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REVIEW ARTICLE

Unraveling DNA helicases

Motif, structure, mechanism and function

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DNA helicases are molecular 'motor' enzymes that use the energy of NTP hydrolysis to separate transiently energetically stable duplex DNA into single strands. They are therefore essential in nearly all DNA metabolic transactions. They act as essential molecular tools for the cellular machinery. Since the discovery of the first DNA helicase in *Escherichia coli* in 1976, several have been isolated from both prokaryotic and eukaryotic systems. DNA helicases generally bind to ssDNA or ssDNA/dsDNA junctions and translocate mainly unidirectionally along the bound strand and disrupt the hydrogen bonds between the duplexes. Most helicases contain conserved motifs which act as an engine to drive DNA unwinding. Crystal structures have revealed an underlying common structural fold for their function. These structures suggest the role of the helicase motifs in catalytic function and offer clues as to how these proteins can translocate and unwind DNA. The genes containing helicase motifs may have evolved from a common ancestor. In this review we cover the conserved motifs, structural information, mechanism of DNA unwinding and translocation, and functional aspects of DNA helicases.

Keywords: crystal structure; DEAD-box protein; DNA helicase; helicase motifs; unwinding enzyme.

DNA helicases are motor proteins that can transiently catalyze the unwinding of energetically stable duplex DNA molecules using NTP hydrolysis as the source of energy [1,2]. They are important enzymatic tools for the cellular DNA machinery. They are known to play essential roles in nearly all aspects of nucleic acid metabolism, such as DNA replication, repair, recombination, and transcription. All helicases share at least three common biochemical properties: (a) nucleic acid binding; (b) NTP/dNTP binding and hydrolysis; (c) NTP/dNTP hydrolysis-dependent unwinding of duplex nucleic acids in the 3′ to 5′ or 5′ to 3′ direction [3]. Therefore, all DNA helicases described to date also have intrinsic DNA-dependent NTPase activity [4,5]. These enzymes usually act in concert with other enzymes or proteins in DNA metabolic activity. Multiple DNA helicases have been isolated from single-cell types because of different structural requirements of the substrate at various stages of the DNA transaction. For example, at least 14 different DNA helicases have been isolated from a simple single-cell organism such as *Escherichia coli*, six from bacteriophages, 12 from viruses, 15 from yeast, eight from plants, 11 from calf thymus, and as many as 24 from human cells. These have been described in the preceding review.

Most helicases from many different organisms contain about nine short conserved amino-acid sequence fingerprints (designated Q, I, Ia, Ib, II, III, IV, V and VI), called 'helicase motifs' [6–10]. These motifs are usually clustered in a region of 200–700 amino acids called the core region. Because of the sequence of motif II (DEAD or DEAH or DEXH), the helicase family is also called the DEAD-box protein family. The crystal structures of some of the DNA helicases are now available and have revealed a 'theme and variations' at the structural level [11]. Despite the ubiquitous presence of DNA helicases, their biological roles have not all been investigated. In the preceding review, we covered history, biochemical assay and properties, and prokaryotic and eukaryotic DNA helicases including Mcm proteins and RecQ family of helicases. This review focuses on the helicase motifs, crystal structures, mechanism of DNA unwinding and translocation, and various functions of the DNA helicases.

Helicase motifs

A computer-assisted amino-acid sequence analysis of helicases from many different organisms has revealed seven to nine short, conserved amino-acid sequence fingerprints or motifs, called 'helicase signature motifs' [6,12]. With this discovery, the helicases were classified into three superfamilies (SF), SF1, SF2 and SF3, based on the extent of similarity and organization of these conserved motifs. This suggests that the genes containing helicase motifs evolved from a common ancestor. These motifs can be used efficiently for the detection and prediction of new helicases in the genome databases. SF1 and SF2 are related, are the largest superfamilies, and contain at least seven conserved

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Abbreviations: eIF-4A, translation initiation factor 4A; ERCC, excision repair cross complementing; NER, nucleotide excision repair; HSV, herpes simplex virus; SF, super family; SSBP, single-stranded DNA-binding protein; SV40, simian virus 40; TFIIH, transcription factor IIH; XP, *Xeroderma pigmentosum*.

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motifs (I, Ia, II–VI), and SF3 has just three motifs (A, B and C) [3]. Motif III differs between SF1 and SF2. In general, SF1 members (Rep, UvrD and PcrA) are considered to be ssDNA translocases, whereas SF2 members (RecG, PriA and HSV NS3) are dsDNA translocases. This property may be the distinction between the SF1 and SF2 family. Another group called family 4 (F4) contains five motifs (1, 1a, 2–4) [3]. The seven motifs of SF1 and SF2 are usually clustered in a region of 200–700 amino acids, the core region. These conserved motifs are separated by stretches of low sequence but high length conservation. In contrast, the N-terminal and C-terminal regions of helicases are characterized by a high degree of sequence and length variability. It has been suggested that the divergent regions are responsible for individual protein functions, whereas the highly conserved domains are involved in ATP binding and hydrolysis or binding and unwinding of nucleic acids.

X-ray crystallographic studies have suggested that the conserved helicase motifs are closely associated in the tertiary structure of the protein and that they may form a large functional domain [3]. Recently, a new motif, the Q motif, was discovered [10]. Figure 1 shows the conserved sequence of all nine motifs (Q, I, Ia, Ib, II, III, IV, V and VI) of the DEAD-box helicase family. The biochemical functions of only some of them (Q, I, Ia, II, III, and VI) have been elucidated and are described below.

Q motif (Gly-Phe-X-X-Pro-X-Pro-Ile-Gln)

This is a newly discovered motif, which is unique to and characteristic of the DEAD-box family of helicases [10]. It consists of a 9-amino-acid sequence containing an invariant glutamine (Q), giving it its name. It is generally present in a range of 15–22 amino acids upstream from motif I. In yeast, this motif has been shown to be essential for viability and site-specific mutagenesis studies and it is involved in ATP binding and hydrolysis in the yeast eIF4A [10]. It has been hypothesized that it is also involved in adenine recognition.

Motif I (Ala/Gly-X-X-Gly-X-Glu-Lys-Thr)

It has been described as the 'A' motif of ATPase (Walker motif A). AXXGXGKT is the consensus sequence for RNA helicase and it forms the closely related SF1, whereas GXXXXGKT, which belongs to SF2 is the consensus sequence for other ATPases, GTPases, and a few DNA helicases [7]. In the consensus sequence, the X could be any amino acid. It was shown that the lysine (K) of GKT binds to β and γ phosphates of the ATP molecule, and the mutation of K to the uncharged amino acid asparagine abolished the binding of ATP [13]. The side chain of the invariant K of motif I occupies the position that will be occupied by the bound Mg^{2+} ion when the NTP–Mg^{2+} complex binds the enzyme. On binding NTP–Mg^{2+}, the lysine side chain contacts the β phosphate of the bound NTP and may act to stabilize the transition state during catalysis. An ATPase-deficient mutant also lacked helicase activity, demonstrating that ATP hydrolysis was required for the unwinding of duplex nucleic acid [3].

Motif Ia (Pro-Thr-Arg-Glu-Leu-Ala)

Structural information on SF1 and SF2 helicases indicate that motif Ia is involved in ssDNA binding. In a recent study, specific mutations were designed in motif Ia of UL9 of HSV-1, and these mutants were analyzed genetically and biochemically [14]. Mutant proteins with residues predicted to be involved in ssDNA binding (R112A and R113A/F115A) exhibited wild-type levels of intrinsic ATPase activity and moderate to severe defects in ssDNA-stimulated ATPase activity and ssDNA binding. All the mutant proteins lacked helicase activity but were able to dimerize and bind the HSV-1 origin of replication as well as wild-type UL9. These results indicate that residues from the Ia motif contribute to the ssDNA-binding and helicase activities of UL9 and are essential for viral growth [14].

