DNA helicases are ubiquitous molecular motor proteins which harness the chemical free energy of ATP hydrolysis to catalyze the unwinding of energetically stable duplex DNA, and thus play important roles in nearly all aspects of nucleic acid metabolism, including replication, repair, recombination, and transcription. They break the hydrogen bonds between the duplex helix and move unidirectionally along the bound strand. All helicases are also translocases and DNA-dependent ATPases. Most contain conserved helicase motifs that act as an engine to power DNA unwinding. All DNA helicases share some common properties, including nucleic acid binding, NTP binding and hydrolysis, and unwinding of duplex DNA in the 3'→5' or 5'→3' direction.

The minichromosome maintenance (Mcm) protein complex (Mcm4/6/7) provides a DNA-unwinding function at the origin of replication in all eukaryotes and may act as a licensing factor for DNA replication. The RecQ family of helicases is highly conserved from bacteria to humans and is required for the maintenance of genome integrity. They have also been implicated in a variety of human genetic disorders. Since the discovery of the first DNA helicase in Escherichia coli in 1976, and the first eukaryotic one in the lily in 1978, a large number of these enzymes have been isolated from both prokaryotic and eukaryotic systems, and the number is still growing. In this review we cover the historical background of DNA helicases, helicase assays, biochemical properties, prokaryotic and eukaryotic DNA helicases including Mcm proteins and the RecQ family of helicases. The properties of most of the known DNA helicases from prokaryotic and eukaryotic systems, including viruses and bacteriophages, are summarized in tables.

**Keywords:** DNA helicase; helicase assay; recombination; repair; replication.

Genetic information is locked in the duplex DNA of the genome. To access this information for many important biological processes, the duplex DNA has to be transiently unwound. For this purpose, a diverse class of enzymes has evolved, known as ‘DNA helicases’. They catalyze the unwinding of duplex DNA and thus play an essential role in many cellular processes, including DNA replication, repair, recombination, and transcription [1–3]. They are also thought to be motor proteins translocating along DNA using nucleoside triphosphate hydrolysis as the source of energy [4]. Multiple DNA helicases have been isolated from different organisms [1–3,5]. Most contain short conserved amino-acid fingerprints called helicase motifs. They are named Q, Ia, Ib, II, III, IV, V and VI. The helicases of this family are also called DEAD/H helicases [6–8]. This review focuses on the general aspects of DNA helicases including historical background, biochemical assays and biochemical properties. In addition, we have also covered the minichromosome maintenance (Mcm) proteins and RecQ family of helicases and summarized the characteristics of most of the known DNA helicases from prokaryotic and eukaryotic systems.

**Historical perspective**

DNA helicase was first discovered in E. coli in 1976 and classified as a ‘DNA unwinding enzyme’ [9]. In 1978 the existence of the first eukaryotic DNA helicase was reported in the lily [10]. Since then, several different DNA helicases have been isolated from many organisms from both prokaryotic and eukaryotic systems. Recently, a new helicase motif, named the ‘Q motif’, has been identified in the DEAD-box family of helicases [8]. The major milestones in the discovery of DNA helicases are summarized in Table 1.

**Biochemical assay**

The most widely accepted and direct assay for measuring helicase activity in vitro was developed almost simultaneously by the Nossal and Richardson groups [11,12]. Helicase partially unwinds duplex DNA substrate (32P-labeled oligonucleotide annealed to a longer ssDNA molecule) yielding two ssDNA molecules of different sizes which are resolved from the starting duplex by electrophoresis followed by autoradiography (Fig. 1). The radioactive label in the DNA permits direct visualization (Fig. 1B) and
quantitation of the results. Several other assays have also been developed but are not commonly used: for example, a rapid quench-flow method [13], fluorescence-based assays [14], filtration assay [15], a scintillation proximity assay [16], a time resolved fluorescence resonance energy transfer assay [17], an assay based on flashplate technology [18], homogeneous time-resolved fluorescence quenching assay and electrochemiluminescence-based helicase assay [19]. Xu et al. [20] recently developed a new method for simultaneous monitoring of DNA binding and helicase-catalyzed DNA unwinding by fluorescence polarization.

Biochemical properties

The basic unwinding reaction catalysed by all of the known DNA helicases is similar. They all share the following common biochemical properties: nucleic acid binding; NTP binding; nucleic acid-stimulated hydrolysis of NTP; NTP/dNTP hydrolysis-dependent unwinding of duplex nucleic acids with specific polarity.

Binding to nucleic acid

Helicases usually need ssDNA as a loading zone where they bind in a sequence-independent manner and translocate unidirectionally. In general, they bind to ssDNA with higher affinity than to dsDNA. However, RecBCD, simian virus 40 (SV40) large antigen, and RuvB helicases preferentially bind to dsDNA. Many helicases need a replication fork-like structure on the substrate for optimum unwinding. Some DNA helicases such as RecBCD, UvrD, Rep and RecQ from E. coli and SV40 large T antigen can also initiate unwinding from the ends of blunt-ended duplex DNA [2]. RecG, RuvA and RuvB helicases of E. coli specifically recognize Holliday junctions [21,22]. Usually oligomeric helicases carry multiple ssDNA-binding sites, although all

Table 1. Historical background of DNA helicases.

