text{ADP} + \text{P}_\text{i} \quad (1)$$

where $\{(\text{dsNA})_{k\ \text{bp}}\}_1$ represents the downstream k bp of dsNA that are closed at the beginning of the single-step reaction and $\{(\text{dsNA})_{k\ \text{bp}}\}_2$ represents the k bp of upstream dsNA that are closed at the end of the reaction. Similarly, $\{(\text{ssNA})_{2k\ \text{nt}}\}_1$ represents the $2k$ nt of upstream ssNA that are open at the beginning of the reaction (and presumably, at least in part, complexed with helicase at that point), and $\{(\text{ssNA})_{2k\ \text{nt}}\}_2$ represents the $2k$ nt of downstream ssNA that are open (and complexed) at the end.

If we assume that the dsNA regions open at the beginning and the end of this single-step intermediate reaction, as well as the helicase-ssNA interactions at these same points, are approximately thermodynamically and structurally equivalent, then the terms on both sides of Equation 1 that represent NA-NA and protein-NA interactions will largely cancel and the equation can be significantly simplified. The (forward) equilibrium constant for this simplified and uncoupled single-step helicase reaction can then be written:

$$K_f \approx [\text{ATP}] / \{[\text{ADP}] \times [\text{P}_\text{i}]\} \quad (2)$$

This formulation, while not entirely correct (as indicated by the ≈ symbol), emphasizes that the overall single-step reaction of the helicase extension reaction is manifested chemically primarily by the hydrolysis of ATP and that, in effect, the helicase acts simply as a catalyst of ATP hydrolysis (and transient dsNA opening) within the overall reaction. We note that writing the reaction this way conceals the fact that this overall intermediate single-step reaction actually comprises a thermodynamic cycle containing a number of substeps, including dsNA opening and closing reactions, protein-NA binding and release reactions, etc., that involve different NA segments. Therefore, the various NA–NA and protein–NA interactions that participate may not entirely cancel across Equation 1, and the overall free energy change for the single-step ATP hydrolysis reaction of Equation 1 may be written:

$$\Delta G^\circ_{\text{overall}} = -RT \ln \{[\text{ATP}] / [\text{ADP}] \times [\text{P}_\text{i}]\} + \Delta G_{\text{other}} \quad (3)$$

where ΔG_{other} represents the algebraic sum of these additional (small) free energy differences across the reaction.

The Coupled Helicase Reaction

An equivalent intermediate (elongation) reaction step within a coupled (for example, to the binding of an ssNA binding protein) overall helicase reaction (Figure 1B) corresponds to a single-step process that may be thermodynamically uphill, downhill, or neutral, depending primarily on the concentration of the ssNA binding protein that both "couples" and drives the overall process in collaboration with the steps that are "fueled" by the binding and hydrolysis of ATP by the helicase. Using the terminology of Equation 1, the single-step elongation reaction of a coupled system can then be written:

$$\{(\text{dsNA})_{k\ \text{bp}}\}_1 + \{(\text{ssNA})_{2k\ \text{nt}}\}_1 + 2\text{P}_{\text{ssNA}} + \text{ATP} \Leftrightarrow \{(\text{ssNA})_{2k\ \text{nt}}\}_2 + 2[\text{P}_{\text{ssNA}}\text{-}\{(\text{ssNA})_{k\ \text{nt}}\}] + \text{ADP} + \text{P}_\text{i} \quad (4)$$

where 2P_{ssNA} represents the two molecules of free ssNA binding protein that bind to the two ssNA segments (k nt long) that result from the opening of the $\{(\text{dsNA})_{k\ \text{bp}}\}_1$ segment to form two $[\text{P}_{\text{ssNA}}\text{-}\{(\text{ssNA})_{k\ \text{nt}}\}]$ complexes (each k nt long), and $\{(\text{ssNA})_{2k\ \text{nt}}\}_1$ and $\{(\text{ssNA})_{2k\ \text{nt}}\}_2$ represent the helicase-bound ssNA intermediates in the overall reaction as defined for Equation 1 (see Endnote 1).

By analogy to the treatment above, Equation 3 can also be simplified by canceling the terms that are essentially equal at the beginning and at the end of the intermediate reaction. The equilibrium constant for the net forward reaction can then be written:

$$K_f = \{[\{(\text{dsNA})_{k\ \text{bp}}\}_1] \times [\text{P}_{\text{ssNA}}]^2 \times [\text{ATP}]\} / \{[\text{P}_{\text{ssNA}} - \{(\text{ssNA})_{k\ \text{nt}}\}]^2 \times [\text{ADP}] \times [\text{P}_\text{i}]\} \quad (5)$$

where Equation 5 also omits the terms of Equation 4 that apply to the transient interactions of the helicase with the NA framework and that largely cancel out across the reaction, but does include the terms that apply to the additional ssNA segments and the ssNA binding proteins that are free at the beginning of this single-step elongation reaction and bound at the end. We note that the terms containing the P_{ssNA} component are raised here to the second power because this ssNA trapping protein binds to *both* of the ssNA strands that result from opening of a single dsNA segment. Other trapping proteins (e.g., a DNA polymerase) may bind to only one of the product ssNA strands.

Coupling ATP Binding and Hydrolysis to Base Pair Opening and Helicase Advance

As indicated above, the reactions represented by Equations 1 and 3 can both be expanded as thermodynamic cycles that explicitly include the sequential intermediate steps of base pair opening and the associated processes of binding and release of the ssNA binding sites of the helicase. An important mechanistic question is where (within the single-step thermodynamic elongation cycle) ATP binding and hydrolysis occur relative to these base pair opening, helicase binding, and helicase release processes.

It is useful to consider that the base pair opening process (in terms of net free energy change over the single-step reaction cycle) need not be directly driven by the helicase advance process that follows within Equations 1 or 3. That is, the binding and hydrolysis of ATP by the helicase may not be directly involved in destabilizing the initial dsNA segment. This issue is discussed extensively later in this review. However, it should be made clear here that even if the thermodynamic cost of the opening of k bp is paid initially by thermal fluctuations, rather than directly by processes induced in the helicase by ATP binding and hydrolysis, this thermodynamic cost must also be "returned" at some point within the single-step helicase reaction (probably as a consequence of the formation within the reaction cycle of new and equally or more favorable protein–NA or NA–NA interactions) to yield a *net* free energy change for the single-step elongation process that is close to zero (except for the substep of ATP hydrolysis).

