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Superfamily 1 helicases

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1. ABSTRACT

Superfamily 1 helicases are nucleic acid motor proteins that couple ATP hydrolysis to translocation along, and concomitant unwinding of, DNA or RNA. This is central to many aspects of cellular DNA and RNA metabolism and, accordingly, they are implicated in a wide range of nucleic acid processing events including DNA replication, recombination and repair as well as many aspects of RNA metabolism. This review discusses our current understanding of the structure, function and mechanism of Superfamily 1 helicases.

2. INTRODUCTION

Following their elucidation of the structure of DNA, Watson and Crick predicted the need for an activity that would prise apart the two DNA strands and expose the bases for replication via a semi-conservative mechanism (1). However, it was not until the 70's that the first DNA helicases were isolated and characterised, initially from *E. coli* cell extracts. Subsequent experiments would show that these enzymes were able to couple the free energy of ATP binding and hydrolysis to the separation of the DNA duplex into its component single-strands. Similarities in the primary structures of helicases led to their formal classification and the identification of a vast number of putative helicases. The first glimpses of helicase structure came from crystallographic studies in the late 90's. These provided a powerful framework for the interpretation of biochemical experiments and led to the proposal of detailed mechanisms for unwinding. However, apparently contradictory conclusions from some biochemical and mechanistic studies have left unresolved questions. We now appreciate that helicases function in a remarkably diverse range of nucleic acid processing pathways, often acting in transient association with partner proteins or as part of stable multi-protein complexes to orchestrate complex nucleic acid manipulations. In this review, we present a concise overview of Superfamily 1 (SF1) helicases with an emphasis on biochemical analysis and enzyme mechanism. We will first explain the classification of SF1 helicases, introduce their known cellular functions and basic biochemical properties, and then discuss our current understanding of their unwinding mechanisms. Finally, we will explore the outstanding questions in the field and highlight promising areas for future research. There have been other excellent reviews of this area, to which the reader is referred for further information and differing opinions (2-6).

3. CLASSIFICATION OF HELICASE-LIKE PROTEINS

3.1. Primary structure and oligomeric state

The work of Koonin and colleagues established that proteins which had been identified as helicases based on their *in vitro* biochemical properties contained characteristic amino acid motifs. Based on primary structure, the sequences available at that time were grouped into two large Superfamilies (SF1 and SF2) and three smaller families (F3, F4 and F5) of putative helicases (7). Superfamilies 1 and 2 were seen to be distinct from the other groups and each contained several identifiable motifs. These motifs were originally considered to be equivalent between the two groups, but structural studies would later show that this was not always the case (5, 8). The proteins in families three to five each contained fewer amino acid motifs within a smaller conserved core region, and some of these motifs were equivalent to those found in SF1 and SF2. A sixth group of nucleic acid motor proteins, related to AAA+ ATPases, was added in an expanded version of the original classification (5). Genomic sequencing data has revealed that proteins containing motifs associated with these different protein families are extremely abundant, accounting for as much as ~2% of the proteome (9). The functional oligomeric state of SF1 and SF2 enzymes has been a matter of extensive debate (3). As will be discussed below, at least some of these enzymes are able to catalyse DNA strand