| Year     | Discovery                                                                 |
|----------|---------------------------------------------------------------------------|
| 1953–76  | Prehelicase years                                                         |
| 1953     | Duplex structure of DNA was solved [55]                                   |
| 1967     | Rep protein was the first helicase in E. coli to be identified by genetic criteria [56] |
| 1976     | Hoffman-Berling isolated first DNA helicase (helicase I, a tral gene product) from E. coli [9] |
| 1978     | Existence of the first eukaryotic DNA helicase was reported from the lily [10] |
| 1979     | E. coli Rep protein was shown to contain DNA helicase activity [57]        |
| 1982     | First bacteriophage protein reported as DNA helicase was T4 gene 41protein [11] |
| 1982–83  | A direct biochemical assay (strand displacement assay) for measuring helicase activity was developed [11,12] |
| 1985     | First mammalian DNA helicase was reported from calf thymus [58]           |
| 1986     | First viral encoded protein reported as DNA helicase was SV40 large tumor antigen [59] |
| 1988     | First yeast protein reported as DNA helicase was ATPase III [60]          |
| 1989     | Two helicase superfamilies (SF1 and SF2) were reported [62]               |
| 1990     | DEAD box helicase family was identified [7]                               |
| 1991     | First human DNA helicase (HDH) reported in the purified form [63]         |
| 1992     | E. coli RecQ protein was reported as DNA helicase [43]                    |
| 1996     | First chloroplast DNA helicase was reported in the purified form from the pea [65] |
| 1997     | Crystalization of First DNA helicase (PcrA from thermophilic bacterium) was reported [66] |
| 1998     | Werner syndrome protein (WRN) was reported as a DNA helicase [67]         |
| 1999     | Bloom’s syndrome (BLM) gene product was reported as a DNA helicase [68]  |
| 2000     | Sgs1 (slow growth suppressor), a RecQ helicase from yeast, was reported as a DNA helicase [69] |
| 2000     | First plant DNA helicase gene (PDHA5) encoding the biochemically active enzyme was cloned [30] |
| 2002     | First eIF-4A (from pea) was reported as a DNA helicase [30]               |
| 2003     | A new helicase motif (Q motif) was identified in DEAD box helicases [8]    |
| 2003     | Presence of Helitron insertion, a DNA helicase bearing transposable element, reported from maize genome [70] |

Fig. 1. Scheme of biochemical assay for measuring unwinding activity of ATP/Mg2+-dependent DNA helicase (A) and autoradiogram of the gel (B). (A) Asterisks denote the 32P-labeled end of the DNA. The partial duplex DNA helicase substrate was prepared by annealing the radiolabeled DNA oligo to M13 ssDNA (circular) as described previously [65]. (B) Lane 1, reaction without enzyme; lane 2, heat-denatured substrate; lane 3, reaction in presence of DNA helicase enzyme. S, Substrate; UD, unwound DNA.
the sites may not simultaneously bind to the DNA. For example, E. coli DnaB [23] and bacteriophage T7gp4 [24] DNA helicases contain six DNA-binding sites even though only one or two subunits interact with ssDNA at a time.

**NTP binding and nucleic acid-stimulated hydrolysis of NTP**

All DNA helicases bind NTP and exhibit nucleic acid-dependent intrinsic NTPase activity necessary for duplex unwinding. In general, replicative hexameric helicases bind ssDNA tightly in the presence of NTP, and weakly in the presence of NDP. This binding results in a conformational change in homo-oligomeric helicases and thereby affects the assembly state of the protein. NTP does not bind with equal affinity to all the sites in hexameric helicases. In general, the DNA helicases are poor NTPases in the absence of ssDNA and their NTPase activities are stimulated by the presence of ssDNA.

**Polarity of DNA unwinding**

DNA helicases exhibit specific polarity, which is defined as the direction of DNA helicase movement on initially bound ssDNA template (i.e. 3’ to 5’ or 5’ to 3’) with respect to the polarity of the sugar phosphate backbone. The polarity of unwinding by DNA helicase can usually be determined by using a DNA substrate consisting of a linear ssDNA template with duplex regions near the end(s). Possible structures of substrates that can be used for directional study *in vitro* are shown in Fig. 2. For helicases involved in DNA replication, the polarity of the reaction is strongly indicative of helicase placement on the leading (3’ to 5’ polarity) or lagging (5’ to 3’ polarity) strands. It has been reported that RecBCD contains a bipartite enzyme activity, where RecB and RecD components of the complex unwind DNA from 3’ to 5’ and 5’ to 3’ directions, respectively [25]. Interestingly, PcrA helicase from *Bacillus anthracis* showed robust 3’ to 5’ as well as 5’ to 3’ helicase activities, with substrates containing a duplex region and a 3’ or 5’ ss poly(dT) tail [26]. Recently, Constantinesco *et al.* [27] have shown that the HerA DNA helicase from thermophilic archaea is able to utilize either 3’ or 5’ ssDNA extensions for loading and subsequent DNA duplex unwinding.

**Monomeric and oligomeric nature of helicases**

The fact that many helicases function as hexamers, as well as the necessity for multiple DNA-binding sites, has led to the suggestion that oligomerization may be necessary for helicase function. The property of an oligomeric helicase is that it possesses multiple DNA-binding sites, a feature that is required for any "active" mechanism of DNA unwinding, because it enables a helicase to bind both ss and duplex DNA or two strands of ssDNA simultaneously at an unwinding fork. On the basis of active assembly of DNA helicases, they can be grouped as monomeric or multimeric helicases. Figure 3 shows the interaction of different forms of DNA helicases with a DNA-unwinding fork. In all these models, at least one subunit always binds to the ssDNA track along which it moves [28]. The other subunit may bind to the dsDNA. On the other hand, the monomeric helicases may contain two different domains, one for ssDNA and the other for dsDNA binding. The bacterial helicases II, IV and PriA ([1] and references cited therein), bacteriophage T4 Dda helicase [29], human DNA helicase IV, V and VI [5], and pea DNA helicase 45 (PDH45) and PDH65 [30,31] are the few examples of monomeric forms. It has been shown that UvrD from *E. coli* is active as a monomer [32].

The functionally active forms of many DNA helicases are oligomeric: *E. coli* DnAB, RuvB, RecBCD and Rho proteins, phage T7 gene 4 helicase/primase, phage T4 gene 41 helicase, SV40 large T antigen. All can assemble to form ring-like toroidal hexamers [1,2,22,23,25,28]. Electron microscopy studies have confirmed that the ssDNA transverses in the center of the hexameric ring. These results show that the hexameric G40P DNA helicase encircles the 5’ tail, interacts with the dsDNA at the ss/dsDNA junction, and excludes the 3’ tail of the forked DNA [33]. Oligomerization of many DNA helicases can be modulated by

**Fig. 2.** Structures of the linear partial duplex substrates commonly used to determine the direction of translocation of the helicase. The 3’ to 5’ directional substrates are on the left and 5’ to 3’ directional substrates are on the right. Asterisks denote the 32P-labeled end.