Thus for an uncoupled helicase reaction, since the

same number of bp must open in front of the helicase as close behind, such intermediate helicase-ssNA binding interactions will *transiently* offset the cost of opening a step-size equivalent of dsNA. In contrast, for a coupled helicase reaction into which we incorporate a "permanent" trapping component (i.e., an ssNA binding protein, see Figure 1B), the unfavorable free energy of opening the dsNA is *permanently* offset (within the context of the overall coupled single-step reaction) by the favorable binding free energy of the ssNA binding proteins to the newly exposed ssNA binding sites. (This binding, of course, accounts for the dependence of the overall coupled reaction on the concentration of the ssNA binding protein.)

If the free energy of ATP hydrolysis is not used to open the dsNA, what is it used for? In both of the two cases described above, in order for the overall helicase reaction to be able to continue into the next elongation cycle, the helicase protein must be released (at least in part) from the ssNA lattice to permit the cycle to begin again. By considering the various ligation states that the protein passes through during a single-step reaction (unliganded, NTP-, and NDP-bound), and the differences in the affinities of these liganded states of the helicase for ssNA, it can be seen that the free energy of hydrolysis of ATP may well be used to drive the release step of the helicase reaction, since this step in the cycle is likely to be that which is thermodynamically "uphill" (unfavorable).

An Upper Limit for the Helicase Unwinding Efficiency

Regardless of whether the free energy of ATP hydrolysis is used to drive the initial dsNA opening process or to drive the subsequent translocation of the helicase across *k* nt of the lattice by releasing a bound helicase subunit, the free energy of hydrolysis of ATP will set an upper limit to the total number of bp (*k*) that can be opened in the single-step reaction cycle. This upper limit is likely to be six to eight bp since, under physiological conditions, the free energy of hydrolysis of ATP is ~ -10 kcal/mol (see Barrante, 1977 and references therein), while it costs only $\sim +1.6$ kcal/mol to open a single base pair of average composition (Daune, 1999). We note that *k* will likely vary (downward) from this range of values for different helicases and for different reaction and coupling conditions as a consequence of reduced helicase efficiency and slippage.

Opening of dsNA Segments by Simple ssNA Binding Ligands and Proteins

The conventional wisdom in thinking about helicase mechanisms has generally held that since the opening (unpairing) of the dsNA lattice is thermodynamically unfavorable at temperatures below the helix-coil transition temperature (T_m), the chemical free energy released by ATP hydrolysis in the helicase reaction must be used to drive this dsNA unpairing process. In an overall sense this is certainly true, but an alternative view states that, if the free energy of activation barrier to unpairing of the dsNA can be overcome by thermal fluctuations (see below), the free energy of interaction between the two strands of the dsNA target of the helicase can be thermodynamically offset by replacing this favorable interaction with the equally or more favorable binding of the helicase subunits to the ssNA products of the helicase reaction. If this is indeed the case, we should be able to model the initial opening and ssNA binding reaction steps of the helicase by examining the interaction of dsNA with ssNA binding proteins and binding protein models that do not hydrolyze ATP.

Consider the opening of a dsNA segment by an ssNA binding protein at an experimental temperature below the unperturbed melting temperature ($T_{m,0}$) of the segment at issue. This dsNA melting process, driven by preferential binding of proteins to the ssNA products of the melting reaction, does not involve ATP and can therefore be used to model an ATP-independent (and, of course, intrinsically nondirectional) helicase reaction. Proteins that bind ssNA preferentially will, of course, lower the melting temperature of dsNA segments with which they are at equilibrium. The binding of the protein to the ssNA (in competition with dsNA formation) provides the free energy to drive the dsNA opening reaction. (In effect, this reaction can be written as an uncatalyzed version of Equations 4 and 5 by removing ATP and its reaction products, as well as the helicase, from the equations.) This process, again assuming that it can proceed to equilibrium, will then result in the melting of the dsNA and the coating of the ssNA products with the ssNA binding protein. For this reason ssNA binding proteins are often called dsDNA (or dsRNA) "melting" proteins (Jensen et al., 1976; Kowalczykowski et al., 1981).

Double-Helix Destabilization by dsNA Melting Proteins

For this *equilibrium* process; i.e., the opening of a dsNA segment *n* bp in length at an ss-dsNA junction adjacent to an ssNA segment coated with ssNA binding protein, where *n* is here the binding site size (in nt) of the ssNA binding protein (McGhee and von Hippel, 1974), we can write:

$$dsNA_{n\ bp} + 2P_{ssNA} \Leftrightarrow 2\{P_{ssNA} - ssNA_{n\ nt}\} \qquad (6)$$

where P_{ssNA} represents the ssNA binding protein. The extent to which this reaction proceeds at a given temperature will depend on the stability (as measured by the $T_{m,0}$) of the dsNA segment involved, the binding affinity of the ssNA binding protein for the ssNA exposed by the reaction, and the concentration of free P_{ssNA}. (The thermodynamics that describe reactions of this type have been written out elsewhere [von Hippel et al., 1982].)

Formaldehyde Melts dsDNA to Equilibrium

It is useful to consider two types of "melting proteins" as helicase models here. The first is formaldehyde (HCHO), which can be considered as a paradigm for a dsNA melting protein since it binds preferentially to the amino and imino groups of the DNA or RNA bases that are otherwise involved in interstrand hydrogen bonds within the base-paired dsNA structure (McGhee and von Hippel, 1977). This reaction is competitive with complementary interstrand base pair formation because the transition state for the HCHO reaction with dsNA requires that the NA residues that interact with HCHO be neither base-paired nor stacked. The binding site size of formaldehyde is one nucleotide residue ($n = 1$ nt). This (reversible)

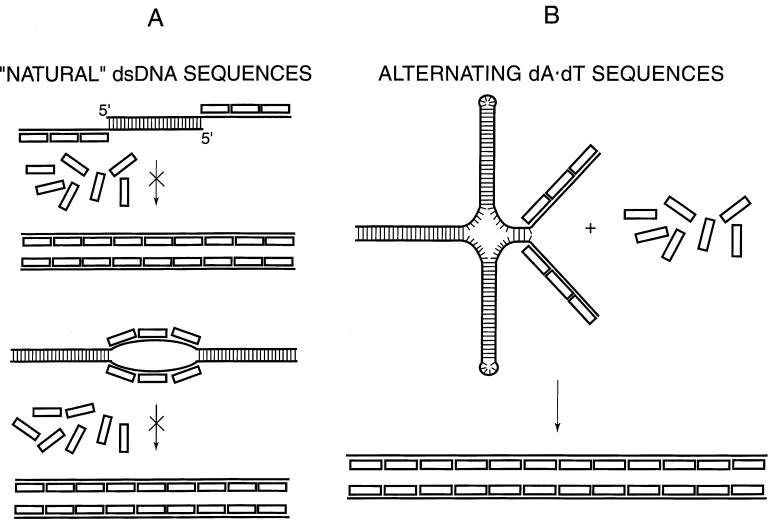

Figure 2. Melting Activity of an Excess of Single-Stranded DNA Binding Protein on dsDNAs of Varying Sequence

(A) "Natural" dsDNA sequences are kinetically blocked from binding single-stranded binding protein (here T4-coded gp32 with a binding site size [*n*] of 7 nt), even when free ssDNA ends (above) or internal (dA·dT-rich) internal loops (below) are precoated with the protein to nucleate the melting process under protein concentration and temperature conditions at which the melting process is thermodynamically favored (J. Newport and M. Young, unpublished experiments).