Motif II (Val-Leu-Asp-Glu-Ala-Asp)

This motif represents a specific form of the ATPase B (Walker B) motif. The proteins containing this motif are also called DEAD-box proteins [15]. The DE of the DEAD sequence is highly conserved and is also present in many proteins, which play a role in DNA and RNA replication [7]. The residue D of DE has been shown to interact with Mg^{2+}, which is required for ATP binding. A mutation in D of DE also affects the ATPase and helicase activities. The ATPase motif seems to be responsible for coupling of ATPase and helicase activity [13]. The eukaryotic initiation factor 4A (elF-4A) is the prototype and the best biochem-

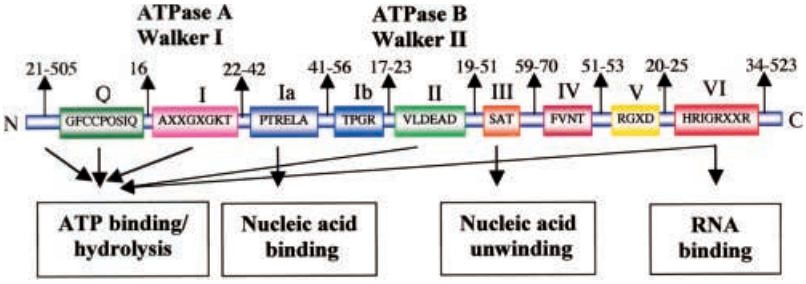

Fig. 1. Schematic diagram representing organization and involvement in the catalytic functions of the best conserved motifs of the DEAD-box helicases. Open boxes represent the conserved helicase motifs, and the consensus amino-acid sequence of each motif is shown by the single-letter code inside the boxes (c = D, E, H, K, R; o = S, T; x = any amino acid). Labels above the open boxes (Q, I, Ia, etc.) are the names assigned to the motifs. The relative positions of the motifs and spacing between motifs are arbitrary. The numbers between the motifs and above the arrows are the typical range of amino-acid residues. The defined functions for some of the motifs (Q, I, Ia, II, III and VI) are also given.

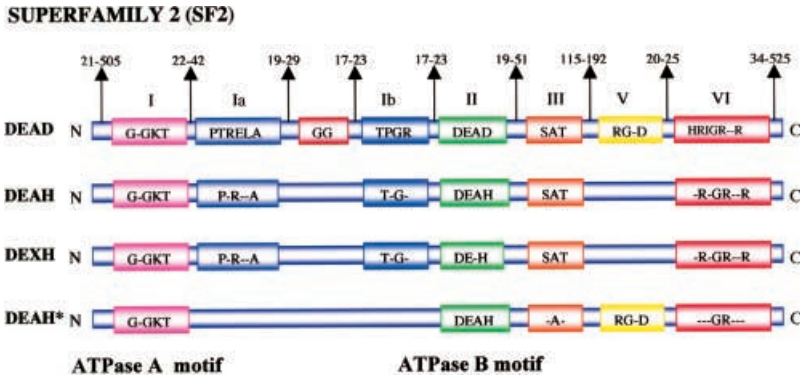

Fig. 2. Organization and variations in the conserved helicase motifs of DEAD-box proteins of SF2. The DEAD, DEAH, and DEXH box proteins mainly belong to the family of RNA helicases, but some DNA helicases also contain these motifs. The proteins of the DEAH* subgroup show the greatest variations in the conserved motifs and are characterized by DNA helicase activity. Numbers above the arrow indicates the range of distances between the conserved domains and the distances of the two flanking domains of the N-terminus and C-terminus of the different proteins. The Q motif is not shown here.

ically characterized member of this family. With the growing number of identified helicases, some variations in motif II have been observed, and these are arranged in different subgroups of SF2 such as DEAD, DEAH, DEXH and DEAH* box proteins (Fig. 2). The proteins of the DEAH* subgroup show the strongest variations in the conserved regions and are characterized by a DNA helicase activity.

Motif III (Ser-Ala-Thr)

This motif of the SF2 family plays an important role in unwinding. In UL5 of HSV-I, a motif III mutant exhibited apparent uncoupling of ATPase and DNA helicase activities as the mutant enzyme retained ATPase activity but lacked significant helicase activity [16]. Mutation in this domain (SAT to AAA) leads to loss of RNA helicase activity in eIF-4A while its ATP binding and hydrolysis as well as RNA binding activities remained intact [13]. This result suggests that motif III of SF2 is involved in the coupling of ATPase to unwinding.

Motif VI (Tyr-Ile-His-Arg-Ile-Gly-Arg)

Several helicases exhibited nucleic acid-binding defects when the motif VI residues were altered [13]. Point mutation in *E. coli* UvrD DNA helicase (SF1 family) has a negative effect on a variety of activities including ssDNA binding, ATP hydrolysis, and ligand-induced conformational changes. An apparent uncoupling of ATPase and helicase activities was observed with a motif VI mutant of HSV1-UL5. Changing the basic residues to H, or R to the uncharged glutamine abolishes RNA binding and reduces ATP hydrolysis, which also results in reduced helicase activity. All these suggest that motif VI, which mediates conformational changes associated with nucleotide binding by virtue of its close proximity to both the NTP-binding site and the DNA-binding site is required for the helicase to move along the DNA substrate.

Motifs I and IV of Rep and PcrA make direct contact with the nucleotide in the enzyme–ADP binary complexes. The residues in motifs Ia, III and V of Rep protein make direct contacts with the bound oligonucleotide [3]. Motifs Ia and V interact primarily with the sugar–phosphate backbone, whereas motif III is involved in hydrogen-bond and stacking interactions with the DNA bases. Mutation of glycine in motif V of UL5 is reported to decrease the affinity of the enzyme for ssDNA as well as the rate of hydrolysis [3]. Most of the mutational studies have been performed in the three members of SF1, *E. coli* UvrD, HSV-1 UL5, and yeast UpfIP. The UvrD is closely related to the Rep and PcrA helicases [3]. The presence of the conserved helicase motifs in a protein is not sufficient for its identification as a helicase. For example, despite the presence of these conserved helicase motifs in the *E. coli* Mfd protein [17] and the human Rad54 protein [18], these proteins do not contain helicase activity. Direct biochemical demonstration of ATP-dependent helicase activity of these proteins is essential before they can be classified as bonafide helicases. Only a few DEAD-box proteins have been shown biochemically to contain unwinding activity. For example, pea DNA helicase 45 [19], yeast Rad3 [20], Dna2 [21], *E. coli* RecQ [22], human REQL helicase [23], *Plasmodium cynomolgi* eIF-4A [24] and ERCC3 [25,26] are DNA helicases (Fig. 3), and human p68, mouse elF-4A, *Xenopus* An3 and *Xeroderma pigmentosum* (XP) 54, *Drosophila* VASA, PRH 75, *Arabidopsis thaliana* DRHl and AtRHI are RNA helicases [20,27,28] (Fig. 3).

