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DNA Helicases

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DNA helicases are proteins that use the energy of NTP hydrolysis to processively denature duplex DNA.[1] As such, helicases can be considered similar to other mechanochemical motors that can catalyze directional movement along a polymeric lattice (e.g., microtubule-based motors as kinesin). Acting during DNA replication, helicases convert the duplex DNA to single strands and thereby activate the DNA for nascent strand synthesis by the replicative DNA polymerases. Because of the fundamental nature of this process, helicases also play essential roles during DNA repair and recombination as well as RNA transcription and are found in virtually all cells (Thömmes and Hübscher 1992; Lohman 1993; Matson et al. 1994).

Our knowledge of the DNA helicases acting during eukaryotic DNA replication is somewhat meager. The replicative helicases functioning during chromosomal replication for any eukaryote are unknown. However, information is available from the study of animal viruses that encode replicative helicases, as well as from prokaryotic replication systems. Here I outline the current understanding of DNA helicases that function in eukaryotic DNA replication.

DNA HELICASE ASSAYS

DNA helicases are often first detected by a DNA-dependent or DNA-stimulated NTPase activity. Verification of DNA helicase activity is then accomplished using an assay that reveals the conversion of double-stranded DNA (dsDNA) into two single strands. The most common as-

[1]Not included in this category are proteins that can denature DNA by binding stoichiometrically to single-stranded DNA (ssDNA) using an NTP-independent mechanism (e.g., ssDNA binding proteins such as bacteriophage T4 gp32 protein; Alberts and Frey 1970), proteins that catalyze movement of a transient bubble through DNA (e.g., RNA polymerases), or topoisomerases that alter the linking number of covalently closed circular DNA molecules.

DNA Replication in Eukaryotic Cells
 0-87969-459-9/96 $5 + .00

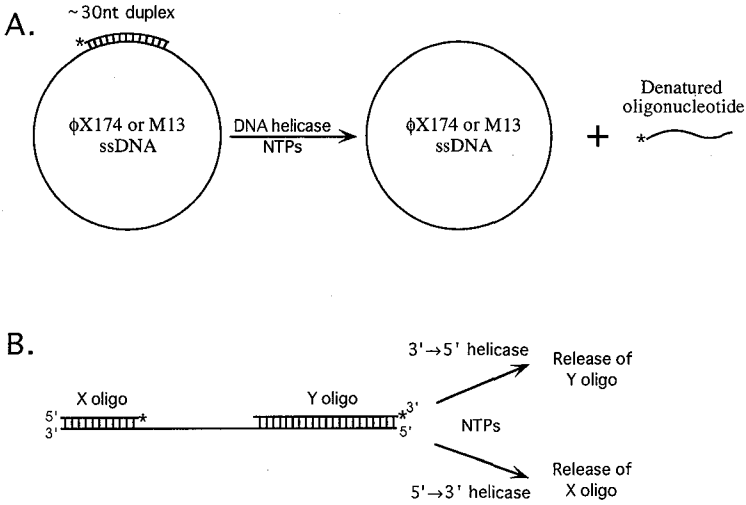

Figure 1 Schematic of assays used to detect DNA helicase activity. (*A*) The basic assay. A ^{32}P-labeled oligonucleotide is annealed to a larger ssDNA molecule such as ϕX174. In an NTP-hydrolysis-dependent reaction, DNA helicases denature the oligonucleotide from the ssDNA circle. Activity can be revealed by subjecting the reaction products to native gel electrophoresis and autoradiography. (*B*) The polarity assay. To determine the polarity of DNA helicase action, a substrate is employed that contains two duplex regions of distinct length flanking a central ssDNA region. Both short DNA strands (designated X oligo and Y oligo) are radioactively labeled. DNA helicase unwinding in the 5′→3′ direction would release the X oligonucleotide, and unwinding in the 3′→5′ direction would release the Y oligonucleotide.

say measures the release of a ^{32}P-labeled oligonucleotide annealed to a larger ssDNA molecule, such as ϕX174 or M13 viral DNA (Fig. 1A) (Venkatesan et al. 1982; Matson et al. 1983). The radioactive substrate and product molecules differ greatly in size so that native gel electrophoresis of the reactive mixture quickly reveals helicase activity.

Upon identification, DNA helicases are most simply characterized with respect to a few basic features, most notably the nucleotide requirements, specificity for DNA and RNA, polarity, and the requirement for ssDNA extensions. Nonhydrolyzable nucleotide analogs such as adenosine 5′-(γ-thio)triphosphate (ATPγS) or adenosine 5′-(β,γ-imido)triphosphate (AppNp) are usually tested to verify the requirement for nucleotide hydrolysis. Polarity, sometimes referred to as directionality, is defined as the direction of helicase movement on ssDNA, i.e., 5′→3′ or 3′→5′. Determination of helicase polarity utilizes a par-

tially duplex substrate in which a central ssDNA region is flanked by two duplex regions of different lengths (Fig. 1B) (Matson 1986). Polarity is ascertained by identifying the duplex region that is denatured by the helicase. For helicases involved in DNA replication, the polarity of the reaction is strongly indicative of helicase placement on the leading (a 3′→5′ polarity) or lagging (a 5′→3′ polarity) strand. Although the assay can determine the polarity of virtually all helicases, certain helicases require or are greatly stimulated by a ssDNA extension on the strand opposite to that used for primary contacts (e.g., *Escherichia coli* DnaB; LeBowitz and McMacken 1986). Optimal activity on helicase substrates containing a DNA fork (i.e., noncomplementary ssDNA extensions on both strands) suggests that the protein interacts with both the leading- and lagging-strand templates during DNA unwinding.

The basic helicase assay has proven extremely useful because of its simplicity. However, it relies on the production of a completely denatured product and thus provides limited information on the mechanism by which the helicase unwinds DNA. The assay has therefore been modified in various ways to reveal information concerning intermediates in the process. For example, synthesis of oligonucleotides containing 2-aminopurine (2-AP) in place of adenine allows the generation of helicase substrates with distinctive fluorescent properties (Raney et al. 1994). Quenching of the 2-AP fluorescence in duplex DNA is alleviated during DNA denaturation. This assay can therefore be used to formulate kinetic models of the unwinding reaction. Other fluorescence-based assays have also been developed (Roman and Kowalczykowski 1989; Houston and Kodadek 1994).

MOLECULAR BIOLOGY OF HELICASES

Comparison of the translated sequences of genes encoding either biochemically characterized or putative DNA helicases has revealed various categories of sequence homology. A significant fraction of prokaryotic and eukaryotic helicases, able to unwind DNA in either the 5′→3′ or 3′→5′ direction, are members of a DNA/RNA helicase superfamily (Hodgman 1988; Gorbalenya et al. 1989). Members of the superfamily are identified by the presence of six conserved protein motifs (I–VI). A seventh motif, termed Ia, is found only in a subset of helicases within the superfamily and has a poorly defined consensus sequence. Variations of motifs I and II are found in numerous proteins able to hydrolyze purine nucleotide triphosphates. Although crystal structures of helicases are currently unavailable, there is abundant high-resolution

structural information from diverse nucleotide-utilizing enzymes (Walker et al. 1982; Fry et al. 1986; Saraste et al. 1990). Such studies reveal that motif I and the closely associated motif Ia form the canonical "A-site" or "P-loop" involved in nucleotide triphosphate recognition. Mutation of motif I, particularly of the lysine in the conserved $GXGK^{T}/_{S}$ sequence, deleteriously affects hydrolysis of the nucleotide with less significant effects on nucleotide binding. Motif II (the "B-site"; ++++$DEX^{H}/_{D}$ where hydrophobic residues [I, L, V, M, F, Y, and W] are indicated by a plus, and X indicates any amino acid) also plays a significant role in nucleotide binding and is thought to interact with the divalent magnesium associated with the nucleotide phosphates. The roles of motifs III–VI are unknown. However, the effects of systematic mutation of each motif (e.g., in the herpes simplex virus type I [HSV] UL5 [Zhu and Weller 1992] and UL9 DNA helicases [Martinez et al. 1992], or the human DNA repair helicase ERCC3 [Ma et al. 1994b]) strongly support the conclusion that the motifs represent structural components that are critical for DNA helicase function.

Although virtually all helicases contain elements similar to the motifs I and II, many lack any recognizable homology with the other motifs found in the helicase superfamily. Comparison of these helicase sequences also does not reveal a second broad grouping of related sequences. However, more limited subsets of homologous sequences have been noted. For example, sequences encoding the large tumor antigen of the papovavirus family, typified by the SV40 T antigen, show significant homology over a broad region to predicted E1 proteins encoded by the papillomavirus family (Clertant and Seif 1984). Whether these helicases are structurally similar to superfamily members is a matter of debate.

REPLICATIVE VIRAL DNA HELICASES

The lack of clearly defined cell-free systems that catalyze chromosomal replication has precluded an unambiguous definition of any cellular replicative helicase (discussed below). However, various animal viruses such as SV40 or HSV encode DNA helicases with known roles in viral DNA replication (Table 1). Study of these viral enzymes has provided a useful avenue to explore the role of helicases in eukaryotic DNA replication. Furthermore, as the replication of certain viruses can be reproduced in vitro, it is possible to biochemically explore the interplay between the DNA helicase and the replication machinery, the latter often of cellular origin.