(B) In contrast, dsDNA consisting of alternating dA·dT sequences *does* melt to equilibrium under these conditions, presumably because this DNA can form transiently palindromic looped-out structures that expose ssDNA binding sites that are long enough to be trapped by gp32 at temperatures close to T_m (see text).

process of formaldehyde monoadduct formation with initially dsNA in the presence of a constant concentration of free HCHO proceeds to equilibrium as described by Equation 5, with the extent to which the $T_{m,0}$ of the dsNA is lowered (ΔT_m) depending on the HCHO concentration.

DNA Melting by T4-Coded Gene 32 Protein Is Kinetically Blocked

Gp32 is the single-stranded DNA binding protein of bacteriophage T4, and has a binding site size on ssNA of seven nucleotide residues ($n = 7$ nt). In contrast to dsNA melting reactions driven by formaldehyde, extensive experiments with gp32 have shown that this protein is *kinetically blocked* from lowering the melting temperature of dsDNA (i.e., the reaction is prevented, by kinetic factors, from reaching binding equilibrium), even at gp32 concentrations that are well in excess of those required thermodynamically to drive the melting process of Equation 6 to completion (Alberts and Frey, 1970; Jensen et al., 1976). Experiments demonstrating this are illustrated in Figure 2A.

What Is the Difference between Reactions in which the T_m-Lowering Component Is Formaldehyde and Those in which It Is gp32?

Clearly the main difference between these two reactions lies in the value of *n* (the binding site size of the ssNA binding ligand), which is 1 nt for HCHO, and 7 nt for gp32. The dsNA opening reaction that provides access to the ssNA "targets" of these destabilizing ligands is largely thermal melting from the ends of the dsDNA (or, for circular dsDNA molecules, preferential interior melting in regions rich in dA·dT bp). It has been estimated that a single bp at the end of a dsDNA segment (Gueron and Leroy, 1995) and at a ss-dsDNA junction in a replication fork (Chen et al., 1992) fluctuates (or "frays") into the open state with a characteristic time of $<<1$ ms at 37°C, and 1–5 ms at 15°C, respectively. This provides formaldehyde (which has a binding site size of one nt) with rapid access to its ssNA target, and since the thermodynamic cost of opening a single base pair at temperatures significantly below $T_{m,0}$ is only $\sim$1.5 times the thermal energy (kT) at room temperature, this ligand drives the melting of dsDNA to equilibrium rapidly in a series of *sequential* single base pair opening steps (see Endnote 2).

The activation barrier for single bp opening to provide access for gp32 to its ssNA targets is, of course, the same. But the binding of a single gp32 molecule appears to require the *simultaneous* opening of up to *seven* bp at the end of a dsDNA hybrid. Since the equilibrium cost for the simultaneous thermally driven "fraying open" of a segment of 7 bp would be $\sim$10 kT, such a fraying reaction would be expected to occur with a characteristic time of many hours, which means virtually never in practical terms.

Gp32 Can Melt Particular dsDNA Sequences

In contrast to natural dsDNA, a double-stranded polynucleotide consisting of complementary repeating dA·dT units *can* be melted to equilibrium by gp32 (Jensen et al., 1976). The likely reason for this is that this particular dsDNA can, at temperatures near its T_m, form alternative (largely base paired) palindromic structures of the sort shown in Figure 2B, which have a much larger probability of exposing ssDNA sequences that are 7 nt in length than does dsDNA of normal sequence. This conjecture may explain why only this form of dsDNA can be rapidly melted to equilibrium in the presence of excess gp32. We note that this kinetic blocking of dsDNA melting by native and unmodified ssNA melting proteins, which applies to *E. coli* ssb protein as well (Williams et al., 1983), may be crucial in preventing these single-stranded binding proteins from acting as "rogue" helicases in vivo (see Endnote 3).

The take-home lesson from our examination of this form of "helicase" activity in the *absence* of a source of chemical free energy from ATP hydrolysis is that such a spontaneous melting reaction can proceed at a reasonable pace if the activation free energy barrier to the opening reaction is not too high to be overcome by thermal fluctuations—i.e., is compatible with the simultaneous opening of at most two base pairs. This can occur for natural DNA if the reaction can proceed in single bp steps that can be sequentially trapped by the ssNA binding ligand, as illustrated in Figure 3.

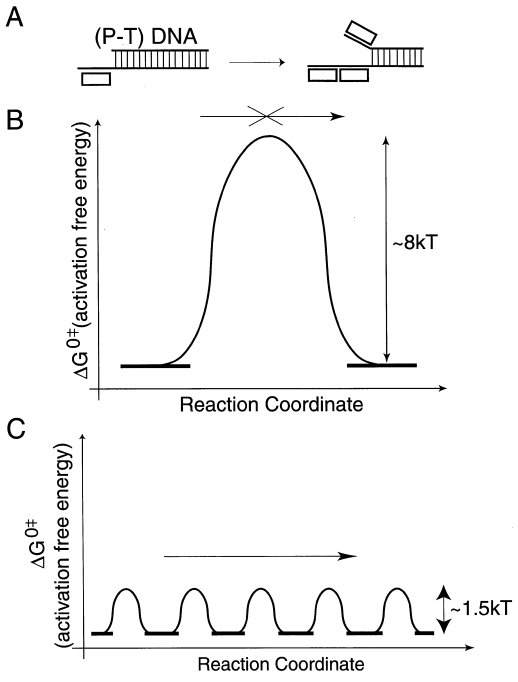

Figure 3. Spontaneous Melting of dsNA Segments Can Occur in the Presence of Melting Proteins if Thermally Unpaired Nucleotide Residues Can Be Trapped Sequentially

(A) Equilibrium unpairing at a primer-template (P-T) DNA junction in the presence of excess DNA melting protein (here a hypothetical single-stranded DNA binding protein with $n = 5$ nt).