Crystal structure

Because of the presence of conserved motifs, it was originally proposed that helicases might generally be organized into a modular structure consisting of a minimum of DNA-binding and NTP-binding domains. The first DNA helicase crystallized was PcrA from a thermophilic bacterium *Bacillus stearothermophilus* [29]. X-ray structures of PcrA, crystallized with or without ADP, were very similar. In both cases, the enzyme crystallizes as a monomer which consists of two parallel domains (1 and 2) with a deep cleft running between them [30]. Each domain contains two additional subdomains (A and B). Subdomain 1A (which carries the Walker A and B motifs) and subdomain 2A structurally resemble the central region of RecA, containing

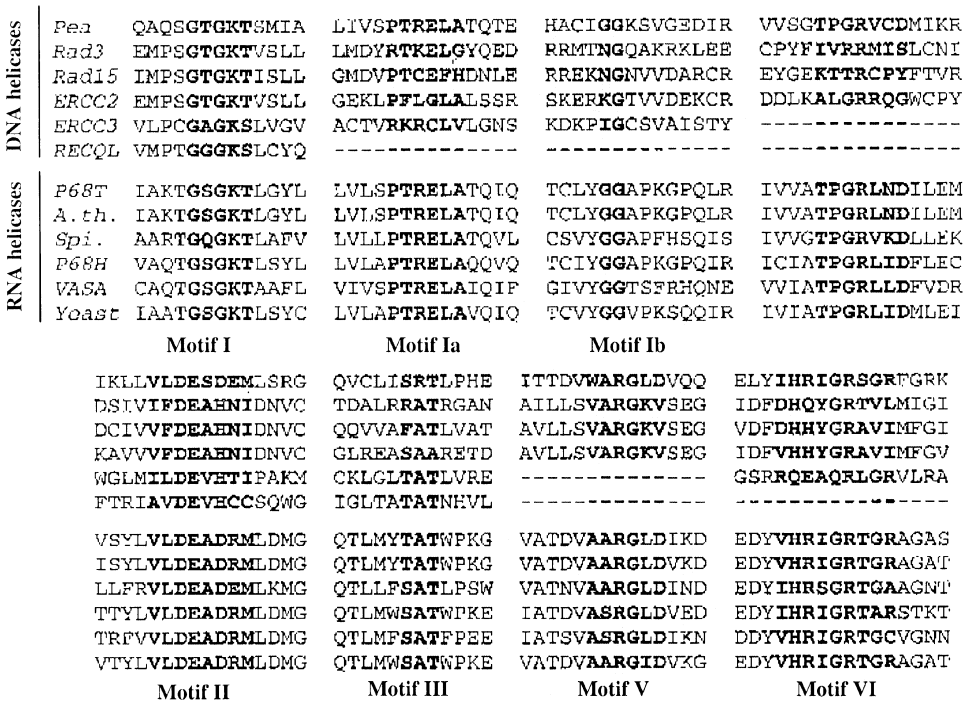

Fig. 3. Alignment of conserved helicase motifs of DNA and RNA helicases of the DEAD/H-box family. The names of the motifs are marked. The amino acids are shown by single-letter code and the letters in bold indicate highly conserved motifs. The accession numbers of aligned sequences of DNA helicases are: Pea (PDH45, Y17186), Rad3 (K03293), Rad15 (X6499), ERCC2 (X52470), ERCC3 (M31899) and RECQL (L36140). The accession numbers of aligned sequences of RNA helicases are: p68T (D16247), A. th (AB010259), Spinach (X99937), p68H (X52104), VASA (X12945) and yeast (X52469).

both a helicase-like strand-separating activity and strand-annealing activity [31]. Both domains contain large insertion sequences that form subdomain 1B and 2B, respectively. The ADP moiety is located at the bottom of the cleft between subdomains 1A and 2A. However, only 1A can bind ADP. This cleft is lined with conserved helicase motifs. No site-bound Mg^{2+} has been found in the two crystal structures. On the basis of structural information for PcrA, a possible mechanism of translocation and unwinding is described below under the subheading 'Mechanism of DNA unwinding and translocation'.

Crystal structures of binary and ternary complexes of the *E. coli* Rep helicase bound to ssDNA [$dT(pT)_{15}$] or ssDNA and ADP were determined to a resolution of 3.0 Å and 3.2 Å, respectively [32]. The asymmetric unit in the crystals contains two Rep monomers differing from each other by a large reorientation of one of the domains, corresponding to swivelling of 130 ° about a hinge region. It was suggested that ATP binding and hydrolysis result in an alteration in the conformation of subdomain Ia, thereby altering the DNA-binding site. Low-resolution structural information is available for the following hexameric helicases: simian virus 40 (SV40) large T antigen, papillomavirus E1, bacteriophage T7gp4, T4gp41, *E. coli* DnaB, RuvB, Rho and RepA encoded by RSF1010 plasmid ([11] and references therein). For some of these, high-resolution structural information has also recently become available. The above helicases act on DNA, except Rho, a transcription terminator protein that acts to separate RNA–DNA hybrids. The *E. coli* Rep helicase and NS3 RNA helicases were also crystallized with ssDNA, giving the first glimpse of how the proteins interact with nucleic acids [32,33]. The structures of T7gp4 and RepA encoded by the RSF1010 plasmid revealed a single-domain protomer with a parallel α-β core topology that includes the RecA core fragment, as well as four β strands extending the β sheet on the carboxy-proximal side, three of which are similar to strands of the RecA protein [34–36].

The crystal structure of UvrB helicase [37] and transcription factor IIH (TFIIH) from human [38] and yeast [39] have been also resolved. The structural organization of yeast and human TFIIH showed that they could be separated into two distinct entities. The human TFIIH contains two complexes: (a) the core TFIIH, which contains five subunits, XPB, p62, p52, p44 and p34; (b) the CAK component, which includes cdk7, cyclinH, and MAT1. The XPD subunit could be associated with either the core TFIIH or CAK component [38]. In yeast, the core TFIIH contains the human counterparts Tfb1 (p62), Tfb2 (p52), and ssl1 (p44), but also Rad3 (XPD) and Tfb3 (MAT1) which are found, respectively, closely associated with and absent from the human core factor. In contrast with the human factor, the yeast ssl2 (XPB) helicase has been reported to be readily dissociated from the other core subunit [40]. The molecular structure of human TFIIH showed that it is $16 \times 12.5 \times 7.5$ nm in size and is organized into a ring-like structure from which a large protein domain protrudes. Within the ring structure, P44 is flanked on either side by the XPB and XPD helicases. Schultz *et al.* [38] suggested a quaternary organizational model for human TFIIH.

The crystal structure of full-length eIF-4A from yeast has been reported [41] to be a 'dumbbell' structure consisting of two compact domains connected by an extended linker. This model also suggests the involvement of the conserved helicase motifs of eIF-4A in its functional activities. Recently the crystal structure of the monomeric-3′-5′ RecG helicase from the thermophilic bacterium *Thermotoga maritima* in a complex with its stalled replication fork substrate (three-way DNA junction, the preferred physiological substrate) has been solved [42]. The study suggested that RecG has three domains: domain 1 is for helicase action, and domains 2 and 3 are motor domains. Their study further suggests that RecG is dsDNA translocase. The crystal structure of RuvB shows that the RuvB protomer is a three-domain structure, and the amino-proximal ATP-binding domain has a parallel α–β fold [43]. Recently, Bernstein *et al.* [44] have determined a 1.8 Å resolution crystal structure of the catalytic core of *E. coli* RecQ in its unbound form and a 2.5 Å resolution structure of the core bound to the ATP analog ATPγS.

In another recent study, the X-ray structure of a hexameric SV40 large T antigen with DNA helicase activity has been presented [45]. The structure identifies the p53-binding surface and reveals the structural basis of hexamerization. The hexamer contains a long, positively charged channel with an unusually large central chamber that binds both ssDNA and dsDNA. The hexamer organizes into two tiers which can potentially rotate relative to each other through connecting α-helices to expand/constrict the channel, producing an 'iris' effect which could be used to distort or melt the origin and unwind DNA at the replication fork [45]. A molecular model for the human nucleotide excision repair protein, XPD, has been developed based on the structural and functional relationship of the protein with a bacterial nucleotide excision repair (NER) protein, UvrB [46]. The validity of using the crystal structure of UvrB as a template for the development of an XPD model was tested by mimicking human disease-causing mutations (XPD: R112H, D234N, R601L) in UvrB (E110R, D338N, R506A) and by mutating two highly conserved residues (XPD, His237 and Asp609; UvrB, H341A and D510A). The XPD structural model can be used to understand the molecular mechanism of XPD human disease-causing mutations. The value of this XPD model demonstrates the generalized approach for the prediction of the structure of a mammalian protein based on the crystal structure of a structurally and functionally related bacterial protein that shares extremely low sequence identity ($< 15\%$) [46].