Table 1 Virally encoded DNA helicases essential for viral DNA replication

Helicase	Deduced m.w.[a]	Oligomeric state (m.w.)[b]	Nucleotide cofactors	K_m (mM)	Polarity	References
SV40 large T antigen	82,000	hexamer (492,000)	ATP, dATP, dTTP>dCTP, UTP	ATP 0.6	3′→5′	Goetz et al. 1988; Wiekowski et al. 1988; Scheffner et al. 1989
Py large T antigen	88,000	n.d.	ATP, dATP>CTP, UTP	ATP 0.64	3′→5′	Seki et al. 1990; Wang and Prives 1991
BPV E1	69,000[c]	n.d.	ATP, dATP, CTP, dCTP, UTP, dTTP, GTP, dGTP	ATP 0.20	3′→5′	Seo et al. 1993; Yang et al. 1993
HSV UL5	114,000	hetero-oligomeric complex (1:1:1) of UL5, UL52, and UL8 (293,000)	site I GTP, ATP site II ATP dATP	site I ATP 1.3 GTP 1.0 site II ATP 0.17	5′→3′	Crute et al. 1991; Earnshaw and Jarvest 1994
HSV UL9	94,000	dimer	ATP, CTP, dATP, dCTP, UTP	ATP 0.54	3′→5′	Fierer and Challberg 1992; Boehmer et al. 1993; Earnshaw and Jarvest 1994
AAV Rep68, Rep78	61,000, 71,000	dimer (Rep78)	ATP>CTP>dATP>GTP> UTP	n.d.	3′→5′	Im and Muzyczka 1990, 1992; X. Zhou and N. Muzyczka, pers. comm.
MVM NS-1	83,000	n.d.	ATP>dATP	n.d.	n.d.	Wilson et al. 1991

(n.d.) Not determined.

[a]Molecular weight deduced from the DNA sequence encoding the helicase.

[b]The most prominent species in solution is indicated. For the SV40 T antigen, the helicase-active species has been found to be a hexamer (Dean et al. 1992; SenGupta and Borowiec 1992; Wessel et al. 1992).

[c]The molecular weight of the E1 protein is assumed from translation of the E1 open reading frame.

Papovavirus

SV40 T Antigen

The viral DNA helicase perhaps best understood is the SV40 large tumor antigen (T antigen; for review, see Fanning and Knippers 1992; see also Weisshart and Fanning; Hassell and Brinton; both this volume). T antigen is an essential initiation and elongation factor for SV40 DNA replication. The protein can also regulate viral transcription and transform various cells by binding to tumor suppressor proteins (e.g., p53), although these activities are not directly involved in the replicative functions of T antigen. During the initiation of replication, T antigen binds as a double hexamer to the viral origin in an ATP-dependent reaction. In the presence of human replication protein A (hRP-A) or heterologous SSB proteins, the origin becomes denatured, and T antigen unwinds the viral DNA bidirectionally outward from the origin. The exposed single strands serve as templates for nascent-strand synthesis by the host DNA polymerase machinery (see Hassell and Brinton, this volume).

T antigen (708 amino acids; predicted M_r 82,000) contains both DNA and RNA helicase activities, each proceeding in the 3′→5′ direction (Stahl et al. 1986; Goetz et al. 1988; Wiekowski et al. 1988; Scheffner et al. 1989). The two activities are distinguishable by their nucleotide requirements. The DNA helicase is most active in the presence of ATP or dATP, with dTTP, UTP, and dCTP functioning to a lesser extent (K_m [ATP] 0.6 mM; Goetz et al. 1988; Wiekowski et al. 1988; Scheffner et al. 1989). In contrast, the RNA helicase is unable to efficiently utilize ATP or dATP, but has significant RNA-unwinding activity in the presence of the other common nucleotides (Scheffner et al. 1989). T antigen most efficiently unwinds DNA substrates containing a 3′overhang 20 nucleotides in length, although a lesser stimulatory effect can be seen using single-strand extensions as small as 5 nucleotides (Wiekowski et al. 1988). The rate of DNA unwinding at 37°C was determined to be in the range of 1.5–3 bp/second (Wiekowski et al. 1988; Murakami and Hurwitz 1993).

SenGupta and Borowiec used nuclease and chemical probing techniques to examine the interaction of T antigen with model replication forks (SenGupta and Borowiec 1992; SenGupta et al. 1992). In the presence of low levels of ATP (≤100 μM), T antigen quantitatively bound the fork without appreciable denaturation of the duplex structure. T antigen significantly protected only one of the two strands from nuclease attack, that containing the 3′single-stranded end (the top strand). Approximately 15 nucleotides of DNA were protected, including the dsDNA/ssDNA junction, a result consistent with the stimulatory effects

of 3′ overhangs on T antigen unwinding (Wiekowski et al. 1988). These results differ in certain aspects from a more limited study of T antigen binding to a synthetic DNA fork (Wessel et al. 1992). However, the latter study used adenosine 5′-(β,γ-methylene)triphosphate (AppCp) rather than ATP and employed conditions in which only a small fraction of the fork substrate was bound by T antigen. The particulars of this discrepancy are not yet understood.

Functional studies were also employed to determine the contacts used by T antigen during fork denaturation (SenGupta and Borowiec 1992). T antigen utilized critical contacts with six phosphates at the dsDNA/ssDNA junction of the top strand during fork denaturation. This result suggests that the T antigen achieves polarity of movement using directional cues within the sugar-phosphate backbone. These and other results also indicate that T antigen has the ability to recognize the fork structure directly, an observation useful in understanding the mechanism of helicase loading onto DNA during the initiation of replication (see, e.g., SenGupta and Borowiec 1994).

T antigen bound to the fork primarily as a hexamer, although double hexameric complexes were also noted (SenGupta and Borowiec 1992). Although T antigen can exist in a variety of oligomeric states, the presence of ATP stimulates the formation of T-antigen hexamers, entities known to be helicase-active (Dean et al. 1992; Wessel et al. 1992), and suggests that the two origin-bound hexamers separate during replication initiation. This question has been addressed by Stahl and colleagues, who examined the products of the origin-dependent DNA-unwinding reaction by electron microscopy (Wessel et al. 1992). In approximately one-quarter of the unwound molecules, T antigen bound at each fork remained joined such that two ssDNA loops ("rabbit ears") were extruded from the complex. This intriguing result implies that SV40 replication on both forks occurs simultaneously within a single mini-factory, a smaller example of the replication centers thought to duplicate the eukaryotic chromosome. Further exploration of this SV40 replication feature is obviously required.

The SV40 T antigen, as well as the polyomavirus (Py) T antigen and the papillomavirus E1 (below), are required for denaturation of the cognate origins during replication initiation. Although the denaturation reactions require ATP hydrolysis, the role of the DNA helicase activity in these processes is unclear. A threonine to alanine mutation at position 124 in the SV40 T antigen gives rise to a protein that has wild-type DNA helicase activity and can bind the origin, yet is unable to initiate the origin-dependent DNA-unwinding reaction (McVey et al. 1993; Moarefi

et al. 1993). Thus, the SV40 T antigen, and by inference E1 and the Py T antigen, likely require functions in addition to the DNA helicase and origin-binding activities for origin denaturation.

T antigen has been subject to extensive analysis to determine critical regions required for the T-antigen DNA helicase and other activities (for review, see Fanning and Knippers 1992). Partial proteolysis demonstrates that the essential helicase domain is contained within amino acids 131 to about 616, although the presence of amino acids to 708 is required for wild-type activity (Wun-Kim and Simmons 1990). This domain contains the core origin-binding domain (amino acids 131–257) with various data indicating that the DNA helicase and origin substrates bind to partially, but not completely, overlapping sites (see, e.g., Mohr et al. 1989; Lin et al. 1992).

Polyomavirus T Antigen

The Py and SV40 large T antigens are approximately 60% similar at the amino acid level and are alike with respect to virtually all replication activities (Seki et al. 1990; Wang and Prives 1991). The Py T antigen (predicted M_r 88,000) is a 3′→5′ DNA helicase with Py origin-binding and origin-unwinding activities. The Py and SV40 helicase activities are equivalent, with only the nucleotide requirements found to differ slightly (Wang and Prives 1991). The Py T antigen is most active in the presence of ATP and dATP, with CTP and UTP substituting to approximately 50% efficiency (K_m [ATP] 0.64 mM).

Papillomavirus: E1

Replication of bovine papillomavirus type 1 (BPV) and most human papillomavirus (HPV) types requires the products of two viral open reading frames (ORFs) designated E1 and E2 (Ustav and Stenlund 1991; Chiang et al. 1992; Del Vecchio et al. 1992; Stenlund, this volume). Analysis of E1 ORF sequences from BPV and HPV type 6 revealed that they bore significant homology with the SV40 and Py T antigen genes over various regions (Clertant and Seif 1984). Thus, it was anticipated that the putative papillomavirus E1 proteins would have similar activities as T antigen, a hypothesis later borne out.

The BPV E1 can bind specifically to the viral origin in a reaction stimulated both by E2, a transcriptional activator, and by ATP (Mohr et al. 1990; Lusky et al. 1993). E1 is a DNA helicase and can also catalyze the large-scale unwinding of DNA molecules containing the viral origin

(Seo et al. 1993; Yang et al. 1993; Hughes and Romanos 1993). In the presence of crude cellular extracts, origin-containing DNA molecules are replicated in a reaction dependent on E1 (Yang et al. 1991). Each of these activities was demonstrated with E1 produced from recombinant baculovirus because of the very low level of the native E1 (predicted M_r 69,000) in infected cells (Santucci et al. 1990; Sun et al. 1990). However, it would be surprising if the native E1 differed significantly from the recombinant form.

E1 is a 3′→5′ DNA helicase that can utilize all common ribo- and deoxyribo- NTPs (K_m[ATP] 200 μM; Seo et al. 1993; Yang et al. 1993). E1 denatures 5′-tailed oligonucleotides annealed to single-stranded ϕX174 fivefold more efficiently than non-tailed oligonucleotides (Seo et al. 1993). The demonstrated 3′→5′ polarity suggests that E1 utilizes primary interactions with the leading strand during DNA denaturation, but possibly also uses significant lagging-strand contacts during viral replication. Although E2 can bind E1 in solution and can stimulate the origin-dependent DNA-unwinding reaction, the presence of E2 does not appear to have detectable effects on the E1 helicase activity in vitro (Seo et al. 1993; Yang et al. 1993).

HSV

HSV encodes two helicases essential for viral replication designated UL5 and UL9 (see Challberg, this volume). Although a bona fide cell-free replication system for HSV is not yet available, much has been learned about these helicases from a combination of biochemical and genetic studies.

UL5

The product of the UL5 gene (predicted M_r 114,000) is found in a heterotrimeric complex with products of the HSV UL8 and UL52 genes in a stoichiometry of 1:1:1 (Crute et al. 1989). In addition to being a 5′→3′ DNA helicase (Crute et al. 1988), the complex (predicted M_r ~293,000) also contains DNA primase activity (Crute et al. 1989). The complex has therefore been suggested to act on the lagging strand during viral replication, using the primase activity to initiate Okasaki strand synthesis.