(B) This opening and binding reaction is kinetically blocked ($\Delta G^{o\ddagger} = \sim 8$kT) if the entire 5 bp segment must open simultaneously.

(C) In contrast, the reaction can proceed to equilibrium if single bp opening events ($\Delta G^{o\ddagger} = \sim 1.5$kT) at the P-T junction can be trapped sequentially. For simplicity of representation the binding of the protein to each newly opened bp is represented here as thermodynamically "neutral" (i.e., the unfavorable free energy of opening is exactly offset by the favorable free energy of sequential [partial] binding of the newly exposed ssDNA nt by the "flexible" ssDNA binding protein).

Motor Proteins Use the Free Energy of ATP Binding and Hydrolysis to Drive Directional Translocation along Cytoplasmic Fibers of Defined Polarity

If the relevant free energy of activation barriers can be overcome, the above reasoning shows that the *net* dsNA opening steps involved in the first stages of the single-step helicase reaction may well be thermodynamically favorable and therefore not require the participation of ATP. If this is so we ask again, what is the free energy of ATP binding and hydrolysis used for? In what follows we will argue that ATP hydrolysis is needed to complete the single-step helicase reaction cycle by driving the *release* of the "lagging" helicase subunit from the ssNA lattice within an oligomeric helicase. This substep then frees this (or another) helicase subunit to participate in the (thermodynamically) favorable initiation of the next single-step reaction cycle of helicase advance. This notion can be more clearly illustrated by considering the ATP-driven reaction cycle for the directional translocation of cellular motor proteins along cytoplasmic fibers.

Structures and Models of Kinesins

Cytoplasmic molecular motors are cargo-carrying proteins that move directionally through the cell by processive polar translocation along specific cytoplasmic fibers composed of actin or tubulin. This directional translocation is driven by sequential cycles of ATP binding and hydrolysis. As a consequence these motor proteins provide useful models for the directional ATP-driven translocation of helicases along ssNA lattices. The molecular structures of several cytoplasmic motors have been solved, and it appears that these proteins generally function as dimers with two lattice binding domains (one per subunit) that bind alternately to the fiber track as the motor dimer advances. More specifically, the movement of dimeric kinesin motors along tubulin fibers has been extensively studied, and the generally accepted mechanism for this process is defined in terms of a "tight-rope walking" model (Hackney, 1994). In this model one "foot" of the kinesin dimer is always bound to the tubulin track to ensure processive translocation. These kinesin domains bind at specific sites on the tubulin fiber, and the "step size" along the tubulin filament that is associated with the consumption of one molecule of ATP under a variety of conditions is ~8 nm. The reaction is tightly coupled (Howard et al., 1989; Block et al., 1990), and involves no slippage of the kinesin on the tubulin track. Studies with kinesins also provide experimental support for the assumption that a kinesin molecule containing only a single subunit (and thus only a single tubulin binding domain) cannot translocate along tubulin fibers in a tightly coupled and processive manner. A kinesin mutant that is unable to dimerize has been tested, and while its movement is still somewhat directionally biased, the overall translocation process is now dominated by slippage and no longer displays a discrete step size (Young et al., 1998).

Reaction Cycles for Motor Proteins

It is useful to consider the thermodynamics and kinetics of a single-step reaction cycle (one that consumes one molecule of ATP) for a dimeric "walking" motor protein such as kinesin, in order to compare it with the equivalent reaction cycle (shown in Figure 1C) of a typical helicase. Such a reaction cycle for a kinesin-like motor protein is shown schematically in Figure 4. Here the binding of the individual "foot" domains of the kinesin dimer to the tubulin track is thermodynamically favorable, with the reaction state in which both kinesin feet are bound to the lattice being substantially more stable than that with only one foot bound. In this scheme the ATP-liganded state of the kinesin binds the tubulin track more tightly than does the ADP-liganded state (Rosenfeld et al., 1996). The unfavorable step that requires an input of free energy must then be the release and repositioning of the lagging foot domain to provide directionality and processivity for the walking of the kinesin dimer along the tubulin track. We therefore argue that it is this substep (lagging foot release and repositioning) in the single-step reaction cycle for kinesin that requires the input of free energy from ATP hydrolysis (see Gilbert et al., 1998).

This situation is slightly different for the movement of myosin along an actin track, where it has been proposed that the free energy of ATP hydrolysis is used to switch

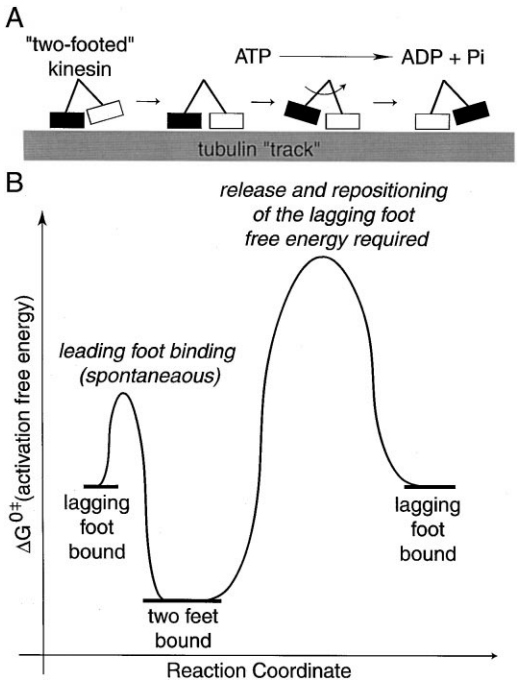

Figure 4. A Dimeric Motor Protein Moving along Its "Track" without Slippage

(A) This schematic represents the "single-step" advance (and ATP binding and hydrolysis) steps in the walking of a dimeric (two-footed) kinesin molecule along a tubulin track.

(B) The free energy of the different reaction states and a postulated free energy of activation profile for the binding of the leading foot and the release and repositioning of the lagging foot is shown. The reaction state in which both feet of the kinesin motor are bound is thermodynamically more stable than the singly bound state, indicating that the release of the lagging foot is likely to be the energy-requiring step in the overall single-step advance reaction.

the myosin into a conformation (the ADP-bound state) that binds the actin filament more tightly than does myosin in the ATP-bound state. Here the free energy derived from the ADP-ATP exchange reaction is used for the release and repositioning of the lagging "foot" of the myosin motor (reviewed by Howard, 1997).

In both systems the free energy derived from ATP hydrolysis is necessary to switch the protein back into a conformation (or state) that has a high affinity (myosin) or a low affinity (kinesin) for its track. Reciprocally, the free energy derived from the ADP-ATP exchange reaction is used to switch the protein into a conformation (or state) that has a low (myosin) or high (kinesin) affinity for the lattice along which it translocates. Thus, depending on the details of the conformational interconversion for a particular motor protein, the ATP binding and hydrolysis steps can drive either release or binding in the single-step reaction cycle (see next section).