**Fig. 3.** Interaction of monomeric or oligomeric DNA helicases with the DNA forked substrate. (A) Monomeric helicase binds to both ssDNA and dsDNA. (B) In homodimeric helicases, one subunit always binds to the ssDNA track along which it moves. (C) Heterodimeric helicase contains two separate domains: one subunit binds/interacts with dsDNA and anchors the helicase to the DNA lattice and the other subunit interacts with ssDNA and translocates along it. (D) Hexameric or oligomeric helicases contain a ring-like structure that enables the proteins to encircle the DNA and thus prevent local reannealing. In this case one or more subunits bind to ssDNA at the ss/dsDNA junction.
interactions with other ligands. For example, the \textit{E. coli} Rep helicase is a monomer up to concentrations of 12 mM in the absence of DNA but forms a dimer on binding to DNA [1].

### Prokaryotic and eukaryotic DNA helicases

DNA helicases have been isolated from many sources and accordingly named as prokaryotic, eukaryotic, bacteriophage, and viral helicases. More than one helicase is present in each system because of a variety of different needs for the duplex DNA to unwind in different DNA metabolisms. For example, at least 14 different DNA helicases have been isolated from a simple single cell organism such as \textit{E. coli} (Table 2), six from bacteriophages (Table 3), 12 from viruses (Table 4), 15 from yeast (Table 5), as many as 25 from human cells (Table 6), and as many as 25 from calf thymus (Table 7). The properties of these DNA helicases are summarized in their respective tables. The first helicase isolated from human cells (Table 8) is called human RecBCD (DNA helicase, can also unwind RNA but not DNA) and is a DNA damage inducible helicase. It is a monomer in solution.

#### Mcm proteins

Mcm proteins (named because they were discovered as the products of genes essential for minichromosome maintenance in yeast) were identified initially for their role in plasmid replication or cell cycle progression. In eukaryotes, Mcm proteins 2–7 are required for initiation and elongation steps of chromosomal DNA replication [36]. The Mcm complex is now known to serve as a replicative helicase. A heterotrimeric complex of human Mcm4/6/7 forms a dimeric structure, contains ATP-dependent DNA helicase activity, binds to ssDNA, and possesses DNA-dependent ATPase activity [37], whereas Mcm2/3/7 serve as regulatory subunits. In the presence of forked DNA structures and single stranded DNA binding protein (SSBP), the Mcm4/6/7 complex possesses processive DNA helicase activity [38]. The six proteins (Mcm2–7) interact with each other to form multiple complexes; however, the predominant form is a hexamer containing all six Mcms, which is relatively stable. Electron microscopy indicates that this complex has a globular structure. \textit{In vivo} studies suggest that all six Mcms are recruited to replication origins during the G1 phase [36]. During G1 phase human Mcm proteins first assemble at or adjacent to bound origin recognition complex along with cell division cycle (Cdc) 6 and Cdc10-dependent transcript 1 (Cdt1) proteins (Fig. 4) and move to other sites during genome replication [39]. These proteins together form a prereplication complex at the origin of DNA replication at the beginning of the S phase. After assembly, the complex is activated by cyclin-dependent kinases (CDKs) and the Cdc7–dumb bell former 4 (Cdc7–Dbf4, DDK) complex in the S phase to promote the initiation of DNA replication (Fig. 4).

A growing list of proteins, including Mcm10 and Cdt1, are involved in the recruitment process. Actually the two protein kinases (CDK and DDK) trigger a chain reaction that results in the phosphorylation of the Mcm complex and finally in the initiation of DNA synthesis [36,39,40]. Recruitment of DDK to the replication origin occurs

#### Table 2. \textit{E. coli} DNA helicases.

| S. No. | Name of helicase | Mol. mass (kDa) | Gene | Polarity | Remarks |
|--------|------------------|----------------|------|----------|---------|
| 1.     | DnaB protein\[^a\] | 52             | dnaB | 5\(^{\prime}\)–3\(^{\prime}\) | Replicative helicase. Moves on lagging strand of replication fork. |
| 2.     | PriA protein\[^b\] | 81.7           | priA | 3\(^{\prime}\)–5\(^{\prime}\) | Replicative helicase. Formed called n\(^{\prime}\)-protein. Binds to ssDNA at primosome assembly sites. |
| 3.     | Rep protein\[^c\] | 72.8           | rep  | 3\(^{\prime}\)–5\(^{\prime}\) | Replicative helicase, unwinds the phage DNA in a highly processive and catalytic manner. |
| 4.     | UvrAB complex\[^d\] | 103           | uvrA | 5\(^{\prime}\)–3\(^{\prime}\) | Repair helicase. Involved in nucleotide excision repair. |
| 5.     | Helicase II\[^e\] (UvrD) | 76             | uvrB | 3\(^{\prime}\)–5\(^{\prime}\) | UvrB is helicase component |
| 6.     | Helicase IV\[^f\] | 78             | helD | 3\(^{\prime}\)–5\(^{\prime}\) | Repair helicase. Involved in nucleotide excision repair. |
| 7.     | RecQ\[^g\] | 80             | recQ | 3\(^{\prime}\)–5\(^{\prime}\) | Recombination helicase. |
| 8.     | RecBCD complex\[^h\] (exo V) | 129       | recC | 3\(^{\prime}\)–5\(^{\prime}\) | Catalyzes the first step in the recombinational repair of dsDNA breaks. Highly processive helicase with bipolar polarity. |
| 9.     | RuvAB\[^i\] | 22             | ruvA | 5\(^{\prime}\)–3\(^{\prime}\) | Recombination helicase. It is an ATP-driven translocase (pump) that promotes branch migration. |
| 10.    | Helicase I\[^j\] | 192            | traI | 5\(^{\prime}\)–3\(^{\prime}\) | First helicase identified. May be involved in site-specific nicking reaction. |
| 11.    | RecG\[^k\] | 76             | recG | 3\(^{\prime}\)–5\(^{\prime}\) | A junction-specific DNA helicase that acts posttranspfactory to drive branch migration of holodale junction. |
| 12.    | Rho\[^l\] | 46             | rho  | 5\(^{\prime}\)–3\(^{\prime}\) | RNA-DNA helicase, can also unwind RNA-RNA but not DNA-DNA. |
| 13.    | Helicase III\[^m\] | 20             | ?   | 5\(^{\prime}\)–3\(^{\prime}\) | Smallest prokaryotic helicase. SSB protein inhibits the ATPase activity of the protein. |
| 14.    | DinG\[^n\] | ?              | DinG | 5\(^{\prime}\)–3\(^{\prime}\) | DNA damage inducible helicase. It is a monomer in solution. |

\[^a\] [71]; \[^b\] [72]; \[^c\] [1]; \[^d\] [73] [74]; \[^e\] [75]; \[^f\] [43]; \[^h\] [76]; \[^i\] [21]; \[^j\] [1]; \[^k\] [77]; \[^l\] [2]; \[^m\] [1]; \[^n\] [78].
Table 3. Bacteriophage DNA helicases. nd, Not determined; PNA, peptide nucleic acid.