The UL5 gene is a member of the DNA/RNA helicase superfamily, although neither recombinant baculovirus-expressed UL5 protein nor UL5 overexpressed in mammalian cells contains detectable helicase ac-

tivity (Calder and Stow 1990; Dodson and Lehman 1991). However, UL5/UL52 complexes isolated from baculovirus-infected insect cells have DNA helicase and primase activities similar to the complete heterotrimeric complex (Calder and Stow 1990; Dodson and Lehman 1991). Indeed, the native complex appears indistinguishable from the complex isolated from insect cells triply infected with baculoviruses expressing the UL5, UL8, and UL52 genes (Dodson et al. 1989; Calder and Stow 1990). In the presence of the ICP8 protein, an HSV-encoded SSB, the rate of duplex DNA unwinding by the heterotrimeric complex has been measured at approximately 2 bp/second (Crute and Lehman 1991). Although this rate is similar to that found for movement of a viral replication fork in vitro initiating from a 5′-tailed duplex substrate (~4 bp/sec; Rabkin and Hanlon 1990), it is considerably slower than the in vivo rate of viral fork movement of the related pseudorabies virus (~50 bp/sec; Ben-Porat et al. 1977). Assuming that the primary helicase during viral replication is the UL5/UL8/UL52 complex, it appears that other viral or host factors have the potential to stimulate the DNA-unwinding rate of this complex by a factor of ten.

Examination of the dimeric UL5/UL52 or heterotrimeric UL5/UL52/UL8 complexes has suggested that two distinct nucleotide triphosphatase (NTPase) sites, distinguishable by their affinity for ATP and GTP, can participate in the helicase reaction (Crute et al. 1991). Moreover, the complex was also seen to contain two effector sites for DNA, each of which independently activates the two NTPase sites. The UL5 complex thus appears to represent an uncommon example of a DNA helicase containing two structurally distinct NTPase sites independently able to drive DNA unwinding, although this conclusion has recently been challenged (Earnshaw and Jarvest 1994). Regardless, information concerning the mechanism of unwinding by the UL5-containing complexes should prove particularly interesting.

UL9

The HSV UL9 protein contains both DNA helicase (Bruckner et al. 1991; Fierer and Challberg 1992) and origin-binding activities (Elias and Lehman 1988; Olivo et al. 1988). UL9 has therefore been proposed to act during the initiation of HSV replication, perhaps facilitating origin denaturation, although such an activity has not yet been demonstrated.

This 3′→5′ helicase is most active in the presence of ATP or dATP, although CTP, dCTP, or UTP can be utilized to a nearly equivalent extent (Boehmer et al. 1993). UL9 contains a single nucleotide-binding site

with K_m(ATP) found to be 0.54 mM (Earnshaw and Jarvest 1994). The protein (predicted M_r 94,000) exists primarily as a dimer in solution and appears to be multimeric as a DNA helicase, although the actual oligomeric species is not yet defined (Boehmer et al. 1993). The rate of unwinding has been measured at approximately 1.25 bp/second, with the processivity of the enzyme limited to roughly 200 nucleotides. ICP8 decreases the amount of UL9 protein required for DNA unwinding and increases the processivity of the reaction to well over 2 kb (Fierer and Challberg 1992; Boehmer et al. 1993). This stimulation is likely due, at least in part, to a physical interaction between UL9 and ICP8 (Boehmer and Lehman 1993).

Parvovirus

Adeno-associated Virus Rep68 and Rep78 Proteins

Transcription from two promoters coupled with alternative splicing leads to the production of four polypeptides from the *rep* region of adeno-associated virus (AAV) (Srivastava et al. 1983; Cotmore and Tattersall, this volume). With predicted molecular weights of 71,000, 61,000, 45,000, and 35,000 (Srivastava et al. 1983), these proteins are referred to as Rep78, Rep68, Rep52, and Rep40 from their mobility on acrylamide gels (Mendelson et al. 1986). The Rep68 and Rep78 proteins are ATP-dependent DNA helicases and can site-specifically cleave the viral DNA upon binding the viral terminal hairpin structures of AAV (*trs*; Im and Muzyczka 1989, 1990, 1992). During the cleavage reaction, the Rep protein becomes covalently attached to the 5′ end of the viral DNA (Im and Muzyczka 1990). These activities are not found associated with either the Rep40 or Rep52 proteins (Im and Muzyczka 1992).

The 3′→5′ helicase activity of Rep68 is most active in the presence of ATP, although other nucleotides can substitute (CTP>dATP> GTP>UTP; X. Zhou and N. Muzyczka, pers. comm.). Mutations within the *rep* gene targeting the putative ATP-binding motif abolish viral replication, *trs* endonucleolytic cleavage, and the viral transcriptional *trans*-activation activity found for *rep* gene products (Labow et al. 1989; Im and Muzyczka 1992). Because Rep68 and Rep78 form covalent linkages with the AAV DNA during *trs* cleavage, it is possible that the Rep helicase remains attached to the 5′ end during DNA unwinding. This mechanism would be similar to the combined action of the *E. coli* rep DNA helicase and bacteriophage ϕX174 A protein during the synthesis of single-stranded circular ϕX174 DNA from the double-stranded replicative form I DNA (Kornberg and Baker 1992). Alterna-

tively, the nicking and DNA-unwinding activities may be partitioned into distinct Rep complexes, so that additional AAV Rep protein binds to the preformed Rep/*trs* complex to unwind the viral DNA.

Minute Virus of Mice NS-1

The organization of the MVM and AAV genomes are similar, so that the MVM NS-1 gene appears to be the AAV *rep* homolog. Subsequent to identification of the DNA helicase activity of AAV Rep68, the NS-1 protein (predicted M_r 83,000) expressed in baculovirus was also found to contain an ATP-dependent DNA helicase (Wilson et al. 1991). Although as yet incompletely characterized, the NS-1 protein can form a covalent linkage with the viral DNA and thus appears to have site-specific endonuclease activity (Chow et al. 1986; Cotmore and Tattersall 1988).

Adenovirus: The Exception to the Rule

Although many viruses encode their own replicative helicases, it is worth noting that the requirement for a DNA helicase is not absolute. Adenovirus appears perfectly capable of replicating its DNA in the absence of a virally encoded helicase (Challberg and Kelly 1989; see Hay, this volume). Adenovirus DNA polymerase is able to elongate through lengthy (5.4 kb) stretches of dsDNA in a reaction requiring the adenovirus DNA-binding protein (the viral SSB; Lindenbaum et al. 1986). The unwinding of duplex DNA during adenovirus replication thus appears to be powered by the hydrolysis of dNTPs as they are incorporated into the growing DNA strand. The lack of need for a DNA helicase may reflect the fact that only leading-strand synthesis occurs during adenovirus DNA replication (Challberg and Kelly 1989). A continuous, unidirectional mechanism is utilized during mitochondrial DNA replication (Clayton, this volume), and thus it is also possible that a DNA helicase activity is not required in the mitochondrial system.

CELLULAR DNA HELICASES

Nuclear DNA Helicases

A handful of DNA helicases have been isolated from eukaryotic cells with defined roles in nucleic acid metabolism (see, e.g., Sung et al. 1987; Drapkin et al. 1994). Because these helicases are not known to function in DNA replication but rather in repair, recombination, or transcription,

their identification has not provided clear information regarding chromosomal replication.

In addition, numerous "orphan helicases" have been isolated whose functions are unknown. In an attempt to ascertain the role of these orphan helicases, some have undergone significant biochemical scrutiny. The primary characteristics of these helicases, and of those with more clearly defined roles in nucleic acid metabolism, are listed in Table 2.

Study of viral and prokaryotic DNA replication systems has indicated that a prominent characteristic of replicative helicases is their ability to specifically interact with other elements of the replication machinery (see also below). By analogy, it can be safely predicted that cellular replicative helicases will be found to physically interact with the cognate DNA polymerases (DNA polymerase-α:DNA primase, DNA polymerase-δ), polymerase accessory factors (e.g., RF-C), or replication protein A (RP-A). Although such standards can serve only as a rough biochemical compass, their use points to various helicases whose properties are consistent with a replicative function. A few examples of such helicases are described below to indicate the state of the field, but any such list must necessarily be interpreted with caution.

Replication Factor-C-associated DNA Helicase

Replication factor C (RF-C) is a multisubunit complex initially isolated from human cells and found essential for SV40 DNA replication (Stillman 1994; see Hübscher et al., this volume). The general function of RF-C, an accessory factor for DNA polymerase-δ and -ε, appears highly conserved in eukaryotes with a RF-C homolog isolated from *Saccharomyces cerevisiae* (Yoder and Burgers 1991; Fien and Stillman 1992). Burger and colleagues have isolated a 5′→3′ DNA helicase from *S. cerevisiae* that copurifies with RF-C through numerous purification steps (Li et al. 1992a). The 60-kD (apparent) helicase, likely a dimer in solution, prefers ATP for DNA unwinding (K_m[ATP] 60 μM). The helicase retains significant activity in the presence of dATP and is only slightly stimulated by *S. cerevisiae* RP-A (scRP-A).