Direct Evidence that ATP Binding and Hydrolysis in Macromolecular Machines Can Drive Conformational Changes that Result in Component Binding and Release

It is an attractive and well-documented notion that ATP binding and hydrolysis drive conformational changes in coupled protein components of macromolecular machines. For example, crystal structures of both PcrA helicase (Dillingham et al., 1999; Velankar et al., 1999) and a truncated form of the bacteriophage T7 DNA helicase called T7E protein (Sawaya et al., 1999), have revealed conformational changes associated with NTP binding and hydrolysis. Furthermore, it has been shown that the T7 DNA helicase (Hingorani and Patel, 1993) binds DNA optimally only in the presence of a nonhydrolyzable analog of dTTP, suggesting that dTTP binding and hydrolysis lead, respectively, to ssDNA binding and release. Crystal structure analysis has shown that a region of this helicase, involving residues implicated in DNA binding, becomes folded upon dTTP binding, consistent with the notion that it might undergo cycles of folding and unfolding in response to cycles of NTP binding and hydrolysis (Sawaya et al., 1999).

Another well studied example is the hexagonal rho helicase, for which ATP hydrolysis results in the release of short RNA oligomers bound to the individual binding sites of the subunits (Wang and von Hippel, 1993). These experiments even suggested that the ATP-catalyzed RNA release process proceeds directionally (5′→3′) along the RNA oligomer. Thus, as suggested above, the release of bound ssRNA from individual rho subunits comprises the ATP-dependent step of the single-step reaction cycle for this helicase.

Yet another clear (though nonhelicase) example for which the structural basis of the ATP-dependent release reaction has been defined is the GroES-GroEL chaperonin system (reviewed by Sigler et al., 1998). Here structural studies have shown that hydrophobic residues of the unfolded protein chain located within chaperonin cavity interact with hydrophobic "patches" that are present on the walls of the cavity at the beginning of the ATPase reaction cycle. ATP hydrolysis catalyzed by chaperonin components then drives a conformational change in the cavity walls, resulting in the burial of these hydrophobic cavity patches within the wall and the concomitant exposure of hydrophilic residues that bind the substrate protein less tightly. As a consequence the substrate protein is released and has a chance to reinitiate folding (Roseman et al., 1996). Subsequently hydrogen exchange techniques were used to directly correlate ATP binding and hydrolysis in these chaperonin complexes with protein unfolding and release (Shtilerman et al., 1999).

Transcription Complexes as Coupled (and Decoupled) Helicases

Transcription complexes that move through dsDNA and use their RNA polymerase activities for the template-directed synthesis of RNA can be considered as efficient and highly coupled helicases. In terms of the nomenclature developed above, the "helicase portion" of the transcription reaction is initiated by binding the RNA polymerase holoenzyme onto the dsDNA genome at a promoter site. The RNA polymerase is then loaded onto the promoter ("melted-in") with the aid of promoter-specific loading factors (σ factor for prokaryotic RNA polymerases; various loading and specificity accessory proteins for eukaryotic RNA polymerases) to form an

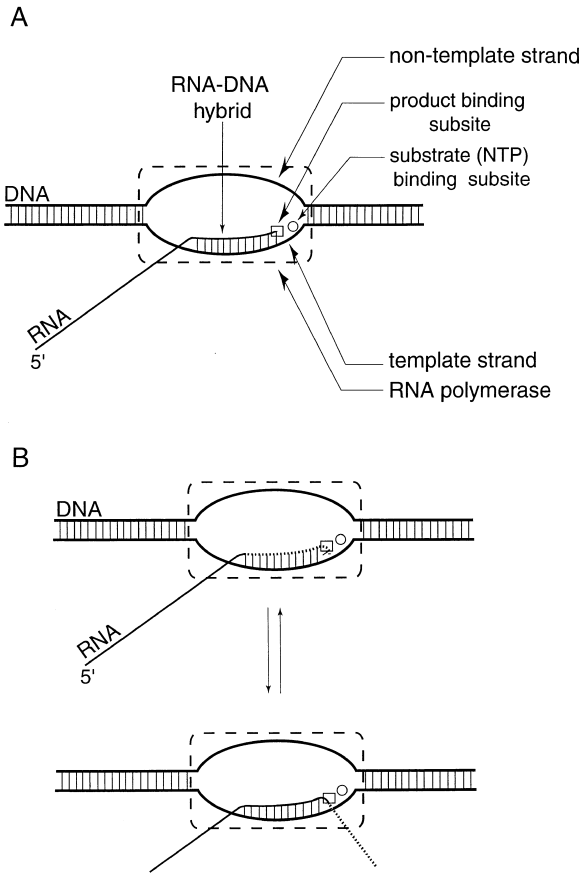

Figure 5. The Transcription Elongation Complex Functions as a Coupled or Uncoupled Helicase

(A) The transcription complex in its actively elongating mode couples dsDNA unwinding and RNA polymerization activities and provides directional translocation, unwinding the dsDNA one bp at a time and concomitantly extending the RNA by one nt. The 3′ terminus of the nascent RNA is shown bound to the product binding subsite of the RNA polymerase and the next required NTP is shown bound to the substrate binding subsite. In this form the polymerase can be considered a tightly coupled helicase.

(B) Here the 3′ end of the nascent RNA has dissociated from the active site of the polymerase and the helicase reaction has been uncoupled from the RNA synthesis reaction. In this uncoupled state the transcription elongation complete can move into a "back-sliding" mode in which it can diffuse randomly along the nucleic acid framework of the complex. The dashed gray transcript sequence, initially within the ds(RNA-DNA) hybrid, is progressively extruded from the front of the complex by back-sliding (see text and von Hippel, 1998).

open promoter-polymerase complex in which the template strand is exposed for template-directed synthesis.

After initiating nascent RNA synthesis and leaving behind the promoter-recognition (specificity) factors, the core transcription complex moves into the very stable and processive elongation phase of the transcription reaction (Figure 5A). Here the nascent RNA chain is extended by one nucleotide residue for every NTP that is hydrolyzed and incorporated (as NMP) into the growing RNA chain. This elongation process involves the formation and translocation (with the polymerase and the transcription bubble) of an 8–9 bp RNA-DNA hybrid that includes the 3′ end of the nascent RNA. The length of the RNA-DNA hybrid in the transcription bubble is maintained constant by a coupled and continuous separation of the hybrid that results in the progressive release of the 5′ end of the nascent RNA into solution as an elongating ssRNA chain. In conjunction with this synthesis, the open transcription bubble (or, in the context of this article, the "helicase bubble") also moves forward along the DNA template and thus into the downstream dsDNA by one bp for every RNA residue incorporated.