| S. No. | Name of helicase | Mol. mass (kDa) | Nucleotide cofactor | Polarity | Remarks |
|--------|------------------|-----------------|---------------------|----------|---------|
| 1.     | T4 gene 41<sup>a</sup> | 58              | dGTP > ATP = dATP > GTP | 5’–3’    | Essential for both the priming and helicase activities. Stimulated by T4 gene 39 protein and forked 3’ tail substrate. |
| 2.     | T4 dda<sup>b</sup>     | 56              | ATP, dATP           | 5’–3’    | Can also unwind DNA-PNA substrate. Inhibited by T4 gene 32 protein involved in the DNA replication. Oligomerization of dda is not required for DNA unwinding. |
| 3.     | T4 UvsW<sup>c</sup>    | 65              | ATP                | Nd       | Catalyzes branch migration and is involved in recombination, repair and the regulation of DNA replication origin. |
| 4.     | T7 gene 4<sup>d</sup>  | 56 and 63       | ATP, dATP dGTP, dTTP | 5’–3’    | 56 kDa protein contains DNA helicase activity, while 63 kDa (with 63 amino acids more) contains both the helicase and primase activities. |
| 5.     | P4 gene α<sup>e</sup>  | 84.9            | ATP, dATP GTP, dGTP, CTP, dCTP | 3’–5’ | Stimulated by forked substrate, contains primase and sequence specific (5’-TAGGAC T –3’) binding activity of ori and crr DNA. |
| 6.     | G40P<sup>f</sup>        | 300             | ATP, GTP, CTP, UTP  | 5’–3’    | Essential for B. subtilis bacteriophage SPP1 replication. |

<sup>a</sup> [11] [79]; <sup>b</sup> [80] [29]; <sup>c</sup> [81]; <sup>d</sup> [82]; <sup>e</sup> [83]; <sup>f</sup> [33].

Table 4. Viral DNA helicases. AAV, Adeno-associated virus; ACNP, *Autographa californica* nuclear polyhedrosis; BPV, bovine papilloma virus; HSV, herpes simplex virus; MVM NS1, minute virus of mice–nonstructural protein; nd, not determined; OBP, origin binding protein; SV40 T-antigen, Simian virus 40 large T antigen; SARS-CoV, severe acute respiratory syndrome coronavirus (Coronaviridae helicase).

| S. No. | Name of helicase | Mol. mass (kDa) | Nucleotide cofactors | Polarity | Remarks |
|--------|------------------|-----------------|---------------------|----------|---------|
| 1.     | SV40 T-antigen<sup>a</sup> | 94              | ATP > dATP > dTTP = UTP | 3’–5’    | Interacts with DNA pol. α; essential for DNA replication; contains both DNA and RNA helicase activities. |
| 2.     | Polyoma T-antigen<sup>b</sup> | 100             | ATP = dATP > CTP > UTP | 3’–5’    | Contains Polyoma ori binding and unwinding activities. |
| 3.     | HSV-1, UL5/UL8/UL52 Complex<sup>c</sup> | 120             | ATP > GTP > CTP > UTP | 5’–3’    | UL5 and UL52 required for helicase-primase activity. |
| 4.     | HSV-1, UL9 protein<sup>d</sup> | 68              | ATP = dATP > CTP > dCTP | 3’–5’    | OBP involved during the initiation of HSV replication. |
| 5.     | BPV-1, E1 protein<sup>e</sup> | 68              | ATP, dATP, CTP, dCTP, UTP, dGTP, GTP | 3’–5’ | OBP, which is stimulated by E2 protein of BPV-1. |
| 6.     | AAV, Rep68, Rep78<sup>f</sup> | 68              | ATP > CTP > dATP > dGTP > UTP | 3’–5’ | Contains site-and strand-specific endonuclease activity. |
| 7.     | AAV-Rep52<sup>g</sup> | 52              | ATP, dATP, CTP, dCTP, UTP, dGTP, GTP | 3’–5’ | Lysine to histidine substitution within motif I was deficient for both DNA helicase and ATPase activities. |
| 8.     | AAV-Rep40<sup>h</sup> | 40              | ATP                 | 3’–5’    | Lysine to histidine mutation in the purine nucleotide-binding site results in a protein that inhibits helicase activity. |
| 9.     | MVM NS-1<sup>i</sup> | 83              | ATP > dATP          | nd       | Appears to have site-specific endonuclease activity. |
| 10.    | ACNP virus P143<sup>j</sup> | 143             | ATP                 | nd       | Stimulated by LEF3/SSB; essential for virus DNA replication. |
| 11.    | Vaccinia virus A18<sup>k</sup> | 57.5            | ATP                 | 3’–5’    | It is a DExH box protein and is involved in transcription. |
| 12.    | SARS-CoV helicase<sup>l</sup> | 70              | ATP, dATP, CTP > all others | 5’–3’ | Attractive target for anti-SARS therapy. |

<sup>a</sup> [84]; <sup>b</sup> [85]; <sup>c</sup> [86]; <sup>d</sup> [87]; <sup>e</sup> [88]; <sup>f</sup> [89]; <sup>g</sup> [89]; <sup>h</sup> [89]; <sup>i</sup> [90]; <sup>j</sup> [91]; <sup>k</sup> [92]; <sup>l</sup> [93].
Table 5. Yeast DNA helicases. nd, Not determined.