Mechanism of DNA unwinding and translocation

The detailed molecular mechanism of DNA unwinding by helicases is still not known. However, there are certain features of unwinding and translocation that are probably common to all helicases. Lohman & Bjornson [47] suggested that the mechanism could be classified as either passive or active. In the passive mechanism, the unwinding protein interacts with ssDNA and translocates unidirectionally. Active mechanisms are described for the oligomeric helicases, which bind both ssDNA and dsDNA. There are two popular models for a general mechanism for helicases, described as 'active rolling' and 'inchworm' models (Fig. 4A,B) [47–49].

In the scheme for the active rolling model (Fig. 4A), initially both the subunits of the dimer are bound to ssDNA. As a consequence of binding ATP, one of the subunits releases the ssDNA and binds to the duplex region at the fork. This is followed by helix destabilization and the release of one of the DNA strands in a process that accompanies the hydrolysis of ATP [47]. It was proposed that the Rep dimer unwinds DNA by an active rolling mechanism in which the two subunits alternate in binding dsDNA and 3′ ssDNA at the ssDNA/dsDNA junction. In this model, translocation along ssDNA is coupled to ATP binding, whereas ATP hydrolysis drives the unwinding of multiple DNA base pairs for each catalytic event (Fig. 4A). The Rep protein exists as a stable monomer in the absence of DNA. Once the Rep monomer binds to DNA, it changes its conformation and forms a homodimer, becoming functionally active in translocation and unwinding [30]. It is proposed that ATP hydrolysis alternates between one subunit and the other as an integral part of the translocation and unwinding reactions [50].

For the inchworm model (Fig. 4B) the enzyme is bound to ssDNA and then translocates along the DNA strand to the fork region, probably on binding ATP. Helix destabilization and release of one of the ssDNA strands takes place as ATP is hydrolysed [48]. This model is consistent with any monomeric or oligomeric state of the protein. Based on the crystal structure of the PcrA protein Velankar *et al.* [49] has proposed a refined model for translocation of the PcrA helicase along ssDNA. This model supports the initial inchworm model proposed for the Rep helicase and suggests that, in the initial stage of the translocation cycle, ssDNA is bound to both domains 1A and 2A of the protein. After ATP binds to the complex, the cleft between domains 1A and 2A closes. It has been suggested that domain 1A releases its grip on ssDNA and slides along it, while domain 2A maintains a tight grip on ssDNA. On ATP hydrolysis, the protein returns to its initial conformation, the inter-domain cleft opens, and domain 2A translocates along the ssDNA.

The distinction between the inchworm and active rolling models is that the former involves two binding sites, an ssDNA-binding site and a dsDNA-binding site, either belonging to a single monomeric enzyme or present on two different subunits of a dimeric enzyme, whereas the latter involves two subunits of a dimeric enzyme which bind alternately to ssDNA and dsDNA. Both mechanisms require ATP hydrolysis, but it is not certain at which step this hydrolysis takes place, although ATP binding appears to be associated with an increased affinity of the enzyme for duplex DNA or RNA.

As Rep protein and UvrD helicases of *E. coli* share about 40% amino-acid sequence identity and several biochemical properties (including formation of dimer in the presence of ssDNA), it has been suggested by Ali & Lohman [51] that the two proteins probably unwind duplex DNA by the same rolling mechanism involving a dimeric helicase. However, Mechanic *et al.* [52] have reported that a mutant lacking the

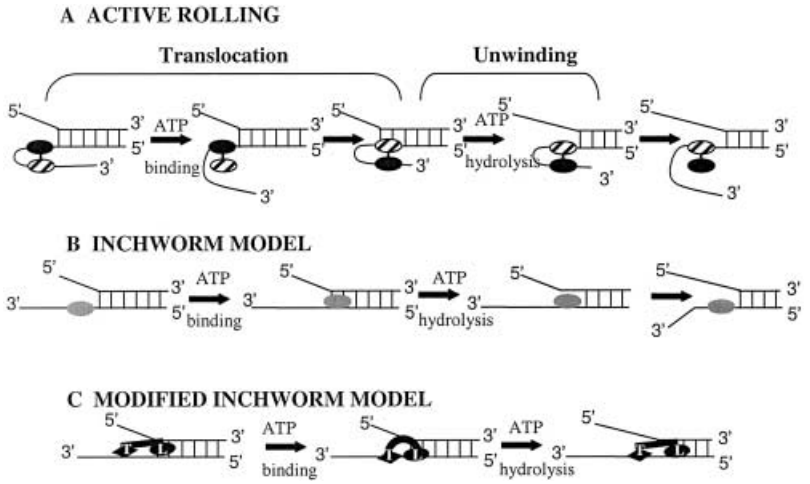

Fig. 4. Models for DNA helicase unwinding and translocation. In both the mechanisms, ATP hydrolysis is required and the helicase protein contacts and translocates on the sugar–phosphate backbone of the strand. (A) Active rolling model. The two subunits of dimeric helicase are shown as an oval shape in which one is black. The dimeric helicase unwinds by interacting directly with both dsDNA and ssDNA. Each subunit alternates binding to dsDNA as the dimer translocates when one subunit releases ssDNA and rebinds to dsDNA [5]. In this model, translocation along ssDNA is coupled to ATP binding, whereas ATP hydrolysis drives the unwinding of multiple DNA base pairs for each catalytic event. (B) Inchworm model. This model is consistent with the monomeric or oligomeric state of the protein (shown as an oval shape). The enzyme monomer first binds to ssDNA and then translocates along the DNA strand. It then binds to the duplex region at the fork. This is followed by unwinding and release of one of the ssDNA strands [48]. If the enzyme is a hexamer, such as the SV40 large T antigen, then one of the subunits (monomer 1) remains associated with the fork through the unwinding cycle. (C) Modified inchworm model. This model is proposed for monomeric UvrD helicase [52,55]. In this model, it is assumed that the monomeric helicase contains two DNA-binding sites: the leading site (L) binds to both dsDNA and ssDNA, and the trailing site (T) binds to only ssDNA. On ATP binding, the enzyme changes its conformation from an extended to a compact state, in which the T site is shifted forward along the ssDNA towards the L site. On ATP hydrolysis, the enzyme changes its conformation from a compact to an extended state. In the extended state the T site is bound to ssDNA, and the L site is extended forward in the duplex region and unwinds the DNA. In all the models the shape of the helicase and location of the DNA are arbitrary.

C-terminal 40 amino acids (UvrDΔ40C) failed to dimerize and yet was as active as the wild-type protein in ATP hydrolysis and helicase assays. Their results show that the monomeric form of UvrD (UvrDΔ40C) is an active helicase both *in vitro* and *in vivo*. Therefore the mechanism of unwinding by monomeric UvrD helicase may be different. Matson's group have proposed a modified version of the inchworm model for DNA unwinding by an active monomeric DNA helicase (Fig. 4C). This model assumes at least two nonequivalent DNA-binding sites on the monomeric protein. The leading site (L) has an affinity for duplex DNA and may also bind ssDNA, whereas the trailing site (T) has an affinity only for ssDNA. A cycle of unwinding begins with the enzyme in an 'extended' conformation in which the T site is bound to ssDNA, and the L site is extended forward in the vicinity of the ssDNA/dsDNA junction. Binding of ATP to the enzyme induces a conformational change in the protein to a more compact state in which the T site is shifted forward along the DNA lattice with respect to the L site. On ATP hydrolysis a distinct number of base pairs are disrupted at the ssDNA/dsDNA junction, and product release is associated with a return of the enzyme to its original conformation by extension of the L site forward with respect to the T site. At this point, the L site is close to the new ssDNA/dsDNA junction, and the cycle is repeated to catalyze further unwinding (Fig. 4C) [52]. Recently, Maluf *et al.* [53] have shown that initiation of DNA unwinding *in vitro* appears to require a dimeric UvrD complex in which one subunit is bound to the ssDNA/dsDNA junction, while the second subunit is bound to the 3′ ssDNA tail.