Thus, in our present terminology, the elongating polymerase serves both as a helicase and (together with the next required NTP) as a helicase coupling factor, maintaining the position of the transcription bubble on the template lattice and driving the directional movement of the transcription complex along it. The dsDNA opening reaction of the transcription complex (i.e., the helicase function itself) operates by a single bp thermally driven "invasion" process, much like that described for the step-wise melting into dsNA of a single-stranded NA binding protein or protein model (e.g., formaldehyde) discussed above. Here the directionality of the helicase reaction is maintained by the coupling of the polymerization of RNA to the advance of the transcription complex. The efficiency of the tightly coupled dsDNA unwinding-RNA polymerization reaction, in this context, can be defined as one bp opened per NTP hydrolyzed and incorporated, since the template-directed extension of the nascent RNA by one NMP residue is accompanied by a forward translocation of the transcription bubble by one nt along the DNA template strand.

When this tight coupling is lost, presumably as a consequence of the dissociation of the 3′ end of the nascent RNA from the polymerase active site, the polymerase and the associated transcription bubble can "diffuse" along the template strand within the dsDNA in a one-dimensional random walk process as described elsewhere (see von Hippel, 1998). This process is illustrated in Figure 5B, and proceeds (when the complex is in a decoupled state) as a thermally driven single bp opening reaction. This single bp reaction is approximately thermodynamically neutral because, as the complex moves forward or backward, one bp opens ahead of the polymerase and one bp closes behind for each translocation step taken (von Hippel, 1998).

In summary, for the purposes of the integrated helicase picture that we are developing here, this brief description should make clear that the initiation and elongation processes of transcription can be considered to involve helicase reactions. Here the dsDNA opening process is directional and is driven by NTP hydrolysis (and by NMP incorporation) when the RNA polymerase is coupled to the movement of the transcription bubble, and nondirectional (i.e., driven by diffusion manifested by random sliding) and independent of NTP binding and hydrolysis when the coupling at the active site is released. Furthermore here the helicase (opening) reaction, like that for the nonkinetically blocked melting reaction seen with single-stranded DNA binding proteins and models (discussed above), clearly depends on the stepwise single bp thermal fraying of the dsDNA ahead of (or behind) the transcription bubble.

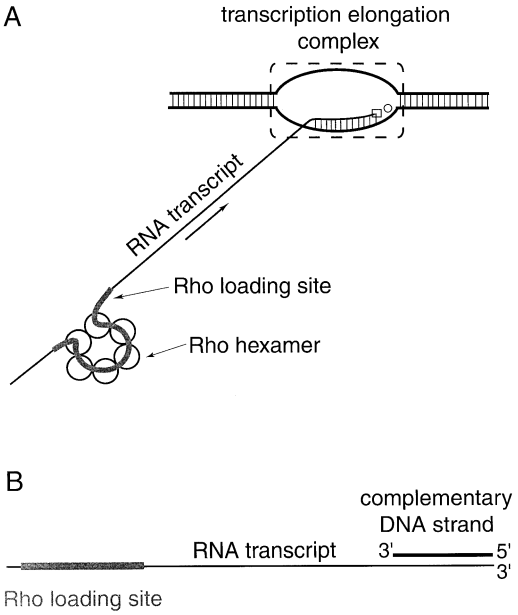

Figure 6. Transcription Termination Factor Rho of *E. coli* as a Model (RNA-DNA) Helicase

(A) The hexameric Rho helicase binds at the (largely unstructured and rC-rich) Rho loading site on the nascent RNA transcript, followed by ATP-dependent unidirectional (5′→3′) translocation along the RNA until it reaches and destabilizes the RNA-DNA hybrid located within the transcription elongation complex, resulting in RNA release.

(B) Rho helicase activity can be assayed directly in a related system in which Rho is initially bound at the Rho loading site of the nascent RNA (isolated from a Rho-dependent termination system such as that shown in Figure 6A) and, on activation of the ATPase, moves directionally along the RNA until it displaces a complementary DNA oligomer that has been annealed to the RNA in an upstream position as shown. The loading, translocation, and helicase activities of Rho can be studied separately using such an assay (see text).

Rho Protein Is an RNA-DNA Helicase for which Loading, Translocation along the ssRNA Lattice, and Opening of the dsNA Lattice, Can Be Separated

The Rho protein of *E. coli* operates, in conjunction with the elongating transcription complex, to terminate transcription at rho-dependent terminator sites along the template. Rho is an ATP-dependent RNA-DNA helicase (Brennan et al., 1987) and appears to trigger Rho-dependent termination by loading onto the nascent RNA transcript at specific rho loading sites that are rich in rC residues and largely devoid of RNA secondary structure. This binding reaction, which may involve all six subunits of the Rho hexamer, activates the cryptic ATPase of Rho and results in directional (5′→3′) translocation of the helicase hexamer along the single-stranded RNA until it reaches ("catches up with") the transcription complex, at which point it translocates into and through the double-stranded RNA-DNA hybrid to release the nascent RNA from the transcription complex (Figure 6A) (Jin et al., 1992; Zhu and von Hippel, 1998).

This process can be demonstrated (in the absence of the transcription complex) with an isolated Rho-dependent RNA transcript to which a complementary DNA oligomer has been annealed at an upstream site, as shown in Figure 6B. Such constructs have been used to characterize the Rho helicase reaction in detail (Walstrom et al., 1997). In these studies the individual steps of the overall helicase reaction can be separated into three sequential processes: (1) loading of Rho onto the nascent RNA; (2) ATP-driven translocation of Rho along the ssRNA transcript; and (3) opening of the upstream ds(RNA-DNA) hybrid segment. These studies showed that the Rho helicase moves processively and directionally along the ssRNA lattice with an efficiency of 1–2 nt translocated per ATP molecule hydrolyzed, and that this translocation continues along the RNA with the *same* efficiency (now defined as bp opened per ATP hydrolyzed) when the ds(RNA-DNA) hybrid is encountered (Figure 6B). The processivity and efficiency of translocation along the ssRNA lattice can be decreased by increasing the salt concentration of the assay solution, presumably reflecting the formation of secondary structure and stacking interactions within the ssRNA lattice that makes Rho advance more difficult. In contrast, the processivity and efficiency of the movement of the helicase through the ds(RNA-DNA) hybrid is not salt concentration dependent (Walstrom et al., 1998).