| S. No. | Name of helicase | Source | Mol. mass (kDa) | Nucleotide cofactors | Polarity | Remarks |
|--------|------------------|--------|-----------------|----------------------|----------|---------|
| 1.     | ATPase III<sup>a</sup> | *S. cerevisiae* | 63 | ATP > dATP | 3<sup>−</sup>–5<sup>+</sup> | Stimulated by yeast Pol I. |
| 2.     | Rad3<sup>b</sup> (XPD) | *S. cerevisiae* | 89 | ATP > dATP > CTP | 5<sup>−</sup>–3<sup>+</sup> | Active at acid pH; involved in DNA excision repair; homologous to XPD gene. |
| 3.     | Rad25<sup>c</sup> (Ssl2) | *S. cerevisiae* | 95 | ATP, dATP | 3<sup>−</sup>–5<sup>+</sup> | Functions in nucleotide excision repair; homologous to XPD; required for Pol II transcription. |
| 4.     | Srs2d | *S. cerevisiae* | 134 | ATP, dATP | 3<sup>−</sup>–5<sup>+</sup> | Involved in error-prone repair; negatively modulates recombination. |
| 5.     | PIF<sup>d</sup> | *S. cerevisiae* | 97 | ATP, dATP | 5<sup>−</sup>–3<sup>+</sup> | Functions in mitochondrial DNA repair and recombination. |
| 6.     | DNA helicase A<sup>e</sup> | *S. cerevisiae* | 90 | ATP, dATP | 5<sup>−</sup>–3<sup>+</sup> | Copurifies with DNA Pol-α- primase; helicase activity stimulated by the yeast RPA. |
| 7.     | DNA helicase B<sup>f</sup> | *S. cerevisiae* | 127 | ATP, dATP > CTP,dCTP, UTP | 5<sup>−</sup>–3<sup>+</sup> | Copurifies with DNA Pol-δ; stimulated by scRPA; encoded by the yorf61 gene. |
| 8.     | DNA helicase C<sup>g</sup> | *S. cerevisiae* | 32 | ATP, dATP, CTP,dCTP > UTP,GTP > dGTP | 5<sup>−</sup>–3<sup>+</sup> | Copurifies with DNA Pol-δ? |
| 9.     | DNA helicase D<sup>h</sup> | *S. cerevisiae* | 60 | ATP,dATP > CTP,dCTP, UTP | 5<sup>−</sup>–3<sup>+</sup> | Copurifies with RF-C. |
| 10.    | ScHel<sup>i</sup> | *S. cerevisiae* | 135 | ATP, dATP | 5<sup>−</sup>–3<sup>+</sup> | Stimulated by *E. coli* SS. |
| 11.    | DNA helicase III<sup>j</sup> | *S. cerevisiae* | 120 | ATP, dATP | 5<sup>−</sup>–3<sup>+</sup> | Encoded by a gene different from Rad3 and RadH. |
| 12.    | Sgs1<sup>k</sup> | *S. cerevisiae* | 39 | ATP, dATP | 3<sup>−</sup>–5<sup>+</sup> | Binds more tightly to a forked DNA substrate than to ss and ds DNA. |
| 13.    | Dna2<sup>m</sup> | *S. pombe* | 65 | ATP, dATP | 5<sup>−</sup>–3<sup>+</sup> | Involved in DNA replication. |
| 14.    | MER3<sup>n</sup> | *S. cerevisiae* | 130 | ATP | 3<sup>−</sup>–5<sup>+</sup> | Meiosis-specific helicase; required for crossing over at time of first meiotic division. |
| 15.    | Hmi1p | *S. cerevisiae* | 80 | ATP | nd | Mitochondrial helicase; required for the maintenance of mitochondrial genome. |

<sup>a</sup> [60]; <sup>b</sup> [94]; <sup>c</sup> [95]; <sup>d</sup> [96]; <sup>e</sup> [97]; <sup>f</sup> [98]; <sup>g</sup> [99]; <sup>h</sup> [100]; <sup>i</sup> [101]; <sup>j</sup> [102]; <sup>k</sup> [98]; <sup>l</sup> [103]; <sup>m</sup> [104]; <sup>n</sup> [105].

during the G1 phase. However, it must function sometimes during the S phase, and this decision is controlled locally at individual origins. Phosphorylation of Mcm2 by DDK results in a conformational change in the Mcm complex. Recruitment of Cdc45 depends on phosphorylation of the Mcm complex and the activity of CDK. The Mcm component of the complex provides the DNA-unwinding function (Fig. 4) to start the DNA replication, which finally requires the concerted action of many enzymes/factors such as replication protein A (RPA), polymerase α, replication factor C, polymerase δ, proliferating cell nuclear antigen (PCNA), flap endonuclease 1, endonuclease DNA2, and DNA ligase I [35,39]. The unwinding of origin DNA is detected only at a later step when the Mcm complex is phosphorylated by DDK at the G1 to S phase transition [40]. These studies suggest an anchoring mechanism other than direct contact with DNA for the Mcm complex immediately after its replication to the origin regions. Recently it has been reported that the Mcm8 protein from HeLa cells, a new member of the Mcm family, forms a complex with Mcm4, Mcm6 and Mcm7 proteins and this complex is involved in initiation of DNA replication [41].

**RecQ family of DNA helicases**

RecQ is a recombination-specific DNA helicase from the SF2 family. Members of the RecQ family of DNA helicases are involved in processes linked to DNA replication, DNA recombination, and gene silencing. It was discovered and named RecQ by H. Nakayama of Kyushu University, Japan (‘Q’ came from Kyushu) [42]. This gene was discovered during a search for genes that control the loss of viability in thymine-starved bacteria, a classic phenomenon known as thymineless death. RecQ1 mutant was resistant to thymineless death, and, in a recBCdsbcB background, it exhibited enhanced UV sensitivity and a deficiency in conjugational recombination, suggesting a role for RecQ in the RecF recombination pathway [42]. RecQ protein was purified to homogeneity and shown to be a helicase that unwinds duplex DNA in the 3' to 5' direction with respect to the single strand to which it binds [43].