Bianco & Kowalczykowski [54] have suggested another inchworm model, the 'quantum inchworm' mechanism for translocation and unwinding of duplex DNA by RecBC DNA helicase. Translocation and unwinding are two separate and consecutive events in the mechanism and are brought about by two different domains [leading (L) and trailing (T)] within the RecBC enzyme. In this model the L domain anchors the enzyme to only one strand of duplex DNA and translocates along it, whereas the T domain is responsible for unwinding. During the translocation and unwinding reaction, the L domain binds up to 23 nt ahead of the T domain, and the T domain uses energy derived from ATP binding and hydrolysis to open the duplex [54,55].

Recently, Ha *et al.* [56] proposed that the Rep monomer uses ATP hydrolysis to move toward the junction between ssDNA and dsDNA but then displays conformational fluctuations that do not lead to DNA unwinding. DNA unwinding is initiated only if a functional helicase is formed via additional protein binding. Partial dissociation of the functional complex during unwinding results in interruptions ('stalls') which lead to either duplex rewinding on complete dissociation of the complex or re-initiation of unwinding on re-formation of the functional helicase. These results suggest that the low unwinding processivity observed *in vitro* for Rep is due to the relative instability of the functional complex.

Functions of DNA helicases

The ubiquitous presence of helicases in prokaryotes and eukaryotes reflect their fundamental importance in DNA metabolism such as replication, repair, recombination and transcription [2,4,5,47,57]. *In vivo*, DNA unwinding is usually coupled to the action of other proteins such as initiator proteins, primases, ssDNA-binding proteins (SSBPs), polymerases and many more factors depending on the associated functions of a particular helicase. SSBPs play many important roles along with helicases such as: (a) increasing the processivity of the helicase; (b) blocking the reannealing of the unwound strands; (c) inhibiting helicase action by blocking access of the helicase. The various helicases and their known function are described below.

Replicative helicases

This section deals with the process of DNA replication (initiation, elongation and termination) and properties of replicative helicases. The synthesis of all genomic DNA involves the highly coordinated action of different types of enzymes including DNA helicases, which melt the DNA duplex to generate the replication fork. Figure 5A shows the schematic representation of progressive DNA unwinding of duplex DNA by a replicative DNA helicase at the replication fork. The replicative DNA helicases occupy a central position at the replicative fork and can unwind duplex DNA quickly and processively in the presence of helicase reporter (trapping agents) such as DNA polymerase and SSBPs [55]. The process of DNA replication can be divided into three phases: initiation, elongation, and termination.

Initiation of DNA replication. In *E. coli*, bacteriophage T7, and SV40 virus, assembly of a preinitiation complex precedes initiation of DNA synthesis at the replication origins. The first step of the initiation process is the melting of the two DNA strands by binding of the subunits of the hexameric helicase to the origin DNA. The separated strands serve as templates for the primase/polymerase for new strand synthesis. The loading of helicases on the origin of replication requires the participation of specific accessory proteins. It has been demonstrated that Rep helicase is loaded on to the replication origin via tight binding with the CisA protein [58]. The T7 gp4 primase/helicase is loaded on to ssDNA by a facilitated ring-opening mechanism, and helicase loading is assisted by the presence of the primase domain [59]. Genetic and biochemical experiments have identified DnaC as the loading factor for DnaB. In the presence of DNA and ATP, the bacteriophage T4 gp41 hexamer binds six T4 gp59 molecules leading to a 1 : 1 helicase/helicase loader molar ratio [60]. Recent studies have shown that the hetero association interactions of T4 gp59 with ssDNA, T4 gp32 and T4 gp41 are all essential in the loading reaction [61].

Elongation of DNA. Elongation of the new DNA chains requires further separation of the DNA templates. In every case, the hexameric helicase responsible for the initial melting of the origin DNA is also the processive helicase that unwinds the growing fork [62]. Additional key proteins are required for melting of the duplex DNA downstream of the replication fork. DNA polymerase on the leading strand and the ssDNA-binding proteins that bind on the lagging strand work with the replicative helicases to complete the elongation mode. It has been proposed that the coupling of

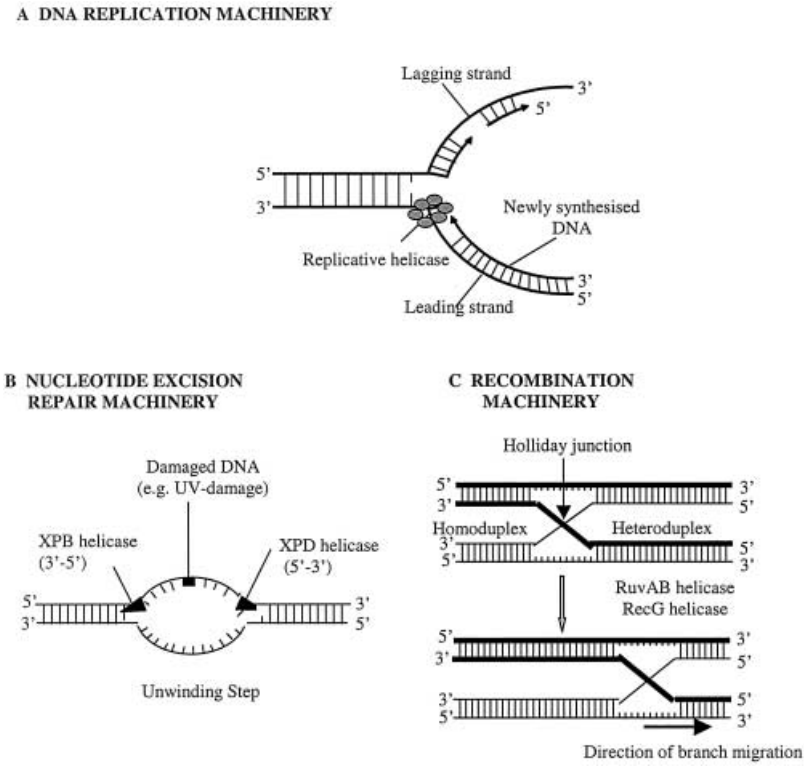

Fig. 5. Models of DNA unwinding. (A) Replicative machinery showing the interaction of replicative DNA helicase at the replication fork. This is a schematic representation of the replication fork showing the unwinding of the DNA by replicative hexameric helicase during fork progression. Both strands of DNA are synthesized in the 5′ to 3′ direction. The leading strand is synthesized continuously, whereas the trailing strand is synthesized in the form of short fragments. (B) Model of DNA unwinding during NER in mammalian cells. In this figure only the unwinding step is shown. The damaged area is unwound by the XPB helicase (3′-5′) and XPD helicase (5′-3′) components of TFIIH. (C) Model of DNA unwinding during recombination. Recombination machinery shows only the steps of unwinding and branch migration of the Holliday junction by *E. coli* RuvAB or RecG helicases.

the helicase to the polymerase during the elongation phase does not require their physical interaction. The helicase is functionally coupled to its homologous SSBP [55].