These results are consistent with a simple helicase mechanism proposed previously for Rho (Geiselmann et al., 1993) that involves (in the terms of this paper) a kinesin dimer-like movement of the multimeric helicase along the ssNA template, with ATP-hydrolysis providing processivity and directionality to the translocation process by driving the release of the lagging helicase subunit at the end of each single-step helicase reaction cycle. Since the six Rho subunits are organized into a hexagonal ring, reorientation of the lagging subunit relative to the RNA lattice may not be required (in contrast to the events of the dimeric kinesin walking cycle), but may follow simply as a consequence of ring translocation along the lattice. However, the Rho reaction, unlike that of dimeric kinesin, must involve some slippage of the helicase on its ssRNA track, as evidenced by the decreased processivity and efficiency of the reaction with increasing salt concentration.

In this view (Geiselmann et al., 1993), the helicase reaction itself corresponds to the ATP-driven translocation of the helicase along the RNA track, with the RNA-DNA hybrid melting ahead of the helicase as a consequence of a sequential single bp thermally driven dsNA opening reaction. The problem for the helicase is then to find a way to capture and accumulate these single bp openings until a full helicase "binding site size-equivalent" of ssRNA has been opened. The details of these intermediate processes are not known, but the end result is that a single Rho subunit moves forward, perhaps in a series of small steps as the bp of the fork open sequentially, and then locks onto and stabilizes the newly open segment when a full binding site size of ssRNA has been exposed. Figure 7 shows a hypothetical intermediate (drawn in the form of a dimeric helicase for simplicity) for such a capture process, with a flexible ssRNA binding site "rolling-in" to the next RNA binding site as the RNA-DNA duplex frays open.

Finally, as described in the second section of this review, the opening reaction of the Rho helicase can be rendered thermodynamically neutral (or even favorable) because the favorable free energy of binding of the heli-

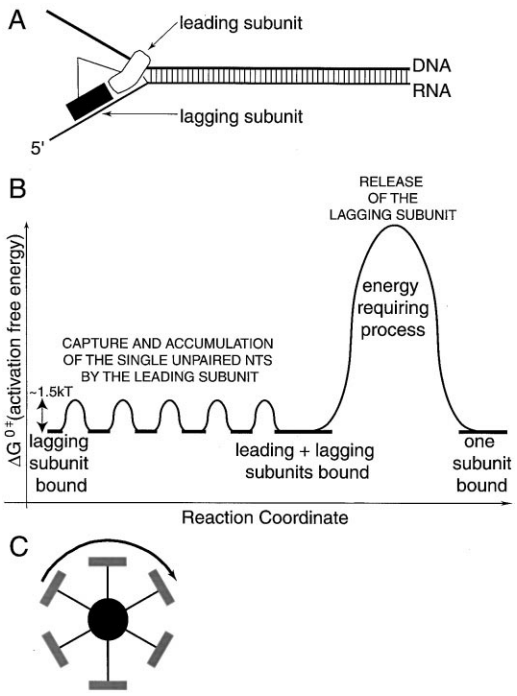

Figure 7. A Model of the dsDNA Unwinding Process Catalyzed by a Dimeric ("Rho-like") Helicase Using a Sequential Single Base Pair Opening and Trapping Process

(A) Here the unpairing reaction is driven by thermal fluctuations and the helicase, drawn as a dimer for simplicity, uses the ssRNA binding site of its leading subunit (drawn curved to suggest conformational flexibility) to capture and accumulate single unpaired nt. Once a full binding site size of RNA has been accumulated (here in a tightly coupled helicase step devoid of slippage), the lagging helicase subunit (black rectangle) is released and relocated to participate in the next single-step helicase reaction cycle. The lagging helicase domain release and reorientation phase is driven by ATP hydrolysis.

(B) Activation free energy diagram for the above single-step process. Note that the entire reaction coordinate shown corresponds to the forward movement into the dsNA of only one helicase binding domain.

(C) A hexameric helicase drawn in the same fashion as the dimeric helicase shown in Figure 7A, emphasizing the possibility that the hexameric helicase might "roll" successive subunit binding domains into the DNA fork sequentially.

case to the newly exposed ssRNA segments offsets the unfavorable free energy of opening of the ds(RNA-DNA) hybrid. The transient release of the lagging rho subunit from the RNA binding track again comprises the only thermodynamically uphill reaction in the overall process and is coupled to (or driven by) ATP hydrolysis, as shown in Figure 4 for the advance of dimeric kinesin.

Mechanisms of the Hexameric Helicases of Replication

Some aspects of the reaction mechanisms of the hexameric helicases associated with DNA replication can be considered in the context developed above for Rho. Clearly the binding, translocation, and coupling mechanisms of these helicases will differ in detail, and furthermore the component parts of the reaction cycle (loading, translocation, and unwinding) are not as neatly separable from one another as are those of Rho. However, the basic mechanistic steps are likely to be similar and can thus be discussed from the same perspective.

DNA Replication Helicases May Also Open dsDNA in Single Base Pair Steps

By analogy with the transcription complex, we can consider the DNA replication fork to represent the upstream "edge" of the transcription bubble, and postulate that DNA replication helicases may also open the dsDNA by sequential (and accumulated) single bp steps. If this single-bp opening process is strictly equivalent (in rate and extent) to the thermal fraying that would occur at an ssDNA–dsDNA junction in the absence of the helicase, the opening reaction would be purely "passive" in terms of the nomenclature developed for helicases by Lohman and coworkers (see Lohman and Bjornson, 1996). In contrast, an "active" process would involve direct participation of the helicase itself in the dsDNA opening process, perhaps by an ATP-dependent protein-driven distortion of the nucleic acid lattice. An intermediate possibility is that the reaction is "largely passive." Here the basic opening process would continue to be driven by thermal fluctuations, but the proximity of the leading edge of the helicase could augment this thermal fraying by effectively "lowering the local melting temperature" of the dsDNA bp at the replication fork, perhaps by means of "shaped" electrostatic interactions involving basic protein residues at the leading edge of the helicase that could stabilize partially open DNA states and thus increase the rate of dsDNA unwinding.