The family name ‘RecQ’ is derived from the *E. coli* RecQ helicase. In general, unicellular organisms express a single RecQ enzyme, whereas more complex organisms express two or more. All helicases of this family share a central seven helicase motifs. RecQ DNA helicases are proposed to function at the interface between DNA replication and recombination to ‘repair’ the damaged replication forks [44]. This family includes at least five members in humans, and the following three are defective in genetic disorders associated with cancer and/or premature aging: WRN, BLM and RECQ4 helicases defective in Werner’s syndrome, Bloom’s syndrome, and Rothmund–Thomson syndrome, respectively [44,45]. RecQ helicases are considered to
be ‘caretaker’ tumor suppressors which suppress neoplastic transformation through control of chromosomal stability [44]. Figure 5 depicts the members of the RecQ family of DNA helicases from humans, *Xenopus*, *Drosophila*, yeast and *E. coli*. The family contains a highly conserved helicase domain which comprises about 400 amino acids. Two signature motifs are present in most RecQ helicases: the RQC domain and HRDC (helicase and RNaseD

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\text{Table 6. Biochemically active DNA helicases from plant cells, CDH, Chloroplast DNA helicase; DSBs, double-strand breaks; HDH, human DNA helicase; nd, not determined; PDH, pea DNA helicase.}
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| S. No. | Name of helicase | Mol. mass (kDa) | Nucleotide cofactors | Polarity | Remarks |
|--------|------------------|-----------------|----------------------|----------|---------|
| 1.     | Lily U-protein\(^a\) | 130 (native) | ATP | nd | Partially purified. Unwinds DNA from ends, gaps and nicks. Its abundance increases during meiosis. |
| 2.     | Soybean chloroplast DNA helicase\(^b\) | nd | dATP > GTP > dCTP | nd | Partially purified. Shows unwinding activity at higher conc. of Mg\(2^+\) (10 mM). |
| 3.     | Pea chloroplast DNA helicase I (CDH I)\(^c\) | 68 | ATP > dATP > NTPs | 3\(’\)–5\(’\) | First plant helicase reported in purified form. It is a homodimer. |
| 4.     | Pea chloroplast DNA helicase II (CDH II)\(^d\) | 78 | ATP = dATP | 3\(’\)–5\(’\) | Stimulated by fork-like structures. |
| 5.     | Pea nuclear DNA helicase I (PDH45)\(^e\) | 45 | ATP > dATP > NTPs | 3\(’\)–5\(’\) | A plant homolog of HDHi; homologous to eIF-4 A; stimulates topo I activity. |
| 6.     | Pea nuclear DNA helicase II (PDH65)\(^f\) | 65 | ATP > dATP | 3\(’\)–5\(’\) | Purified to homogeneity. Present at extremely low abundance and contain the high specific activity. |
| 7.     | Arabidopsis Ku DNA helicase (AtKu70/80)\(^g\) | 70.3 | ATP | nd | Functions as heterodimer that binds to dsDNA. Gene expression is induced by DSBs, may be involved in DSB repair. |
| 8.     | Pea nuclear DNA helicase III (PDH120)\(^h\) | 54 | ATP > NTPs | 3\(’\)–5\(’\) | Purified to homogeneity. Present at extremely low abundance and contain the high specific activity. |

\[^a\] [10]; \[^b\] [106]; \[^c\] [65]; \[^d\] [3]; \[^e\] [30]; \[^f\] [31]; \[^g\] [107]; \[^h\] [3].

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\text{Table 7. Calf thymus/bovine DNA helicases.}
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| S. No. | Name of helicase | Mol. mass (kDa) | Nucleotide cofactors | Polarity | Remarks |
|--------|------------------|-----------------|----------------------|----------|---------|
| 1.     | DNA helicase A\(^a\) | 47 | ATP = dATP > CTP > dCTP | 3\(’\)–5\(’\) | Copurifies with calf thymus DNA Pol-\(\alpha\)/?Primase; stimulated 20-fold by RPA. |
| 2.     | DNA helicase B\(^b\) | 100 | dATP = ATP > all other | 5\(’\)–3\(’\) | Binds to dsDNA also. |
| 3.     | DNA helicase C\(^c\) | 40 | dATP = ATP > all other | 5\(’\)–3\(’\) | Stimulated by 100 mM KCl on short substrates. |
| 4.     | DNA helicase D\(^d\) | 100 | dATP = ATP | 5\(’\)–3\(’\) | Stimulated 10-fold by RPA and forms large aggregates in low salt. |
| 5.     | DNA helicase E\(^e\) | 104 | dATP = ATP | 3\(’\)–5\(’\) | Copurifies with DNA Pol-\(\epsilon\) and dependent on unspecific SSB; probably involved in DNA repair. |
| 6.     | DNA helicase F\(^f\) | 72 | ATP, dATP, dCTP, UTP, CTP, GTP, dGTP, dTTP | 5\(’\)–3\(’\) | Copurifies with RPA. In presence of RPA, this helicase can unwind longer duplexes |
| 7.     | DNA helicase I\(^g\) | 200 | ATP = dATP | 3\(’\)–5\(’\) | Stimulated by 150 mM NaCl. |
| 8.     | DNA helicase I\(^h\) | 130 | ATP = dATP > all other | 3\(’\)–5\(’\) | Can unwind dsRNA also. |
| 9.     | \(\delta\) helicase\(^i\) | 57 | ATP = dATP > CTP | 5\(’\)–3\(’\) | Copurifies with DNA Pol. \(\delta\) and acts as strand-displacement factor for Pol.\(\delta\) |
| 10.    | Cytosolic DNA helicase\(^j\) | 110 | ATP = dATP > CTP, dCTP | 3\(’\)–5\(’\) | All the three subunits bind to ATP. |
| 11.    | Bovine mitochondrial helicase\(^k\) | 57 | ATP = dATP | 3\(’\)–5\(’\) | Possible role in mitochondrial DNA replication. |

\[^a\] [108]; \[^b\] [108]; \[^c\] [108]; \[^d\] [108]; \[^e\] [109]; \[^f\] [110]; \[^g\] [111]; \[^h\] [111]; \[^i\] [100]; \[^j\] [112]; \[^k\] [64].
C-terminal) domain (Fig. 5). The RQC domain is unique to RecQ family helicases and probably has a role in mediating interactions with other proteins. The HRDC domain is found in a number of nucleases that digest RNA, and may be involved in DNA binding in RecQ proteins [45]. The members of this family of helicases can unwind many different kinds of structurally diverse DNA substrates, including G4-DNA (G-quadruplex DNA) and synthetic Holliday junctions. The BLM and WRN proteins have been shown to promote branch migration of *bona fide* Holliday