DNA Helicases Associated with DNA Polymerases

DNA polymerase-α-associated DNA helicases. Thömmes and Hübscher identified a 3′→5′ DNA helicase, termed DNA helicase A (apparent M_r 47,000), that followed the activity of the calf thymus DNA polymerase-α:DNA primase complex during purification (Thömmes and Hübscher 1990; Thömmes et al. 1992). The helicase efficiently utilizes ATP (K_m

Table 2 Eukaryotic nuclear DNA helicases

Helicase	Source (cell line)	Apparent (m.w.)[a]	Nucleotide cofactors[e]	Polarity	Remarks[g]	References
Human DNA helicase I (HDH I)	human (HeLa)	65,000	ATP, dATP	3′→5′	can unwind DNA:RNA hybrids	Tuteja et al. 1990
Human DNA helicase II (HDH II) Ku autoantigen	human (HeLa)	87,000, 72,000[b]	ATP, dATP	3′→5′	functions in double-strand break repair and V(D)J recombination; regulator of DNA-dependent protein kinase	Tuteja et al. 1994; Taccioli et al. 1994; Smider et al. 1994
Human DNA helicase III (HDH III)	human (HeLa)	46,000	ATP, dATP	3′→5′	—	Tuteja et al. 1992
Human DNA helicase IV (HDH IV)	human (HeLa)	100,000	ATP, dATP	5′→3′	can unwind DNA:RNA hybrids	Tuteja et al. 1991
Human DNA helicase V (HDH V)	human (HeLa)	92,000	ATP, dATP	3′→5′	—	Tuteja et al. 1993
XPD/ERCC2	human	87,000[c]	ATP, dATP	5′→3′	functions in nucleotide excision repair; component of BTF2-TFIIH transcription factor	Sung et al. 1993; Drapkin et al. 1994
XPB/ERCC3	human	89,000[c]	ATP	3′→5′	functions in nucleotide excision repair; component of BTF2-TFIIH transcription factor	Schaeffer et al. 1993; Drapkin et al. 1994; Ma et al. 1994a
DNA helicase ε	human (HeLa)	72,000[d]	ATP, dATP > CTP	3′→5′	helicase activity is dependent on hRP-A	Seo et al. 1991

DNA helicase α	human (HeLa)	110,000, 90,000	ATP, dATP > CTP, dCTP	3′→5′	—	Seo and Hurwitz 1993
RIP100	human (HeLa)	100,000	ATP, dATP	3′→5′	associated with RIP60; RIP60 binds to replication origin region of DHFR[h]	Dailey et al. 1990
DNA helicase Q1	human (HeLa)	73,000	ATP, dATP	3′→5′	gene homologous to *E. coli* RecQ gene; identical to human DNA helicase I?	Seki et al. 1994
DNA helicase Q2	human (HeLa)	100,000	ATP	5′→3′	identical to human DNA helicase IV?	Seki et al. 1994
DNA helicase A	calf thymus	47,000[d]	ATP, dATP > CTP	3′→5′	copurifies with ct DNA pol-α:primase; stimulated ~20-fold by ctRPA	Thömmes and Hübscher 1990; Thömmes et al. 1992
DNA helicase B	calf thymus	100,000	dATP, ATP > GTP, CTP, dGTP, UTP, dCTP, dTTP	5′→3′	—	Thömmes et al. 1992
DNA helicase C	calf thymus	40,000[d]	dATP, ATP	5′→3′	—	Thömmes et al. 1992
DNA helicase D	calf thymus	100,000, 45,000	dATP, ATP	5′→3′	stimulated ~10-fold by ctRPA	Thömmes et al. 1992
DNA helicase E	calf thymus	104,000	dATP, ATP	3′→5′	copurifies with DNA pol-ε	Siegal et al. 1992
DNA helicase F	calf thymus	72,000	ATP, dATP, dCTP, UTP, CTP, GTP, dGTP, dTTP	5′→3′	copurifies with RPA	Georgaki et al. 1994

Table 2 (continued)

Helicase	Source (cell line)	Apparent (m.w.)[a]	Nucleotide cofactors[e]	Polarity	Remarks[g]	References
DNA helicase I	calf thymus	200,000	ATP, dATP	3′→5′	—	Zhang and Grosse 1991
DNA helicase II	calf thymus	130,000	ATP, dATP > CTP, dCTP > GTP, dGTP > UTP, dTTP	3′→5′	can unwind dsRNA	Zhang and Grosse 1991, 1994
δ Helicase	fetal calf thymus	57,000	ATP, dATP	5′→3′	copurifies with DNA pol-δ	Li et al. 1992b
DNA helicase B	mouse (FM3A)	58,000	ATP > dATP, dGTP, GTP, CTP, UTP	5′→3′	—	Seki et al. 1988
DNA helicase C1	mouse (FM3A)	? (5.2 S)	ATP	5′→3′	—	Yanagisawa et al. 1992
DNA helicase I	*Xenopus laevis*	62,000 and 75,000?, 107,000?	ATP, dATP	3′→5′	—	Poll et al. 1994
Rad3	*S. cerevisiae*	90,000[c]	ATP, dATP	5′→3′	critical in DNA excision repair; homologous to the XPD gene; constituent of pol II transcription factor b	Sung et al. 1987; Feaver et al. 1993
Rad25	*S. cerevisiae*	95,000[c]	ATP, dATP	n.d. (3′→5′?)[f]	functions in nucleotide excision repair; homologous to XPB; required for pol II transcription	Guzder et al. 1994

Srs2	*S. cerevisiae*	130,000	ATP, dATP	3′→5′	mutants have a hyperrecombination phenotype; involved in error-prone repair?	Rong and Klein 1993
PIF1	*S. cerevisiae*	98,000[c]	ATP, dATP	5′→3′	functions in mtDNA repair and recombination; affects chromosomal telomere length	Lahaye et al. 1991, 1993; Schulz and Zakian 1994
RF-C-associated DNA helicase	*S. cerevisiae*	60,000[d]	ATP > dATP	5′→3′	copurifies with RF-C	Li et al. 1992a
DNA helicase A	*S. cerevisiae*	90,000?	ATP, dATP	5′→3′	copurifies with DNA pol-α:primase	Biswas et al. 1993a,b
DNA helicase B	*S. cerevisiae*	127,000[c]	ATP, dATP	5′→3′	stimulated by scRPA; encoded by the yORF61 gene	Biswas et al. 1995
scHelI	*S. cerevisiae*	135,000	ATP, dATP	5′→3′	—	Bean et al. 1993
DNA helicase III	*S. cerevisiae*	120,000	ATP, dATP	5′→3′	—	Shimizu and Sugino 1993

(n.d.) Not determined.
[a]Determined by SDS-PAGE, unless otherwise indicated.
[b]Exists as a heterodimer in solution.
[c]Molecular weight predicted from the sequence of the cloned gene.
[d]Exists as a homodimer in solution.
[e]Nucleotides that give rise to ≥50% maximal helicase activity are shown.
[f]The polarity of the XPB/ERCC3 DNA helicase, a human homolog of Rad25, has been reported to be 3′→5′ (Drapkin et al. 1994).
[g]"Copurifies with . . ." indicates that the helicase is found to initially copurify with the indicated factor through several chromatographic steps.
[h]RIP60 can be photocrosslinked to a region containing an origin of DNA replication near the dihydrofolate reductase gene from Chinese hamsters.

0.2 mM), dATP (K_m 0.25 mM), and CTP (K_m 0.45 mM). The isolated helicase A is poorly processive, but this can be greatly stimulated, along with the DNA helicase activity, by calf thymus RP-A (ctRP-A; Thömmes et al. 1992). As human RP-A inhibited the activity of helicase A, a physical interaction between ctRP-A and helicase A is suggested.

Similarly, purification of *S. cerevisiae* DNA polymerase-α:DNA primase, using immunoaffinity chromatography, indicated that a 5′→3′ DNA helicase could associate with the bound polymerase up to approximately 0.35 M NaCl (Biswas et al. 1993a). Photoaffinity cross-linking with radiolabeled ATP suggests that the helicase is approximately 90,000 in molecular weight. The helicase activity was stimulated up to threefold by scRP-A.

DNA polymerase-δ-associated DNA helicase. A 5′→3′DNA helicase, termed the δ helicase, cofractionated with fetal calf thymus DNA polymerase-δ through five chromatographic steps (Li et al. 1992b). Upon separation from DNA polymerase-δ, the DNA helicase activity was found associated with an approximately 57-kD polypeptide that could utilize ATP and dATP for DNA unwinding. The use of a preformed DNA fork significantly stimulated δ helicase activity.

DNA polymerase-ε-associated DNA helicase. DNA helicase E was found by Bambara and colleagues to coelute with calf thymus DNA polymerase-ε through four chromatographic steps (Siegal et al. 1992). The 104-kD helicase unwinds DNA in the 3′→5′direction and is most active in the presence of dATP and ATP.

RP-A-associated DNA Helicase

Calf thymus DNA helicase F has been observed to copurify with the cognate RP-A through various chromatographic steps (Georgaki et al. 1994). The 5′→3′activity appears to reside in a 72-kD polypeptide, although gel filtration studies suggest that DNA helicase F can also exist in a dimeric form. The helicase is somewhat unusual in that it can efficiently utilize all common NTPs and dNTPs. Although the enzyme was not active on substrates containing lengthy duplex regions (e.g., 400 bp in length), unwinding was observed in the presence of ctRP-A, whereas the *E. coli* or bacteriophage T4 SSBs had no significant effect.

A variety of DNA helicases are known that either require the cognate RP-A for activity or are greatly stimulated by its addition. These include human DNA helicase ε (Seo et al. 1991), calf thymus DNA helicases A and D (Thömmes et al. 1992), and yeast DNA helicase B (Biswas et al. 1995).

Mitochondrial DNA Helicases

Two mitochondrial DNA helicases have been identified, although neither is unambiguously associated with mitochondrial DNA replication (see Clayton, this volume). The nuclear *PIF1* gene of *S. cerevisiae* encodes a 5′→3′ DNA helicase that is more likely involved in mitochondrial DNA repair and recombination than replication (Lahaye et al. 1991, 1993). More recently, the *PIF1* gene product has also been implicated as an inhibitor of both chromosomal telomere elongation and de novo telomere formation, indicating that the PIF1 helicase has an additional nuclear function (Schulz and Zakian 1994). Bovine mitochondria contain a 3′→5′ DNA helicase, indicating that it is not the mammalian counterpart of PIF1 protein (Hehman and Hauswirth 1992). The role of the bovine enzyme in mitochondrial DNA metabolism is currently unknown.