Structural Requirements for Strand Displacement by the Translocating Helicase

The model for Rho helicase action described above (Geiselmann et al., 1993) suggested that the opening of the ssNA segments ahead of the helicase might be effectively passive (i.e., driven by thermally induced fraying of the dsNA), with the helicase simply moving directionally along the ssNA strand onto which it had been initially loaded. If these ideas are substantially correct, one would predict that the displaced strand in the reaction is effectively "shouldered aside" as a consequence of the translocation of the helicase along the strand to which it binds, and therefore that the structure, sequence, and composition of the *displaced strand* might be relatively unimportant for helicase function. This expectation is consistent with the results of an earlier study of UvrD mechanisms that showed that this helicase could catalyze the unwinding of an "unnatural" RNA-DNA hybrid (Matson, 1989), as well as with similar results obtained in a recent study of the T4 gp41 replication helicase (Tackett et al., 2001). Of course, a mechanism is still required to prevent reannealing of the displaced strand, ultimately by the stable trapping of at least one of the ssNA strands produced by the helicase reaction. One method that may be used by hexagonal replication helicases (and perhaps by Rho) to prevent transient reannealing is to thread one of the separate ssNA strands through the helicase ring to hold it away from the complementary ssNA strand at the level of the helicase (e.g., see Bujalowski and Jezewska, 1995; Yu et al., 1996a; Ahnert and Patel, 1997).

Indirect Measurements Show that Replication Helicases Can Translocate along an ssNA Lattice

Because its helicase substrate can be designed with a separate loading site, translocation region, and dsNA

hybrid sequence, ssNA translocation driven by ATP hydrolysis could be demonstrated directly for Rho (Figure 6B; for details see Walstrom et al., 1997). This is more difficult (or, at least, less unambiguous) for replication helicases, since their ssNA loading sites are only transiently present at the fork and thus not spacially separated from the dsDNA to be unwound. Nevertheless, indirect experiments have shown that replication helicase can translocate unidirectionally along an ssDNA track. Early experiments with the T4 gp41 helicase (Liu and Alberts, 1981) demonstrated that the rate of ATP consumption by this enzyme could be made to depend on the length of the ssDNA lattice to which the helicase was bound. Subsequently a more quantitative approach to this system (Young et al., 1994) showed that the rate of ATP hydrolysis catalyzed by gp41 bound to ssDNA could be modeled in terms of a lattice length–dependent, directional, and processive translocation of this helicase along the ssDNA template. A similar demonstration has recently been made for PcrA helicase (Dillingham et al., 2000).

Helicase Mechanisms in Transcription and Replication Have Different Structural and Thermodynamic Requirements

As indicated above, the fork at which DNA replication helicases operate can be viewed as the structural and transition state equivalent of the junction between open and closed bp encountered by the RNA polymerase at the front edge of the transcription bubble. As discussed above, the RNA polymerase moves forward in the coupled RNA synthesis process of transcription in single-bp opening steps involving the hydrolysis of a single NTP and the incorporation of a single NMP into the nascent RNA. Concomitantly the transcription bubble closes behind the RNA polymerase and thus the bubble remains essentially constant in size. The net process of single bp opening and closing is therefore effectively energetically coupled and thus approximately thermodynamically neutral, although the opening and closing processes need not be simultaneous if the transcription complex has some flexibility.

This is not the case for semi-conservative DNA replication. The net process of opening one bp at the replication fork requires the hydrolysis of three NTPs: one to drive the helicase reaction itself (if we assume an efficiency of one bp opened per ATP hydrolyzed for a typical replicative helicase) and two more that are required for the synthesis of the two new bp that result, one on the leading and one on the lagging template strand. The lack of energy conservation and coupling between the base pair opening and closing reactions in replication reflects the structural complexity of the replication "trombone" (eg., see von Hippel and Jing, 2000) and means that a separate helicase activity, with its own source of free energy, is required at the replication fork. The ATP-driven replication helicases serve this function, operating as an integrated entity with their homologous leading and lagging strand DNA polymerases (Dong et al., 1996; E. D. and P. H. v. H., submitted) and primases.

Generality of the Basic Helicase Mechanism

In this article we have attempted to lay out a unified overall mechanism for helicase reactions that is based on demonstrated reaction principles and mechanisms for NA binding proteins and models, motor proteins, and transcription complexes. Clearly such a unitary mechanism can only go so far in explaining the detailed properties of helicases integrated into macromolecular machines, and a variety of special structural features and coupling mechanisms will be required in real cases. The structural and mechanistic literature in these areas is growing exponentially, and cannot be reviewed in this article.

Instead, our intention here has been to provide a general thermodynamic and kinetic framework for such more detailed studies of helicase mechanisms. In our view a reaction model of the sort proposed here—which is based on (1) the spontaneous opening, in single bp steps, of the dsNA ahead of the helicase; (2) the trapping of open single bp intermediates by special structural mechanisms to permit the surmounting of fairly high free energy of activation barriers to helicase-ssNA binding reactions that are themselves essentially thermodynamically neutral or downhill; and (3) a thermodynamically unfavorable (and ATP hydrolysis requiring) release reaction to permit repetition of the overall cycle—can provide a starting point for mechanistic analysis of many biological processes that require the transient or permanent opening of dsNA sequences.

In closing we stress again that helicase reactions in general, and the central roles that these components play in the functioning of macromolecular machines, will vary widely in their molecular and structural details. However, we hope that the overall approach outlined here can be helpful in providing an initial mechanistic context within which to analyze each specific case.

Acknowledgments

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Endnotes

(1) For simplicity, Equation 4 is set up in terms of a hypothetical ssNA binding protein with a binding site size defined (below) as *n* (McGhee and von Hippel, 1974), which is here set equal to the apparent step size *k* of the single-step helicase cycle. Of course in real coupled helicase-ssNA binding protein reactions (see Equation 5), *k* need not be equal to *n*.

(2) Transition state theory tells us that a reaction substep (such as single bp fraying at the end of a dsDNA helix) that is thermodynamically uphill by only one to two times *k*T (*k*T is ~ -0.6 kcal/mol at room temperature; *k* is the Boltzmann constant and T is the absolute temperature) will proceed at a rapid rate (μs) if the concentration of the binding ligand (here HCHO) that will bind and stabilize the open state is sufficient to make the overall reaction thermodynamically favorable.

(3) Creating a protein with a structure that permits sequential melting into dsNA in a series of single bp steps may not be as difficult as this description of the gp32 system suggests. Thus, it has been shown that removal of $\sim$60 residues from the C terminus of gp32 (to form gp32*I) can also remove the kinetic block to the melting of natural dsDNA and permit this melting to go to equilibrium in the presence of an excess of the modified protein (Greve et al., 1978; Kowalczykowski et al., 1981; Lonberg et al., 1981). It appears that the C-terminal domain of gp32 comprises a “flap” that masks the electrostatic subsite of the gp32 ssDNA binding site, and removal of this flap exposes this subsite (Wu et al., 1999), giving the gp32*I protein the ability to trap single open bp sequentially as the duplex DNA frays open (see below).