Table 8. Human DNA helicases. nd, Not determined.

| S. No. | Name of helicase | Mol. mass (kDa) | Nucleotide cofactors | Polarity | Remarks |
|--------|-----------------|-----------------|----------------------|----------|---------|
| 1.     | HDH I\(^a\)     | 65              | ATP, dATP            | 3'−5'    | Can also unwind DNA/RNA and RNA/RNA hybrids; may be involved in rDNA transcription. |
| 2.     | HDH II\(^a\)    | 87              | ATP, dATP            | 3'−5'    | Functions in dsDNA break repair and V(D)J recombination; regulator of DNA-dependent protein kinase |
| 3.     | HDH III\(^a\)   | 46              | ATP, dATP            | 3'−5'    | Prefers replication fork-like structure of substrates |
| 4.     | HDH IV\(^a\)    | 100             | ATP, dATP            | 3'−5'    | Can unwind DNA/RNA hybrids |
| 5.     | HDH V\(^a\)     | 92              | ATP, dATP            | 3'−5'    | Has highest turnover rate |
| 6.     | HDH VI\(^a\)    | 128             | ATP, dATP            | 3'−5'    | Prefers replication fork-like structure of substrates |
| 7.     | HDH VII\(^a\)   | 36              | nd                   | nd       | Trimer of one molecule of hnRNP A1 and two molecules of annexin II |
| 8.     | HDH VIII\(^b\)  | 68              | ATP                  | 5'−3'    | A DNA and RNA helicase corresponding to G3 bp protein, an element of the RAS transduction pathway, similar to E. coli RecBCD |
| 9.     | HDH IX\(^b\)    | 45              | nd                   | nd       | A Gty-Arg rich protein identified as ribonuclear protein DO |
| 10.    | XPD/ERCC2\(^b\) | 87              | ATP, dATP            | 5'−3'    | Functions in nucleotide excision repair; component of BTF2-TFIIH transcription factor. |
| 11.    | XPB/ERCC3\(^b\) | 89              | ATP                  | 3'−5'    | Functions in nucleotide excision repair; component of BTF2-TFIIH transcription factor. |
| 12.    | Helicase\(^a\)  | 72              | ATP, dATP, CTP       | 3'−5'    | Helicase activity is dependent on HRP-A |
| 13.    | Helicase\(^a\)  | 110             | ATP, dATP, CTP       | 3'−5'    | Stimulated by 5'-tailed fork and SSB. |
| 14.    | RIP 100\(^b\)   | 100             | ATP, dATP            | 3'−5'    | Associated with RIP60; RIP60 binds to replication origin region of DHFR\(^b\) |
| 15.    | Helicase Q1\(^a\) | 73           | ATP, dATP            | 3'−5'    | Gene homologous to E. coli RecQ gene; identical to human DNA helicase I |
| 16.    | Helicase Q2\(^a\) | 100           | ATP                  | 5'−3'    | Identical with DNA helicase IV |
| 17.    | HchlR1\(^a\)    | 112             | ATP                  | 5'−3'    | Can unwind RNA/DNA substrates. Unlike others it can translocate along ssDNA in both directions when substrate have a very long ssDNA region. |
| 18.    | HHcsA\(^a\)     | 116             | ATP, dATP            | 5'−3'    | Hexameric protein. |
| 19.    | WRN helicase\(^c\) | 163        | ATP, dATP ≥ DCTP, CTP | 3'−5'    | Mutated in Werner syndrome, homologous to RecQ and contains 3'-5' exonuclease activity |
| 20.    | BLM helicase\(^c\) | ≈ 160   | ATP                  | 3'−5'    | Mutated in cells of Bloom's syndrome patient and belongs to RecQ family. |
| 21.    | Mcm4/6/7 complex\(^c\) | ≈ 600 | ATP, dATP            | 3'−5'    | The DNA unwinding activity is stimulated by SSB and forked DNA structures; can function as a replication helicase. |
| 22.    | HEL308\(^d\)    | 124.5           | ATP, dATP            | 3'−5'    | Homologous to DNA crosslink sensitivity protein Mus308 of D. melanogaster. Stimulated by RPA. |
| 23.    | HFDH1\(^d\)     | ≈ 120           | ATP                  | 3'−5'    | First F-box protein that possesses enzyme activity. |
| 24.    | Human RECP1\(^d\) | 75           | ATP                  | 3'−5'    | Needs 3' tail of 10 nt on the substrate to open the duplex; can unwinds blunt end substrate with bubble of 25 nt; stimulated by hRPA. |
| 25.    | BACH1\(^d\)     | 130             | ATP                  | 5'−3'    | A nuclear phosphoprotein interacts with tumor suppressor, BRCA1. Involved in DSB repair and contain tumor suppression activity. |

\(^{a}[63]; ^{b}[113]; ^{c}[5]; ^{d}[5]; ^{e}[5]; ^{f}[6]; ^{g} A. Falaschi & A. Ochem, unpublished data; ^{h}[114]; ^{i}[115]; ^{j}[116]; ^{k}[117]; ^{l}[118]; ^{m}[119]; ^{n}[120]; ^{o}[120]; ^{p}[121]; ^{q}[122]; ^{r}[123]; ^{s}[124]; ^{t}[37]; ^{u}[125]; ^{v}[126]; ^{w}[51]; ^{x}[127].\)
BLM makes numerous contacts with other proteins. It is a large T antigen and belongs to the family of ring helicases, which includes SV40 containing exonuclease activity as well. The BLM helicase differs from the other family of RecQ helicases in the roles of the different conserved and are important in functionally distinguishing carboxy ends (Fig. 5). These flanking ends are poorly members of this family are large with extended amino and to be a key function of the RecQ helicases.

Intermediates formed during DNA replication is proposed absence of cofactors. This ability to process recombination moderately processive and limited to 75–150 bp in the unwinding that is catalysed by these enzymes is only occurs over several kilobases of DNA, whereas the regular significantly, this RecQ-helicase-catalysed branch migration junctions generated by the RecA protein [46,47]. Significantly, this RecQ-helicase-catalysed branch migration occurs over several kilobases of DNA, whereas the regular unwinding that is catalysed by these enzymes is only moderately processive and limited to 75–150 bp in the absence of cofactors. This ability to process recombination intermediates formed during DNA replication is proposed to be a key function of the RecQ helicases.