Termination of DNA replication. Termination of replication involves a protein that forms a tight complex with the termination site (called Ter), acting as a 'contra-helicase'. In *E. coli* and *Bacillus subtilis*, terminators are organized into two sets of five or three Ter sites which, when complexed with terminator proteins, cause replication arrest in a polar fashion. These replication termination proteins operate by blocking the ATP-dependent unwinding activity of helicases in a polar fashion [63]. Ter sequences (acting in *cis*) and terminator proteins (acting in *trans*) are the two components that dictate replication arrest. It has been postulated that the protein–Ter complex thus comprises a trap for replication forks that arrive at the site, and, as a consequence, also serves to prevent forks from meeting at other template positions.

Only some of the *E. coli*, bacteriophage, and viral DNA helicases are reported to play clear cut roles in DNA replication. However, owing to the lack of clearly defined cell-free systems that catalyze chromosomal replication, it is difficult to define the role of any cellular replicative helicase. Nonetheless, the study of viral replicative helicases has helped to define the role of helicases in eukaryotic DNA replication. The best characterized replicative helicases are DnaB, PriA and Rep proteins from *E. coli*, T7gp4 and T4gp41 proteins from bacteriophage, and SV40 large T antigen, polyoma virus T antigen, bovine papillomavirus E1, herpes simplex virus (HSV) UL5 and UL9, adeno-associated virus (AAV) Rep 68 and Rep 78, and minute virus of mice (MVM) NS1 proteins from viral sources [4,47,55]. A comparison of the properties of the replicative DNA helicases is given in Table 1. Indirect evidence also suggests that yeast Dna2P, *Xenopus laevis* FFA-1 and Werner syndrome helicase (WRN) proteins may be candidates for DNA replicative helicases [64–68]. Liao *et al.* [69] have reported the first direct evidence that *Xenopus* Bloom's syndrome protein homolog (XBLM) plays an important role in DNA replication, suggesting that Bloom's syndrome may be the consequence of defective DNA replication.

Repair helicases

DNA repair is a fundamental cellular process for protecting cells against damaged DNA. The helicases are some of the first proteins that encounter DNA damage and thereby play an important role in its repair. During DNA repair, especially in NER, the damaged area on the DNA has to unwind first in order be repaired, because most of the DNA repair machinery enters through ssDNA. The unwinding step during NER in mammalian cells is shown in Fig. 5B.

Examples and some properties of repair helicases are described in Table 2. In *E. coli* the UvrAB complex and UvrD are the repair DNA helicases involved in NER. The structure of UvrB suggests that it functions as a helicase adapted to the unique requirements of DNA repair [37]. It has been reported that the UvrA-mediated loading of UvrB is an asymmetric process which starts on the 5′ side of the lesion, and the strand-separating activity of the UvrAB complex is used to create an 'entry site' for UvrB at this position [70]. In general, the UV-induced lesions or other helix-distorting intrastrand adducts are better tolerated by DNA helicases than those produced by intercalating agents. However, in the yeast *Saccharomyces cerevisiae* the Rad3 protein, a repair helicase, is absolutely required in NER and is found to be inhibited by UV damage and intrastrand cisplatin adducts located on the strand along which the enzyme translocates, whereas lesions on the opposite strand had no effect [71]. DNA helicases involved in the initial stages of NER are more sensitive to DNA damage (e.g. UvrAB complex, Rad3 or XPD) than those that are involved in the subsequent removal of the damaged DNA (e.g. UvrD). ERCC6 (excision repair cross complementing), which corrects the repair of Cockayne syndrome complementation group B, encodes a protein containing seven conserved helicase motifs [72]. The repair proteins ERCC3 (XPB) and ERCC2 (XPD) contain conserved helicase motifs and DNA-unwinding activity. The function of these helicases is to open up the structure around the damaged site to enable the structure-specific nucleases to excise the DNA [73]. The human XRCC5 DNA repair gene has been shown to encode an 80-kDa subunit of the Ku protein [74] and together with the 70-kDa subunit possesses ATP-dependent DNA helicase activity [75].

Recombination and other functions of the DNA helicases

The properties of recombination helicases and some other functional helicases are summarized in Table 3. During genetic recombination, the pairing of homologous regions of DNA requires both the unwinding of duplex DNA by helicases for strand invasion and the generation of extended single-stranded segments. Recombination in *E. coli* requires the co-ordinated action of at least 25 proteins, which include RecA, RecBCD, RecF, RecG, RecN, RecO, RecQ, RecR, RuvAB, RuvC, PriA, SSBP, DNA polymerases, DNA topoisomerases and DNA ligases [76]. Of these proteins, only RecBCD, RecG, RecQ, RuvAB, and PriA act as DNA helicases (Table 3). Homologous recombination can be divided into four sequential processes: (a) initiation; (b) homologous pairing and strand exchange; (c) DNA heteroduplex extension or branch migration; (d) resolution. DNA helicases are centrally involved in initiation and branch migration. The role of recombination helicases (RuvAB and RecG) in branch migration is shown in Fig. 5C. The properties of some of the recombination helicases are briefly described below.

E. coli RecBCD carries a number of diverse enzymic activities such as ATP-dependent ssDNA endonulcease, ssDNA exonuclease, dsDNA exonuclease, RecA-loading, DNA-dependent ATPase and ATP-dependent helicase activities [55,57,77]. It has sets of helicase signature motifs in both RecB and RecD, two of its three subunits. RecA can serve as a reporter of RecBCD helicase activity [78]. RecB and RecD contain 3′ to 5′ and 5′ to 3′ DNA helicase activity, respectively [79]. These findings point to a bipolar translocation model for RecBCD in which the two DNA helicases are complementary, travelling with opposite polarities, but in the same direction, on each strand of the antiparallel DNA duplex. Recently, by electron microscopy of individual molecules, it has been shown that RecD is a fast helicase

Table 1. Replicative helicases. nd, Not determined; nr, not required; OBP, origin binding protein; AAV, adeno-associated virus; BPV E1, bovine papillomavirus E1; MCM, minichromosome maintenance; MVM, minute virus of mice; TAG, T antigen; WRN, Werner syndrome helicase.

S. No.	Name of helicase	Source	Mol. mass (kDa)	Nucleotide requirement	Fork requirement	Remarks
1.	DnaB[a]	*E. coli*	52.2	ATP > GTP, CTP	3′ ss tail of ~90 nts	Stimulated by SSB and DnaG; stimulates rolling circle replication, and unwinding is ori-dependent.
2.	PriA[b]	*E. coli*	81.7	ATP = dATP	nr	Stimulated by primosome assembly site sequence.
3.	Rep[c]	*E. coli*	72.8	ATP = dATP ≫ GTP, dGTP	3′ ss tail of > 4 nts	Discovered first as replicative helicase; required for replication of ϕX174, fd and M13 phages.
4.	T7gp4A[d] and 4B	Phage	A, 63 B, 56	dTTP > ATP, all others	3′ ss tail of 6–7 nts	Required for phage DNA replication; interacts specifically with T7 DNA polymerase.
5.	T4gp41[e]	Phage	59	GTP > ATP, dATP, dGTP	3′ ss tail of 29 nts	Stimulated by T4 gp61; also contains primase activity.
6.	G40P[f]	Phage	300	ATP > GTP > CTP > UTP	5′ ss tail of 25 nts	Essential for *B. subtilis* bacteriophage SPP1 replication.
7.	SV40 TAG[g]	Virus	82	ATP, dATP, dTTP > dCTP, UTP	3′ ss tail of 5–10 nts	OBP essential for SV40 replication; contains both DNA and RNA helicase activities.
8.	Polyoma TAG[h]	Virus	88	ATP, dATP > CTP, UTP ≫ GTP	nr	Similar to SV40 TAG. OBP required for Polyoma virus DNA replication.
9.	BPV E1[i]	Virus	69	All NTPs and dNTPs	5′ ss tail	Required for bovine papilloma virus DNA replication.
10.	HSV UL5[j]	Virus	114	ATP > GTP,CTP,UTP	3′ ss tail	UL5 and UL52 required for helicase-primase activities.
11.	HSV UL9[k]	Virus	94	ATP = dATP > CTP, dCTP, UTP	5′ ss tail	Stimulated by ICP8 and initiates origin-specific DNA replication.
12.	AAV Rep68, Rep78[l]	Virus	61 71	ATP > CTP > dATP > GTP > UTP	nd	Contains site and strand specific endonuclease activity; required for viral DNA replication.
13.	MVM NS-1[m]	Virus	83	ATP > dATP,	nd	Homolog of AAV Rep helicase.
14.	MCM4/6/7[n]	Yeast	80–100 (each)	ATP = dATP	5′ and 3′ tails	Required for initiation and elongation steps of chromosomal DNA replication.
15.	Dna2p[o]	Yeast	172	ATP = dATP	nd	Indirect evidence shows its role in DNA replication.
16.	FFA-1[p]	*Xenopus laevis*	170	ATP	nd	Required for the formation of replication foci; homologous to the human WRN.