MECHANISM OF DNA UNWINDING

The mechanism of DNA unwinding by helicases is not currently understood, even though the area is one of vigorous investigation. Studies with various helicases, however, have suggested two distinct models by which DNA unwinding could occur. The two models are distinguished by the manner of helicase movement along the DNA, namely, "rolling" or "creeping" (SenGupta and Borowiec 1992; Wong and Lohman 1992). As demonstrated in Figure 2 for the SV40 large T antigen, each model postulates a DNA helicase entity that utilizes at least two distinct binding sites for the DNA substrate, and incorporates a cycle of NTP binding and hydrolysis that induces changes in the relative affinity of the binding sites for DNA.

The rolling model is most consistent with an oligomeric DNA helicase (Fig. 2A). All DNA helicases are apparently oligomeric (generally dimers or hexamers; Lohman 1993), allowing at least two DNA- and two NTP-binding sites per helicase complex. A basic postulate of this model is that monomers in the helicase complex can bind separately to the ssDNA and dsDNA behind and ahead of the fork, respectively, or to dsDNA and the ssDNA-dsDNA junction itself. The helicase denatures the DNA duplex between these two sites using an active mechanism dependent on NTP hydrolysis. In the process, the general protein/DNA complex formed by the downstream monomer is shifted forward to the upstream monomer. The cycle is completed by rolling of the helicase complex to bind a new helicase monomer to the dsDNA ahead of the fork. The model is supported by studies with the dimeric rep helicase of *E. coli*, indicating that the state of the bound nucleotide (e.g.,

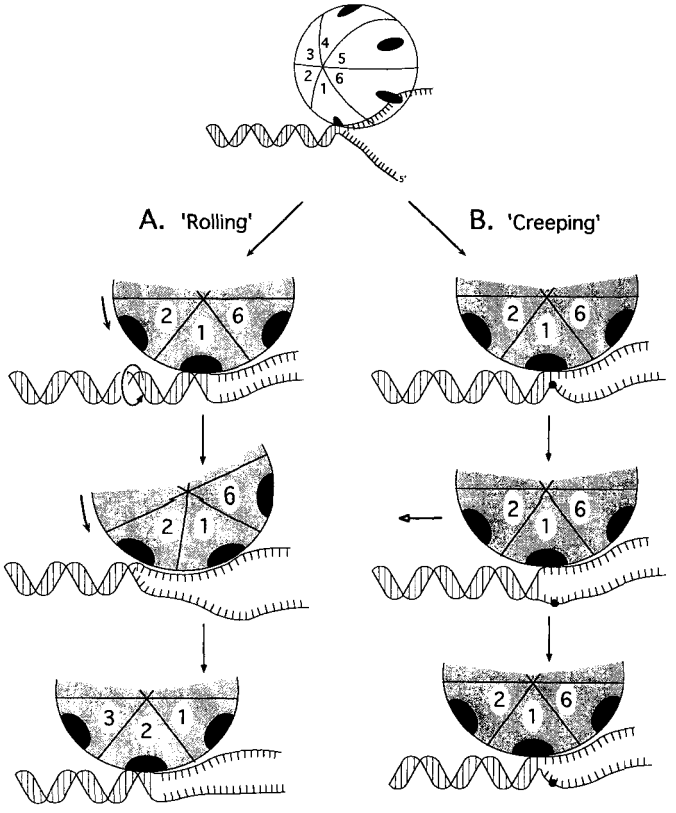

Figure 2 Models of DNA helicase action shown with the SV40 large T antigen. T antigen binds to synthetic replication forks as a hexamer, using contacts with the sugar-phosphate backbone of the strand containing the 3′ ssDNA end (SenGupta and Borowiec 1992). The primary DNA-binding site for each monomer is indicated by a dark patch. Other orientations of T antigen with the fork are possible (see, e.g., SenGupta et al. 1992), but are not shown for the sake of brevity. (*A*) The rolling model. The T-antigen hexamer is initially bound to the fork using the DNA-binding site of monomer 1. In an ATP-dependent process, monomer 2 interacts with the dsDNA roughly 10–15 bp ahead of the fork, denaturing the duplex DNA between the DNA-binding sites of monomers 1 and 2. During this step, DNA rotation is required to allow complete untwisting of the duplex DNA. The hexameric helicase then rolls to regenerate the initial state, except that monomer 2 is now bound to the fork. (*B*) The creeping model. The T-antigen hexamer binds to the fork using the DNA-binding site of monomer 1. Either by an active (ATP-dependent) or passive (ATP-independent) process, a few base pairs (e.g., one or two) denature at the fork. The T-antigen hexamer moves forward along the DNA in an ATP-dependent process and prevents DNA reannealing. T-antigen movement with respect to a black marker on the DNA is shown. In contrast to the rolling model, monomer 1 remains associated with the fork through the unwinding cycle.

ATP or ADP) can alter the relative affinity of the rep subunits for ssDNA or dsDNA (Lohman 1993). The rolling model makes the prediction that the step size of helicase movement is approximately equivalent to the distance between the two DNA-binding sites used sequentially within the multimeric DNA helicase. One potential complication of the rolling model is a requirement for a significant number of DNA base pairs to be unwound per helicase step. For the *E. coli* rep helicase, the step size is predicted to be about 16 nucleotides (Wong and Lohman 1992). Determination of the dimensions of the T-antigen hexamer (see, e.g., Wessel et al. 1992) also leads to a predicted step size of about 10–15 nucleotides, assuming equivalent spacing of DNA-binding sites around the hexameric surface. A single unwinding step for each of the helicases would therefore require the hydrolysis of multiple ATP molecules and torsional rotation of the DNA helix of over 360°.

The creeping (or inchworm) model of DNA unwinding posits that the helicase has contacts with both the ssDNA and dsDNA on each side of the fork. NTP utilization changes the affinity of the dsDNA-binding site to favor the binding of ssDNA. Either by a passive or active process, one or a small number of base pairs become denatured and the helicase (and fork) advance (Fig. 2B). Alternatively, the helicase need only bind to the ssDNA behind the fork. Statistical analysis indicates that the average lifetime of a GC base pair at a replication fork is <0.1 msec (millisecond) at room temperature (Chen et al. 1992). The rate of DNA unwinding for the most rapid helicases is about 1 msec/bp (~1000 bp/sec) and, thus, DNA helicases are not required to forcibly denature the DNA duplex, but rather move passively while thermal forces cause the DNA to "breathe" at the fork. The creeping model does not require the DNA helicase to be oligomeric and instead suggests that the non-DNA-bound subunits serve to interact with other proteins at the fork. Variations of both models are possible and it would be foolhardy to predict that all helicases unwind by identical mechanisms.

A large fraction of the eukaryotic DNA is in the form of chromatin and is bound by factors regulating nucleic acid enzymology (e.g., transcription factors) and structural proteins such as histones. Such proteins have the potential to alter the dynamic properties of the DNA, and it is conceivable that protein/DNA complexes can significantly reduce the rate of duplex denaturation. Although eukaryotic DNA replication complexes have the potential to destabilize protein/DNA complexes ahead of the fork (Wolffe and Brown 1986), chromatin is a relatively inefficient template for SV40 DNA replication in vitro compared to naked DNA (see, e.g., Cheng and Kelly 1989; see Wolffe, this volume). In vitro pro-

gression of a minimal bacteriophage T4 replication fork, powered by the T4 gene 41 helicase, is halted by various protein/DNA complexes such as those formed by histones (Bonne-Andrea et al. 1990) or prokaryotic RNA polymerases or repressors (Bedinger et al. 1983). This inhibition can be relieved by addition of the T4 dda DNA helicase, which allows movement through these barriers (Bedinger et al. 1983; Bonne-Andrea et al. 1990). Thus, one area of potentially fruitful investigation in the study of eukaryotic chromosomal replication is the search for specialized helicases or helicase accessory factors that can overcome barriers caused by common protein/DNA complexes.

INTERACTION OF DNA HELICASES WITH OTHER FACTORS

DNA helicases act to open the DNA duplex for nascent-strand synthesis, and thus it is not unexpected that prokaryotic and eukaryotic replicative helicases physically interact with the cognate DNA polymerase(s), DNA primase, or SSB. In eukaryotic viral systems, both the SV40 T antigen and the BPV E1 protein bind to the human DNA polymerase-α:DNA primase complex (Collins et al. 1993; Dornreiter et al. 1993; Park et al. 1994) and T antigen also interacts with hRP-A (Dornreiter et al. 1992). As noted above, the HSV UL5 protein is contained within a heterotrimeric complex that also contains DNA primase activity and can bind the HSV ICP8. Similarly, the bacteriophage T7 gene 4 protein contains both DNA helicase and DNA primase activities in its 63-kD form and can interact with the T7 DNA polymerase (Nakai and Richardson 1986) and T7 SSB (Kim and Richardson 1994). Comparable interactions between the DNA helicases and the polymerase machinery of *E. coli* and bacteriophage T4 also occur (Marians 1992).

The observation that DNA helicases generally form complexes with the DNA polymerase machinery suggests a requirement for tight coupling of DNA unwinding and DNA synthesis. In lieu of such coupling, a block to DNA polymerase action by template damage could conceivably lead to the generation of lengthy regions of ssDNA between the moving fork and stalled polymerase. This notion suggests that the rate of replication fork progression is generally modulated primarily through the rate of nucleotide incorporation rather than the rate of DNA unwinding. Furthermore, Richardson and colleagues have demonstrated that primer synthesis by the T7 gene 4 helicase/primase slows the rate of DNA unwinding (Debyser et al. 1994). This study indicates that the modulation of the rate of DNA unwinding is also critical for the coordination of leading- and lagging-strand DNA synthesis.

Various viral DNA helicases have also been demonstrated to interact with a variety of other factors (e.g., T antigen and the tumor suppressor p53 protein), although none of these interactions yet appears to be involved in the DNA-unwinding reaction per se. Studies with prokaryotic systems indicate that termination factors exist which bind to specific DNA sites, preventing DNA unwinding by helicases through the site (Kuempel et al. 1989). Replication fork barriers are also found in higher eukaryotes, suggesting that similar factors may exist in chromatin (see Bastia and Mohanty, this volume).