Except for E. coli RecQ, human RECQ5 and RECQL, members of this family are large with extended amino and carboxy ends (Fig. 5). These flanking ends are poorly conserved and are important in functionally distinguishing the roles of the different ‘isofoms’ in humans. WRN helicase differs from the other family of RecQ helicases in containing exonuclease activity as well. The BLM helicase belongs to the family of ring helicases, which includes SV40 large T antigen and E. coli DnaB and RuvB proteins [47]. BLM makes numerous contacts with other proteins. It is a component of ATM kinase, BASC (BRCA1-associated genome surveillance complex), a multi-enzyme complex that contains BRCA1 and MLH1, the MRE11–RAD50–NBS1 complex [44]. It has also been shown in a number of studies that WRN interacts physically and functionally with proteins required for DNA replication, including DNA polymerase δ, flap endonuclease 1, PCNA, and RPA [44]. A physical and functional interaction has also been reported for WRN protein and the Ku heterodimer complex, suggesting that WRN protein is also involved in double-strand break repair [48]. The connection between RecQ homologs and Ku appears to be relevant for telomere maintenance as well. It has been shown that primary fibroblasts from patients with Werner’s syndrome, like Ku-deficient mouse cells, display excessive telomere shortening and premature replicative senescence, which is thought to contribute to the early onset of aging seen in Werner’s syndrome [49]. However, yeast Sgs1p is also required for a telomere maintenance pathway that is independent of telomerase and dependent on recombination [50]. Thus the RecQ helicases help to define two mechanically distinct telomere maintenance pathways that are both telomerase-independent and recombination-dependent.

In a recent study it has been shown that only short DNA duplexes (< 30 bp) can be unwound by RECQ1 alone, but the addition of human replication protein A (hRPA) increases the processivity of the enzyme (> 100 bp). These findings suggest that RECQ1 and hRPA may also interact in vivo and function together in DNA metabolism [51]. RECQ5 is one of the five RecQ helicase homologs identified in humans. Drosophila RECQ5 helicase is capable of unwinding 3’ Flap, three-way junction, fork and three-strand junction substrates at lower protein concentrations than 5’ Flap, 12 nucleotide bubble and synthetic Holliday junction structures, which can be unwound efficiently by WRN and BLM [52]. Taken together these findings provide evidence that RecQ helicases facilitate smooth ‘replisome’ progression through the genome. Six cDNAs of RecQ-like proteins (AtRecQ1, AtRecQ2, AtRecQ3, AtRecQ4A, AtRecQ4B and AtRecQ5m) and one gene (AtWRNexo) homologous to the exonuclease domain of human Werner protein have also been isolated from plants [53].

**Concluding remarks**

The DNA helicases are known to play essential roles in unwinding of duplex strands in almost every aspect of nucleic acid metabolism, which makes them very important molecules. Functional helicases always work as an integrated component of a large macromolecular complex which is ‘designed’ to carry out a particular function on its genomic DNA target. The functions of all reported DNA helicases have not yet been investigated. It will be important to identify and characterize their specific substrates, interacting proteins, and cofactors in order to precisely elucidate their specific functions in nucleic acid metabolism. Despite the diversity of their functions and the large range of organisms in which these proteins have been identified, high sequence conservation is maintained, suggesting that all helicase genes evolved from a common ancestor. The family of DEAD-box helicases differ mainly in the N-terminal and C-terminal sequences, which contain different targeting signals. The
different regulatory mechanisms at both the level of expression and post-transcription may explain the wide spectrum of functions involving DEAD-box helicases. The crystal structures of DNA helicases suggest that the helicase motifs are clustered together for the overall unwinding function. The motifs, crystal structure, mechanism of unwinding, and various functions of DNA helicases are described in detail in the following review.

The Mcm proteins play an important role in replication by acting as licensing factors, ensuring that replication is not re-initiated until the first round is completed in one cell cycle. These proteins initiate unwinding at the origin, leading to the formation of a replication bubble. They create a replication fork and move along the fork, at the same time bringing the origin to the unlicensed state. The conservation of Mcms from Archaea to humans suggests that several of these proteins may have evolved from a single progenitor.

Helicases of the RecQ family catalyse critical genome maintenance reactions in bacterial and eukaryotic cells, playing key roles in several DNA metabolic processes. Mutations in RecQ genes are linked to genome instability and human disease. As mutants in RecQ family genes have unstable chromosomes, it was proposed that members of the RecQ helicase family play a central role in the maintenance of genomic stability and thereby the prevention of tumorigenesis. A connection between the action of RecQ homologs and gene expression was demonstrated by the involvement of QDE3 of *Neurospora crassa* in post-transcriptional gene silencing [54]. However, only one RecQ homolog was found in *E. coli* and yeast genomes, but multiple homologs are found in the animal and plant kingdom. These homologs of RecQ seem to have evolved first by duplication and then by consecutive shuffling or fusion with other protein domains [54]. Careful genomic studies and further biochemical analysis are needed to understand fully how RecQ DNA helicases influence the processing and/or prevention of recombination intermediates.

Despite considerable progress overall in the helicase field in the last two decades, a large gap in our knowledge persists which prevents complete understanding of the complex genetic processes. Considering the multiplicity of helicases, their tissue specificity, functional diversity, and control of activity by phosphorylation/dephosphorylation, it is reasonable to assume that they also play an important role in cell division, growth, and development. Progress in plant helicases is slow. A better understanding of these proteins will have to await the breakthrough that hopefully will be provided by the high-resolution structure to be solved by X-ray crystallography. Furthermore, the RNAi approach and/or transgenic antisense plant technology should help in understanding the detailed role of helicases in plant development.

The number of DNA helicases isolated from all systems is continuously growing. This has created the problem of a bewildering array of different names and classification. Therefore it is important that a clear, scientific system for nomenclature and classification of helicases is formulated. However, this class of proteins does not, at this point, lend itself to a simple method of classification.

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