[a] [90]; [b] [91]; [c] see [4]; [d] see [4]; [e] [92]; [f] [93]; [g] [94]; [h] [95]; [i] [96]; [j] [97]; [k] [98]; [l] [99]; [m] [100]; [n] [101]; [o] [21] [p] [68].

Table 2. DNA repair helicases. nd, Not determined.

S. No	Name of helicase	Mol. mass (kDa)	Nucleotide requirements	Remarks
1.	*E. coli* helicase II (UvrD)[a]	82	ATP > dATP	Involved in methyl-directed mismatch repair.
2.	*E. coli* UvrAB complex[b]	180	ATP = dATP	Involved in NER. UvrB is the helicase component.
3.	Yeast Rad3[c]	90	ATP = dATP	Absolutely required for NER, inhibited by DNA damage.
4.	Calf thymus helicase E[d]	104	ATP, dATP	Can unwind DNA from nicks and has been predicted to play a role in DNA repair.
5.	Mammalian ERCC2[e] (XPD)	87	ATP, dATP	Component of transcription factor (TFIIH). Unwinds DNA in 5′ to 3′ direction and involved in NER.
6.	Mammalian ERCC3[f] (XPB)	89	ATP	Component of TFIIH, unwinds DNA in 3′ to 5′ direction and involved in NER.
7.	Mammalian ERCC6[g] (putative helicase)	168	nd	Contains helicase motifs involved in DNA repair of Cockayne's syndrome complementation group B.
8.	Human HDH II/Ku[h] (XRCC5)	70/80	ATP = dATP	Involved in double-strand break repair and V(D) J recombination of immunoglobulins.

[a] [57]; [b] [102]; [c] [103]; [d] [104]; [e] [105]; [f] [106]; [g] [72]; [h] [75].

acting on the 5′-ended strand and RecB is a slow helicase acting on the 3′-ended strand on which the single-stranded loop accumulates [80].

RecG helicase translocates on duplex DNA, driving a wedge through the branch point of a fork or Holliday junction structure, thus catalysing both strand unwinding and strand annealing (Fig. 5C). RuvAB carries out a similar reaction. The RecG helicase is necessary for efficient recombination and repair of DNA *in vivo* and is also called as junction-specific DNA helicase [81]. RecG protein differs from other helicases analysed in that it mediates strand separation via translocation on dsDNA rather than ssDNA. The RecG helicase of *E. coli* has been postulated to act on stalled replication forks to promote replication restart via the formation of a four-stranded (Holliday) junction. McGlynn & Lloyd [82] have shown that RecG can actively unwind the leading and lagging strand arms of model replication fork structures *in vitro*. Thus, RecG translocates simultaneously along two DNA strands, one with 5′ to 3′ and the other with 3′ to 5′ polarity. The unwinding of both nascent strands at a damaged fork, and their subsequent annealing to form a Holliday junction, may explain the ability of RecG to promote replication restart.

E. coli RuvAB helicase is an ATP-driven translocase (pump) that promotes branch migration of the Holliday junction [83]. These are four-way branched DNA structures formed during recombination, replication and repair and are processed in *E. coli* by the RuvA, RuvB and RuvC proteins. RuvA targets the junction and facilitates loading of RuvB helicase and RuvC endonuclease to form complexes that catalyse junction branch migration (RuvAB) and resolution (RuvABC resolvasome) [84]. RuvAB-catalyzed branch migration involves the unwinding and rewinding of duplex DNA near the base of the Holliday junction, with the transient unwinding reaction taking place within the interior of the RuvB ring. RuvA plays a scaffolding role in preparing the homoduplex DNA strand for strand separation and the recombining strands of the heteroduplex for reannealing [85]. Recently, Kaplan & O'Donnell [86] demonstrated that DnaB can drive branch migration and dislodges proteins while encircling two DNA strands, its *in vivo* role in DNA recombination. The other helicases that play a role in recombination are helicase II (UvrD), RecQ from *E. coli*, bacteriophage T4 UvsW protein, Ku autoantigen, and WRN helicase (Table 3). Some other functions of DNA helicases are also described in Table 3.

Role of DNA helicases in transcription

DNA helicases are known to alter the DNA or DNA/RNA structures, remodeling chromatin and modulating access to the DNA template by transcriptional machinery. The RNA polymerases that catalyze the template-dependent elongation of RNA transcripts can be considered to be helicases that unwind the dsDNA genome in order to 'expose' the ssDNA strand that will serve as the template for RNA synthesis by the transcription complex. Generally for RNA polymerases in the elongation-competent state, these helicase and synthesis activities are 'tightly coupled' with the extension of the RNA transcript by one nucleotide involving the consumption of one molecule of NTP and the concomitant unwinding of a single base pair at the downstream end of the transcription bubble [55]. DNA helicases thus play an important role as part of the transcription complex in most if not all transcriptional processes including activation (e.g. TFIIH), initiation (e.g. TFIIH, SNF2), maintenance (e.g. SW1), DNA repair (e.g. TFIIH, ERCC6/RAD26) and termination (e.g. Factor 2, Rho) [72,73,87–89]. Transcription activation involves the formation of a preinitiation complex, its association with ssDNA activation, and promoter clearance. TFIIH is an RNA polymerase II transcription factor that performs ATP-dependent functions in both transcription initiation, where it catalyzes formation of the open complex, and in promoter escape, where it suppresses arrest of the early elongation complex at promoter-proximal sites. TFIIH possesses three known ATP-dependent activities: a 3′ to 5′ DNA helicase activity catalyzed by its XPB (ERCC3, RAD25) subunit, a 5′ to 3′ DNA helicase activity catalyzed by its XPD (ERCC2, RAD3) subunit, and a kinase activity specific for the C-terminal domain of RNA polymerase II [87,88]. Moreland *et al.* [88] reported that the TFIIH XPB

Table 3. Recombination and other functional helicases. HDH, Human DNA helicase; PDH65, pea DNA helicase 65; nd, not determined; RPA, replication protein A; WRN, Werner syndrome helicase.