CONCLUDING REMARKS

Significant information is known concerning eukaryotic DNA helicases, but much more remains to be discovered. There are numerous aspects of DNA helicase function that are worthy of study, but four of the most critical are: (1) isolation and characterization of cellular DNA helicases functioning in chromosomal DNA replication; (2) acquisition of knowledge concerning the three-dimensional structure(s) of DNA helicases; (3) characterization of the molecular mechanism(s) by which DNA helicases unwind duplex DNA; and (4) development of agents that target viral or cellular DNA helicase function. The essential nature of DNA helicase function during replication indicates that they potentially represent an Achilles' heel toward which to direct antiviral and anticancer therapy. Advancement toward each of these four points will allow a test of this possibility.

ACKNOWLEDGMENTS

I thank Leonard Blackwell, Natalia Smelkova, Eda Kapsis, and Thomas Gillette for critical comments on the manuscript, and X. Zhou and N. Muzyczka for communicating results prior to publication. Work from the author's laboratory was supported by National Institutes of Health grants AI-29963 and CA-62198, the Pew Biomedical Scholars Program (T88-00457-063), and Kaplan Cancer Center Developmental Funding and Kaplan Cancer Center Support Core Grant (NCI P30CA-16087).

REFERENCES

Alberts, B.M. and L. Frey. 1970. T4 bacteriophage gene 32: A structural protein in the replication and recombination of DNA. *Nature* **227:** 1313–1318.

Bedinger, P., M. Hochstrasser, C.V. Jongeneel, and B.M. Alberts. 1983. Properties of the

T4 bacteriophage DNA replication apparatus: The T4 dda DNA helicase is required to pass a bound RNA polymerase molecule. *Cell* **34:** 115–123.

Bean, D.W., W.E. Kallam, Jr., and S.W. Matson. 1993. Purification and characterization of a DNA helicase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 21783–21790.

Ben-Porat, T., M.L. Blankenship, J.M. DeMarchi, and A.S. Kaplan. 1977. Replication of herpesvirus DNA. III. Rate of DNA elongation. *J. Virol.* **22:** 734–741.

Biswas, E.E., P.H. Chen, and S.B. Biswas. 1993a. DNA helicase associated with DNA polymerase alpha: Isolation by a modified immunoaffinity chromatography. *Biochemistry* **32:** 13393–13398.

Biswas, E.E., C.M. Ewing, and S.B. Biswas. 1993b. Characterization of the DNA-dependent ATPase and a DNA unwinding activity associated with the yeast DNA polymerase α complex. *Biochemistry* **32:** 3020–3026.

Biswas, E.E., P.-H. Chen, J. Leszyk, and S.B. Biswas. 1995. Biochemical and genetic characterization of a replication protein A dependent DNA helicase from the yeast, *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* **206:** 850–856.

Boehmer, P.E. and I.R. Lehman. 1993. Physical interaction between the herpes simplex virus 1 origin-binding protein and single-stranded DNA-binding protein ICP8. *Proc. Natl. Acad. Sci.* **90:** 8444–8448.

Boehmer, P.E., M.S. Dodson, and I.R. Lehman. 1993. The herpes simplex virus type-1 origin binding protein. DNA helicase activity. *J. Biol. Chem.* **268:** 1220–1225.

Bonne-Andrea, C., M.L. Wong, and B.M. Alberts. 1990. In vitro replication through nucleosomes without histone displacement. *Nature* **343:** 719–726.

Bruckner, R.C., J.J. Crute, M.S. Dodson, and I.R. Lehman. 1991. The herpes simplex virus 1 origin binding protein: A DNA helicase. *J. Biol. Chem.* **266:** 2669–2674.

Calder, J.M. and N.D. Stow. 1990. Herpes simplex virus helicase-primase: The UL8 protein is not required for DNA-dependent ATPase and DNA helicase activities. *Nucleic Acids Res.* **18:** 3573–3578.

Challberg, M. and T. Kelly. 1989. Animal virus DNA replication. *Annu. Rev. Biochem.* **58:** 671–717.

Chen, Y.Z., W. Zhuang, and E.W. Prohofsky. 1992. Energy flow considerations and thermal fluctuational opening of DNA base pairs at a replicating fork: Unwinding consistent with observed replication rates. *J. Biomol. Struct. Dyn.* **10:** 415–427.

Cheng, L. and T.J. Kelly. 1989. Transcriptional activator nuclear factor I stimulates the replication of SV40 minichromosomes in vivo and in vitro. *Cell* **59:** 541–551.

Chiang, C.M., M. Ustav, A. Stenlund, T.F. Ho, T.R. Broker, and L.T. Chow. 1992. Viral E1 and E2 proteins support replication of homologous and heterologous papillomaviral origins. *Proc. Natl. Acad. Sci.* **89:** 5799–5803.

Chow, M., J.W. Bodnar, M. Polvino-Bodnar, and D.C. Ward. 1986. Identification and characterization of a protein covalently bound to DNA of minute virus of mice. *J. Virol.* **57:** 1094–1104.

Clertant, P. and I. Seif. 1984. A common function for polyoma virus large-T and papillomavirus E1 proteins? *Nature* **311:** 276–279.

Collins, K.L., A.A. Russo, B.Y. Tseng, and T.J. Kelly. 1993. The role of the 70 kDa subunit of human DNA polymerase alpha in DNA replication. *EMBO J.* **12:** 4555–4566.

Cotmore, S.F. and P. Tattersall. 1988. The NS-1 polypeptide of minute virus of mice is covalently attached to the 5′ termini of duplex replicative form DNA and progeny single strands. *J. Virol.* **62:** 851–860.

Crute, J.J. and I.R. Lehman. 1991. Herpes simplex virus-1 helicase-primase. Physical and

catalytic properties. *J. Biol. Chem.* **266:** 4484–4488.

Crute, J.J., E.S. Mocarski, and I.R. Lehman. 1988. A DNA helicase induced by herpes simplex virus type 1. *Nucleic Acids Res.* **16:** 6585–6596.

Crute, J.J., R.C. Bruckner, M.S. Dodson, and I.R. Lehman. 1991. Herpes simplex-1 helicase-primase. Identification of two nucleoside triphosphatase sites that promote DNA helicase action. *J. Biol. Chem.* **266:** 21252–21256.

Crute, J.J., T. Tsurumi, L.A. Zhu, S.K. Weller, P.D. Olivo, M.D. Challberg, E.S. Mocarski, and I.R. Lehman. 1989. Herpes simplex virus 1 helicase-primase: A complex of three herpes-encoded gene products. *Proc. Natl. Acad. Sci.* **86:** 2186–2189.

Dailey, L., M.S. Caddle, N. Heintz, and N.H. Heintz. 1990. Purification of RIP60 and RIP100, mammalian proteins with origin-specific DNA-binding and ATP-dependent DNA helicase activities. *Mol. Cell. Biol.* **10:** 6225–6235.

Dean, F.B., J.A. Borowiec, T. Eki, and J. Hurwitz. 1992. The simian virus 40 T antigen double hexamer assembles around the DNA at the replication origin. *J. Biol. Chem.* **267:** 14129–14137.

Debyser, Z., S. Tabor, and C.C. Richardson. 1994. Coordination of leading and lagging strand DNA synthesis at the replication fork of bacteriophage T7. *Cell* **77:** 157–166.

Del Vecchio, A.M., H. Romanczuk, P.M. Howley, and C.C. Baker. 1992. Transient replication of human papillomavirus DNAs. *J. Virol.* **66:** 5949–5958.

Dodson, M.S. and I.R. Lehman. 1991. Association of DNA helicase and primase activities with a subassembly of the herpes simplex virus 1 helicase-primase composed of the UL5 and UL52 gene products. *Proc. Natl. Acad. Sci.* **88:** 1105–1109.

Dodson, M.S., J.J. Crute, R.C. Bruckner, and I.R. Lehman. 1989. Overexpression and assembly of the herpes simplex virus type 1 helicase-primase in insect cells. *J. Biol. Chem.* **264:** 20835–20838.

Dornreiter, I., W.C. Copeland, and T.S. Wang. 1993. Initiation of simian virus 40 DNA replication requires the interaction of a specific domain of human DNA polymerase alpha with large T antigen. *Mol. Cell. Biol.* **13:** 809–820.

Dornreiter, I., L.F. Erdile, I.U. Gilbert, D. von Winkler, T.J. Kelly, and E. Fanning. 1992. Interaction of DNA polymerase alpha-primase with cellular replication protein A and SV40 T antigen. *EMBO J.* **11:** 769–776.

Drapkin, R., J.T. Reardon, A. Ansari, J.C. Huang, L. Zawel, K. Ahn, A. Sancar, and D. Reinberg. 1994. Dual role of TFIIH in DNA excision repair and in transcription by RNA polymerase II. *Nature* **368:** 769–772.

Earnshaw, D.L. and R.L. Jarvest. 1994. Characterisation of the nucleotide and DNA coeffector binding sites of the herpes simplex virus type 1 (HSV-1) encoded helicase-primase complex and UL9 origin binding protein. *Biochem. Biophys. Res. Commun.* **199:** 1333–1340.

Elias, P. and I.R. Lehman. 1988. Interaction of origin binding protein with an origin of replication of herpes simplex virus 1. *Proc. Natl. Acad. Sci.* **85:** 2959–2963.

Fanning, E. and R. Knippers. 1992. Structure and function of simian virus 40 large tumor antigen. *Annu. Rev. Biochem.* **61:** 55–85.

Feaver, W.J., J.Q. Svejstrup, L. Bardwell, A.J. Bardwell, S. Buratowski, K.D. Gulyas, T.F. Donahue, E.C. Friedberg, and R.D. Kornberg. 1993. Dual roles of a multiprotein complex from *S. cerevisiae* in transcription and DNA repair. *Cell* **75:** 1379–1387.

Fien, K. and B. Stillman. 1992. Identification of replication factor C from *Saccharomyces cerevisiae*: A component of the leading-strand DNA replication complex. *Mol. Cell. Biol.* **12:** 155–163.

Fierer, D.S. and M.D. Challberg. 1992. Purification and characterization of UL9, the herpes simplex virus type 1 origin-binding protein. *J. Biol. Chem.* **66:** 3986–3995.

Fry, D.C., S.A. Kuby, and A.S. Mildvan. 1986. ATP-binding site of adenylate kinase: Mechanistic implications of its homology with *ras*-encoded p21, F_1-ATPase, and other nucleotide-binding proteins. *Proc. Natl. Acad. Sci.* **83:** 907–911.

Georgaki, A., N. Tuteja, B. Sturzenegger, and U. Hübscher. 1994. Calf thymus DNA helicase F, a replication protein A copurifying enzyme. *Nucleic Acids Res.* **22:** 1128–1134.

Goetz, G.S., F.B. Dean, J. Hurwitz, and S.W. Matson. 1988. The unwinding of duplex regions in DNA by the simian virus 40 large tumor antigen-associated DNA helicase activity. *J. Biol. Chem.* **263:** 383–392.

Gorbalenya, A.E., E.V. Koonin, A.P. Donchenko, and V.M. Blinov. 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair, and expression of DNA and RNA genomes. *Nucleic Acids Res.* **17:** 4713–4730.

Guzder, S.N., P. Sung, V. Bailly, L. Prakash, and S. Prakash. 1994. RAD25 is a DNA helicase required for DNA repair and RNA polymerase II transcription. *Nature* **369:** 578–581.

Hehman, G.L. and W.W. Hauswirth. 1992. DNA helicase from mammalian mitochondria. *Proc. Natl. Acad. Sci.* **89:** 8562–8566.

Hodgman, T.C. 1988. A new superfamily of replicative proteins. *Nature* **333:** 22–23.

Houston, P. and T. Kodadek. 1994. Spectrophotometric assay for enzyme-mediated unwinding of double-stranded DNA. *Proc. Natl. Acad. Sci.* **91:** 5471–5474.

Hughes, F.J. and M.A. Romanos. 1993. E1 protein of human papillomavirus is a DNA helicase/ATPase. *Nucleic Acids Res.* **21:** 5817–5823.

Im, D.-S. and N. Muzyczka. 1989. Factors that bind to adeno-associated virus terminal repeats. *J. Virol.* **63:** 3095–3104.

———. 1990. The AAV origin binding protein Rep68 is an ATP-dependent site-specific endonuclease with DNA helicase activity. *Cell* **61:** 447–457.

———. 1992. Partial purification of adeno-associated virus Rep78, Rep52, and Rep40 and their biochemical characterization. *J. Virol.* **66:** 1119–1128.

Kim, Y.T. and C.C. Richardson. 1994. Acidic carboxyl-terminal domain of gene 2.5 protein of bacteriophage T7 is essential for protein-protein interactions. *J. Biol. Chem.* **269:** 5270–5278.

Kornberg, A. and T.A. Baker. 1992. *DNA Replication*. W.H. Freeman, New York.

Kuempel, P.L., A.J. Pelletier, and T.M. Hill. 1989. Tus and the terminators: The arrest of replication in prokaryotes. *Cell* **59:** 581–583.

Labow, M.A., P.L. Hermonat, and K.I. Berns. 1989. Positive and negative autoregulation of the adeno-associated virus type 2 genome. *J. Virol.* **60:** 251–258.

Lahaye, A., S. Leterme, and F. Foury. 1993. PIF1 DNA helicase from *Saccharomyces cerevisiae*. Biochemical characterization of the enzyme. *J. Biol. Chem.* **268:** 26155–26161.

Lahaye, A., H. Stahl, D. Thines-Sempoux, and F. Foury. 1991. PIF1: A DNA helicase in yeast mitochondria. *EMBO J.* **10:** 997–1007.

LeBowitz, J.H. and R. McMacken. 1986. The *Escherichia coli* dnaB replication protein is a DNA helicase. *J. Biol. Chem.* **261:** 4738–4748.

Li, X., B.L. Yoder, and P.M. Burgers. 1992a. A *Saccharomyces cerevisiae* DNA helicase associated with replication factor C. *J. Biol. Chem.* **267:** 25321–25327.

Li, X., C.-K. Tan, A.G. So and K.M. Downey. 1992b. Purification and characterization of

δ helicase from fetal calf thymus. *Biochemistry* **31:** 3507–3513.

Lin, H.-J., R.H. Upson, and D.T. Simmons. 1992. Nonspecific DNA binding activity of simian virus 40 large T antigen: Evidence for the cooperation of two regions for full activity. *J. Virol.* **66:** 5443–5452.

Lindenbaum, J.O., J. Field, and J. Hurwitz. 1986. The adenovirus DNA binding protein and adenovirus DNA polymerase interact to catalyze elongation of primed DNA templates. *J. Biol. Chem.* **261:** 10218–10227.

Lohman, T.M. 1993. Helicase-catalyzed DNA unwinding. *J. Biol. Chem.* **268:** 2269–2272.

Lusky, M., J. Hurwitz, and Y.S. Seo. 1993. Cooperative assembly of the bovine papilloma virus E1 and E2 proteins on the replication origin requires an intact E2 binding site. *J. Biol. Chem.* **268:** 15795–15803.

Ma, L., E.D. Siemssen, H.M. Noteborn, and A.J. van der Eb. 1994a. The xeroderma pigmentosum group B protein ERCC3 produced in the baculovirus system exhibits DNA helicase activity. *Nucleic Acids Res.* **22:** 4095–4102.

Ma, L., A. Westbroek, A.G. Jochemsen, G. Weeda, A. Bosch, D. Bootsma, J.H. Hoeijmakers, and A.J. van der Eb. 1994b. Mutational analysis of ERCC3, which is involved in DNA repair and transcription initiation: Identification of domains essential for the DNA repair function. *Mol. Cell. Biol.* **14:** 4126–4134.

Marians, K.J. 1992. Prokaryotic DNA replication. *Annu. Rev. Biochem.* **61:** 673–719.

Martinez, R., L. Shao, and S.K. Weller. 1992. The conserved helicase motifs of the herpes simplex virus type 1 origin-binding protein UL9 are important for function. *J. Virol.* **66:** 6735–6746.

Matson, S.W. 1986. *Escherichia coli* helicase II (urvD gene product) translocates unidirectionally in a 3′ to 5′ direction. *J. Biol. Chem.* **261:** 10169–10175.

Matson, S.W., D.W. Bean, and J.W. George. 1994. DNA helicases: Enzymes with essential roles in all aspects of DNA metabolism. *BioEssays* **16:** 13–22.

Matson, S.W., S. Tabor, and C.C. Richardson. 1983. The gene 4 protein of bacteriophage T7. Characterization of helicase activity. *J. Biol. Chem.* **258:** 14017–14024.

McVey, D., S. Ray, Y. Gluzman, L. Berger, A.G. Wildeman, D.R. Marshak, and P. Tegtmeyer. 1993. cdc2 phosphorylation of threonine 124 activates the origin-unwinding functions of simian virus 40 T antigen. *J. Virol.* **67:** 5206–5215.

Mendelson, E., J.P. Trempe, and B.J. Carter. 1986. Identification of the *trans*-acting Rep proteins of adeno-associated virus by antibodies to a synthetic oligopeptide. *J. Virol.* **60:** 823–832.

Moarefi, I.F., D. Small, I. Gilbert, M. Höpfner, S.K. Randall, C. Schneider, A.A.R. Russo, U. Ramsperger, A.K. Arthur, H. Stahl, T.J. Kelly, and E. Fanning. 1993. Mutation of the cyclin-dependent kinase phosphorylation site in simian virus 40 (SV40) large T antigen specifically blocks SV40 origin DNA unwinding. *J. Virol.* **67:** 4992–5002.

Mohr, I.J., M.P. Fairman, B. Stillman, and Y. Gluzman. 1989. Large T-antigen mutants define multiple steps in the initiation of simian virus 40 DNA replication. *J. Virol.* **63:** 4181–4188.

Mohr, I.J., R. Clark, S. Sun, E.J. Androphy, P. MacPherson, and M.R. Botchan. 1990. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. *Science* **250:** 1694–1699.

Murakami, Y. and J. Hurwitz. 1993. Functional interactions between SV40 T antigen and other replication proteins at the replication fork. *J. Biol. Chem.* **268:** 11008–11017.

Nakai, H. and C.C. Richardson. 1986. Interactions of the DNA polymerase and gene 4 protein of bacteriophage T7. Protein-protein and protein-DNA interactions involved in RNA-primed DNA synthesis. *J. Biol. Chem.* **261:** 15208–15216.

Olivo, P.D., N.J. Nelson, and M.D. Challberg. 1988. Herpes simplex virus DNA replication: The UL9 gene encodes an origin-binding protein. *Proc. Natl. Acad. Sci.* **85:** 5414–5418.

Park, P., W. Copeland, L. Yang, T. Wang, M.R. Botchan, and I.J. Mohr. 1994. The cellular DNA polymerase alpha-primase is required for papillomavirus DNA replication and associates with the viral E1 helicase. *Proc. Natl. Acad. Sci.* **91:** 8700–8704.

Poll, E.H., J. Harrison, A. Umthun, D.L. Dobbs, and R.M. Benbow. 1994. *Xenopus laevis* ovarian DNA helicase I: A 3′ to 5′ helicase that unwinds short duplexes. *Biochemistry* **33:** 3841–3847.

Rabkin, S.D. and B. Hanlon. 1990. Herpes simplex virus DNA synthesis at a preformed replication fork in vitro. *J. Virol.* **64:** 4957–4967.

Raney, K.D., L.C. Sowers, D.P. Millar, and S.J. Benkovic. 1994. A fluorescence-based assay for monitoring helicase activity. *Proc. Natl. Acad. Sci.* **91:** 6644–6648.

Roman, L.J. and S.C. Kowalczykowski. 1989. Characterization of the helicase activity of the *Escherichia coli* RecBCD enzyme using a novel helicase assay. *Biochemistry* **28:** 2863–2873.

Rong, L. and H.L. Klein. 1993. Purification and characterization of the SRS2 DNA helicase of the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 1252–1259.

Santucci, S., E. Androphy, C. Bonne-Andréa, and P. Clertant. 1990. Proteins encoded by the bovine papillomavirus E1 open reading frame: Expression in heterologous systems and in virally transformed cells. *J. Virol.* **64:** 6027–6033.