S. No.	Name of helicase	Mol. mass (kDa)	Nucleotide requirement	Remarks
1.	*E. coli* helicase II (UvrD)[a]	82	ATP > dATP	Indirect evidence suggests its role in recombination as an antirecombinase; also unwinds blunt end duplex DNA and nicked circle DNA substrates.
2.	*E. coli* Rec BCD[b]	330	ATP, dATP	Involved in recombination; generates ssDNA that can be utilized by RecA protein in homologous strand exchanges.
3.	*E. coli* RecQ[c]	74	ATP = dATP	Involved in RecF recombination pathway; functions as suppressor of illegitimate recombination; also unwinds blunt end duplex DNA.
4.	*E. coli* RuvAB[d]	22 37	ATP	Required for recombination and repair; helps in branch migration of a Holliday junction.
5.	*E. coli* RecG[e]	76	ATP, dATP	Required for normal levels of recombination and repair; junction specific helicase and involved in branch migration of Holliday junction.
6.	Bacteriophage T4[f] UvsW protein	nd	ATP	Involved in phage recombination and is functional analog of RecG helicase. Catalyzes branch migration and also dissociation of RNA–DNA hybrids.
7.	HDH II/Ku[g]	70 80	ATP = dATP	Involved in V(D)J recombination of immunoglobulins and also in dsDNA break repair.
8.	WRN helicase[h]	163	ATP = dATP ≫ dCTP, CTP	A member of RecQ family of DNA helicases; may be involved in DNA recombination, replication and repair. WRN helicase also accelerates the transcription of ribosomal RNA.
9.	HDH IV/nucleolin[i]	100	ATP, dATP	Can unwind DNA–DNA, RNA–RNA and RNA–DNA hybrids. Involved in preribosomal assembly and ribosome biogenesis.
10.	*E. coli* helicase 1 (Tra I)[j]	192	ATP, dATP > all others	Required for DNA transfer during conjugation. Also contains nicking activity.
11.	*E. coli* Rho[k]	50	ATP > NTPs	Specific for RNA–DNA hybrids functions in transcription termination at specific sites; contains ssRNA-dependent NTPase activity.
12.	Yeast MER3[l]	130	ATP	Involved in meiotic crossing over and stimulated by yeast RPA.
13.	PDH65[m]	65	ATP > dATP	Homologous to HDHI, may be involved in rDNA transcription.

[a] [57]; [b] [107]; [c] [22]; [d] [83]; [e] [81,108]; [f] [109]; [g] [75], see [110]; [h] [65]; [i] [111], see [110]; [j] [57]; [k] [57]; [l] [112]; [m] [113].

DNA helicase was primarily responsible for preventing premature arrest of early elongation intermediates during the exit of the polymerase from the promoter. TFIIH is also required for transcription-coupled nucleotide excision repair [87,88]. Finally in *E. coli*, the Rho protein, a RNA/DNA helicase, and in *Drosophila*, Factor 2 are required for transcription termination [57,87].

Concluding remarks

DNA helicases are involved in almost every aspect of nucleic acid metabolism, which makes them very important components of the cell. High sequence conservation has been maintained in the large group of helicases, suggesting that all helicase genes evolved from a common ancestor. The DEAD-box helicases contain common conserved helicase signature sequences but they differ mainly in their N-terminal and C-terminal sequences, which contain different targeting signals. Different regulatory mechanisms at the level of both expression and post-transcription explain the wide spectrum of functions involving DEAD-box helicases. The crystal structures of SF1 and SF2 helicases have shown that the DNA helicase motifs are clustered together in the tertiary structure, forming an NTP-binding pocket and a portion of the nucleic acid-binding site. According to Hall & Matson [3], the conserved helicase motifs can be envisioned as the engine, generating energy by the consumption of fuel (NTPs) which is used to perform

work. The nonconserved portions of helicase structure may contain specific domains such as protein–protein interaction domains, cellular localization signals, site-specific DNA-recognition domains, and oligomerization interfaces, which may be unique to individual helicases. The crystal structures of a few helicases have been resolved, which will greatly help us to understand the mechanism by which ATP hydrolysis and conformational changes are coupled to allow these proteins to carry out their varied enzymatic activities. Despite the available crystallographic data, many questions remain unanswered. For example, the mechanism of strand separation remains a mystery. It is also unclear why hexameric helicases generally prefer substrates with two tails (i.e. a real fork) rather than a simple tailed duplex, which is sufficient for other enzymes such as PcrA and Rep. These matters should be resolved by future structure studies, and the structure of helicases complexed with other modulator proteins should help us to understand other details of their mechanism of action.

Although several DNA helicases have now been isolated from different sources, the role of only a few have been elucidated to date. As the basics of DNA metabolism are very similar in all eukaryotes from yeast to mammals, the combination of genetic studies in yeasts, *in vitro* systems, and biochemical characterization should help us to clarify the roles of the various DNA helicases *in vivo*. It now appears that oligomeric helicases (mainly hexameric) are often integral components of larger protein complexes, and their role within the complex is to provide molecular motor function. Therefore, it would perhaps be naïve to perceive helicases merely as nucleic acid-unwinding enzymes. To gain a complete picture of helicase function, it is important to study these enzymes *in situ* as components of the macromolecular machines in which they have a central function. It will be interesting to see how they are integrated into the overall processes in which they function. These studies will provide insight into the mechanisms of action of helicases themselves, and also into the overall biological systems within which they operate.

During the elongation phase of DNA replication the base pairs of dsDNA at the replication fork must be opened continuously and sequentially to expose the ssDNA templates, which will be copied by the DNA polymerase. However, DNA at replication forks is synthesized at rates of ≈ 500 bp·s^{-1}. This means that helicases at these forks must be opening dsDNA at this rate or faster. It has been suggested that enhanced thermal fluctuation may be responsible for sequential base-pair opening by replicative helicases at the replication fork. This also suggests that replicative helicases may advance by a 'passive' unwinding mechanism rather than by some mechanical application of energy from NTP hydrolysis. In such a case, ATP hydrolysis may serve only to generate the ADP-bound form of the helicase and to initiate the release of protein from the ssDNA product in order to complete the ATP-binding and hydrolysis cycle.

A detailed view of the energetics of the translocation process and the mechanism of unwinding is also lacking. Low-resolution structural analysis, by electron microscopy, is beginning to provide clues to some of the functions and mechanisms of action of these remarkable proteins in prokaryotes. On the basis of electron-microscopy studies, a 'dual helicase' model has recently been proposed for the *E. coli* RecBCD enzyme complex. It will be interesting to see whether such bipolar, nonidentical helicase "motors" are used by other DNA-processing enzymes. We still need to understand the molecular details of how other proteins modulate the activity of helicases or how helicases function within larger macromolecular complexes such as the primosome. Structures of helicases complexed with other modulator proteins will help us to understand in greater detail their mechanism of action. A better understanding of these proteins in plant will have to await breakthroughs on their high-resolution structures using X-ray crystallography. Furthermore, in plants, the RNAi approach and/or transgenic antisense/sense plant technology will be very helpful in understanding the detailed role of helicases in plant growth and development and therefore will have important biotechnological applications.

Overall this field is now progressing fast, and accordingly the number of isolated DNA helicases in both prokaryotic and eukaryotic systems are increasing. This has created the problem of a bewildering array of different names and their classification. Therefore it is important that a clear, scientific system for nomenclature and classification of helicases is formulated. This is a complicated problem and does not seem to have a straightforward solution. However, there may be several possible ways of classifying DNA helicases, which may not be convenient. For example, they can be classified on the basis of the kind of duplex-strand unwinding (e.g. DNA or RNA or RNA/DNA or DNA/RNA helicase), source of identification (e.g. prokaryotic or eukaryotic DNA helicases), oligomeric nature of protein (e.g. monomeric or oligometic DNA helicases), polarity of translocation (e.g. 3′–5′ or 5′–3′ DNA helicases), associated functions (e.g. replicative, repair, recombination, or other functions) or on the basis of helicase motifs present in the superfamily (e.g. SF1, SF2, SF4 and F4). We believe that this class of proteins does not, at this point, lend itself to a simple scheme of classification. Overall, the progress in the DNA helicase field is further extending our understanding of DNA metabolism and is also providing us with a dizzying array of molecular tools for cellular machinery.

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