Saraste, M., P.R. Sibbald, and A. Wittinghofer. 1990. The P-loop—Common motif in ATP- and GTP-binding proteins. *Trends Biochem. Sci.* **15:** 430–434.

Schaeffer, L., R. Roy, S. Humbert, V. Moncollin, W. Vermeulen, J.H.J. Hoeijmakers, P. Chambon, and J.-M. Egly. 1993. DNA repair helicase: A component of BTF2 (TFIIH) basic transcription factor. *Science* **260:** 58–63.

Scheffner, M., R. Knippers, and H. Stahl. 1989. RNA unwinding activity of SV40 large T antigen. *Cell* **57:** 955–963.

Schulz, V.P. and V.A. Zakian. 1994. The *Saccharomyces PIF1* DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell* **76:** 145–155.

Seki, M., T. Enomoto, J. Yanagisawa, F. Hanaoka, and M. Ui. 1988. Further characterization of the DNA helicase activity of mouse DNA-dependent adenosinetriphosphatase B (DNA helicase B). *Biochemistry* **27:** 1766–1771.

Seki, M., J. Yanagisawa, T. Kohda, T. Sonoyama, M. Ui, and T. Enomoto. 1994. Purification of two DNA-dependent adenosinetriphosphatases having DNA helicase activity from HeLa cells and comparison of the properties of the two enzymes. *J. Biochem.* **115:** 523–531.

Seki, M., T. Enomoto, T. Eki, A. Miyajima, Y. Murakami, F. Hanaoka, and M. Ui. 1990. DNA helicase and nucleoside-5′-triphosphatase activities of polyoma virus large tumor antigen. *Biochemistry* **29:** 1003–1009.

SenGupta, D. and J.A. Borowiec. 1992. Strand-specific recognition of a synthetic DNA replication fork by the SV40 large tumor antigen. *Science* **256:** 1656–1661.

———. 1994. Strand and face: The topography of interactions between the SV40 origin of replication and T antigen during the initiation of replication. *EMBO J.* **13:** 982–992.

SenGupta, D.J., L.J. Blackwell, T. Gillette, and J.A. Borowiec. 1992. Recognition of

model DNA replication forks by the SV40 large tumor antigen. *Chromosoma* **102:** S46–S51.

Seo, Y.-S. and J. Hurwitz. 1993. Isolation of helicase α, a DNA helicase from HeLa cells stimulated by a fork structure and single-stranded DNA binding proteins. *J. Biol. Chem.* **268:** 10282–10295.

Seo, Y.-S., S.-H. Lee, and J. Hurwitz. 1991. Isolation of a DNA helicase from Hela cells requiring the multisubunit human single-stranded DNA-binding protein for activity. *J. Biol. Chem.* **266:** 13161–13170.

Seo, Y.-S., F. Müller, M. Lusky, and J. Hurwitz. 1993. Bovine papilloma viral (BPV)-encoded E1 protein contains multiple activities required for BPV DNA replication. *Proc. Natl. Acad. Sci.* **90:** 702–706.

Shimizu, K. and A. Sugino. 1993. Purification and characterization of DNA helicase III from the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 9578–9584.

Siegal, G., J.J. Turchi, C.B. Jessee, T.W. Myers, and R.A. Bambara. 1992. A novel DNA helicase from calf thymus. *J. Biol. Chem.* **267:** 13629–13635.

Smider, V., W.K. Rathmell, M.R. Lieber, and G. Chu. 1994. Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. *Science* **266:** 288–291.

Srivastava, A., E.W. Lusby, and K.I. Berns. 1983. Nucleotide sequence and organization of the adeno-associated virus 2 genome. *J. Virol.* **45:** 555–564.

Stahl, H., P. Dröge, and R. Knippers. 1986. DNA helicase activity of SV40 large tumor antigen. *EMBO J.* **5:** 1939–1944.

Stillman, B. 1994. Smart machines at the DNA replication fork. *Cell* **78:** 725–728.

Sun, S., L. Thorner, M. Lentz, P. MacPherson, and M. Botchan. 1990. Identification of a 68-kilodalton nuclear ATP-binding phosphoprotein encoded by bovine papillomavirus type 1. *J. Virol.* **64:** 5093–5105.

Sung, P., L. Prakash, S.W. Matson, and S. Prakash. 1987. RAD3 protein of *Saccharomyces cerevisiae* is a DNA helicase. *Proc. Natl. Acad. Sci.* **84:** 8951–8955.

Sung, P., V. Bailly, C. Weber, L.H. Thompson, L. Prakash, and S. Prakash. 1993. Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature* **365:** 852–855.

Taccioli, G.E., T.M. Gottlieb, T. Blunt, A. Priestley, J. Demengeot, R. Mizuta, A.R. Lehmann, F.W. Alt, S.P. Jackson, and P.A. Jeggo. 1994. Ku80: Product of the *XRCC5* gene and its role in DNA repair and V(D)J recombination. *Science* **265:** 1442–1445.

Thömmes, P. and U. Hübscher. 1990. DNA helicase from calf thymus. Purification to apparent homogeneity and biochemical characterization of the enzyme. *J. Biol. Chem.* **265:** 14347–14354.

———. 1992. Eukaryotic DNA helicases: Essential enzymes for DNA transactions. *Chromosoma* **101:** 467–473.

Thömmes, P., E. Ferrari, R. Jessberger, and U. Hübscher. 1992. Four different DNA helicases from calf thymus. *J. Biol. Chem.* **267:** 6063–6073.

Tuteja, N., K. Rahman, R. Tuteja, and A. Falaschi. 1991. DNA helicase IV from HeLa cells. *Nucleic Acids Res.* **19:** 3613–3618.

———. 1993. Human DNA helicase V, a novel DNA unwinding enzyme from HeLa cells. *Nucleic Acids Res.* **21:** 2323–2329.

Tuteja, N., R. Tuteja, K. Rahman, L.Y. Kang, and A. Falaschi. 1990. A DNA helicase from human cells. *Nucleic Acids Res.* **18:** 6785–6792.

Tuteja, N., K. Rahman, R. Tuteja, A. Ochem, D. Skopac, and A. Falaschi. 1992. DNA helicase III from HeLa cells: An enzyme that acts preferentially on partially unwound

DNA duplexes. *Nucleic Acids Res.* **20:** 5329–5337.

Tuteja, N., R. Tuteja, A. Ochem, P. Taneja, N.W. Huang, A. Simoncsits, S. Susic, K. Rahman, L. Marusic, J. Chen, J. Zhang, S. Wang, S. Pongor, and A. Falaschi. 1994. Human DNA helicase II: A novel DNA unwinding enzyme identified as the Ku autoantigen. *EMBO J.* **13:** 4991–5001.

Ustav, M. and A. Stenlund. 1991. Transient replication of BPV-1 requires two viral polypeptides encoded by the E1 and E2 open reading frames. *EMBO J.* **10:** 449–457.

Venkatesan, M., L.L. Silver, and N.G. Nossal. 1982. Bacteriophage T4 gene 41 protein, required for the synthesis of RNA primers, is also a DNA helicase. *J. Biol. Chem.* **257:** 12426–12434.

Walker, J.E., M. Saraste, M.J. Runswick, and N.J. Gay. 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1:** 945–951.

Wang, E.H. and C. Prives. 1991. DNA helicase and duplex DNA fragment unwinding activities of polyoma and simian virus 40 large T antigen display similarities and differences. *J. Biol. Chem.* **266:** 12668–12675.

Wessel, R., J. Schweizer, and H. Stahl. 1992. Simian virus 40 T-antigen DNA helicase is a hexamer which forms a binary complex during bidirectional unwinding from the viral origin of DNA replication. *J. Virol.* **66:** 804–815.

Wiekowski, M., M.W. Schwarz, and H. Stahl. 1988. Simian virus 40 large T antigen DNA helicase. *J. Biol. Chem.* **263:** 436–442.

Wilson, G.M., H.K. Jindal, D.E. Yeung, W. Chen, and C.R. Astell. 1991. Expression of minute virus of mice major nonstructural protein in insect cells: Purification and identification of ATPase and helicase activities. *Virology* **185:** 90–98.

Wolffe, A.P. and D.D. Brown. 1986. DNA replication in vitro erases a *Xenopus* 5S RNA gene transcription complex. *Cell* **47:** 217–227.

Wong, I. and T.M. Lohman. 1992. Allosteric effects of nucleotide cofactors on *Escherichia coli* Rep helicase-DNA binding. *Science* **256:** 350–355.

Wun-Kim, K. and D.T. Simmons. 1990. Mapping of helicase and helicase substrate-binding domains on simian virus 40 large T antigen. *J. Virol.* **64:** 2014–2020.

Yanagisawa, J., M. Seki, T. Kohda, E. Enomoto, and M. Ui. 1992. DNA-dependent adenosinetriphosphatase C1 from mouse FM3A cells has DNA helicase activity. *J. Biol. Chem.* **267:** 3644–3649.

Yang, L., R. Li, I.J. Mohr, R. Clark, and M.R. Botchan. 1991. Activation of BPV-1 replication *in vitro* by the transcription factor E2. *Nature* **353:** 628–632.

Yang, L., I. Mohr, E. Fouts, D.A. Lim, M. Nohaile, and M. Botchan. 1993. The E1 protein of bovine papilloma virus 1 is an ATP-dependent DNA helicase. *Proc. Natl. Acad. Sci.* **90:** 5086–5090.

Yoder, B.L. and P.M. Burgers. 1991. *Saccharomyces cerevisiae* replication factor C. I. Purification and characterization of its ATPase activity. *J. Biol. Chem.* **266:** 22689–22697.

Zhang, S. and F. Grosse. 1991. Purification and characterization of two DNA helicases from calf thymus nuclei. *J. Biol. Chem.* **266:** 20483–20490.

———. 1994. Nuclear DNA helicase II unwinds both DNA and RNA. *Biochemistry* **33:** 3906–3912.

Zhu, L.A. and S.K. Weller. 1992. The six conserved helicase motifs of the UL5 gene product, a component of the herpes simplex virus type 1 helicase-primase, are essential for its function. *J. Virol.* **66:** 469–479.