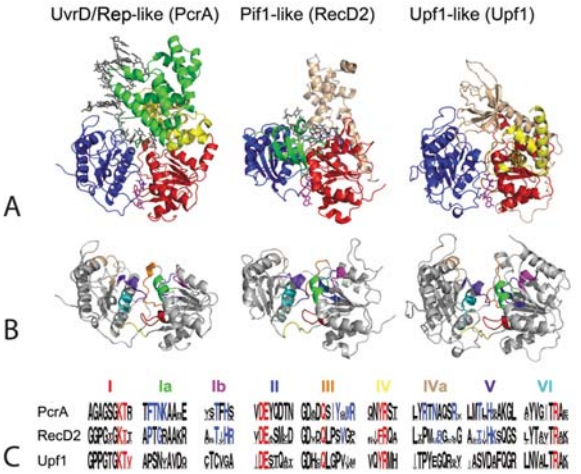

Figure 1. Representative structures and amino acid sequence motifs for Superfamily 1 helicases. (A) The structures shown are B. stearothermophilus PcrA (PDB code 3PJR (69); a member of the UvrD/Rep-like family), Deinococcus radiodurans RecD2 (PDB code 3GPL (71); a member of the Pif1-like family) and the core of human Upf1 (PDB code 2GJK (65); a member of the Upf1-like family). In the upper panel, the helicase core domains, N-core and C-core, are shown in red and blue respectively for all three proteins and a non-hydrolysable ATP analogue (magenta) is bound at their interface. Where present, DNA is shown in gray. Accessory domains are shown in different colors and are specific to each helicase shown. In PcrA, the 1B and 2B accessory domains are shown in yellow and green respectively. In RecD2, an N-terminal flanking domain is shown in wheat, and the 2B accessory domain is shown in green. In Upf1, domains 1B and 1C are shown in wheat and yellow respectively. (B) The core domains of each helicase are shown, with the helicase signature motifs highlighted in different colors according to the key in Roman numerals below. (C) The lower panel shows sequence motifs for each class of SF1 helicase in Weblogo format (149). Where the information is available, residues known to be involved in ATP binding, hydrolysis or sensing are colored red, and residues involved in single-stranded nucleic acid binding are shown in blue. The scope of the motifs for PcrA is loosely based on the definitions of Koonin and co-workers (7). The scope of the motifs for RecD2 and Upf1 is based on structural alignment of their core domains with PcrA. Sequence motifs were determined with WebLogo from multiple sequence alignments that were implemented in COBALT (150). Either ~100, ~250 or ~500 proteins that were most similar, but not identical, to the Upf1, RecD2 and PcrA proteins shown (respectively) were used in the alignments. Note that motif Ib has, in some cases, been referred to as motif Ic.

separation as monomers, but others have been characterised as either dimers or oligomers. In distinct contrast, all of the enzymes in the remaining families form hexameric (or double-hexameric) rings that encircle the DNA substrate (10).

3.2. Motor properties

Many proteins defined as helicases on the basis of primary structure are indeed motor proteins that catalyse the processive separation of duplex nucleic acids. In general, those enzymes translocate on single-stranded DNA (or RNA) with a defined polarity, either 3′-5′ (referred to as class A enzymes) or 5′-3′ (class B enzymes), and displace the non-translocated strand at the ss-dsDNA junction (5). When analysed using *in vitro* assays with purified enzymes that may lack targeting factors, many such helicases are found to require a region of ssDNA from which to bind and initiate unwinding. In that case, the underlying polarity of the ssDNA motor produces a strong bias in the nature of the substrates that will be unwound. Class A enzymes commonly require a 3′-terminated ssDNA flanking the duplex, whereas class B enzymes require a 5′-terminated overhang. However, biochemical studies have also established that many putative "helicases" do not possess *bona fide* strand separation activity but are instead "translocases"; enzymes that couple ATP hydrolysis to directional motion along the nucleic acid without strand separation. Based on this distinction, "helicase-like" motor proteins that move along single-stranded or double-stranded DNA are classified as belonging to the α- or β-class respectively (5). Although the DNA duplex is quasi-symmetrical, it appears that β-class dsDNA translocase enzymes mainly engage one strand of the intact duplex to promote translocation (11-12). Therefore, they effectively still display a polarity and can be classified as either class A or class B. Furthermore, some enzymes do not move processively along nucleic acids at all and are not strictly motor proteins, but instead act as "switches" that couple the energy derived from ATP binding and hydrolysis to promote conformational changes in nucleic acids or nucleoprotein complexes (13-14). A final complexity is that all of these different motor functionalities can be applied to either DNA or RNA lattices, and there are even examples of helicase-like proteins that translocate peptides (15). Translocases and switches will not be discussed further in this review because, to the best of our knowledge, all of the SF1 enzymes that have been characterised to date are SF1α enzymes and true helicases: they translocate on single-stranded nucleic acids and catalyse strand separation *in vitro*.

4. SUPERFAMILY 1 HELICASES

Members of helicase SF1 are defined by the presence of several specific amino acid motifs (Figure 1). Certain motifs are diagnostic of SF1; for example the highly conserved motif III sequence GDxxQ is a useful hallmark of a SF1 enzyme. Many motifs are shared with SF2 enzymes, and a few are found in all classes of DNA motor proteins. Universal helicase motifs include I and II (equivalent to Walker A and B motifs) involved in nucleotide binding and hydrolysis and a region equivalent to motif VI that contains an "arginine finger", which promotes ATP hydrolysis and energy transduction. Based upon primary structure analyses of bacteria, archaea and eukarya, three distinct families of enzyme within SF1 have been defined; UvrD/Rep-like helicases, Pif1-like enzymes and the Upf1-like family (2). UvrD-like enzymes are (almost) always SF1Aα DNA helicases, whereas Pif1- and Upf1-like helicases are SF1Bα enzymes and include examples of proteins that act on both DNA and RNA.

Interestingly, slight variations are observed between the helicase motifs of the three families (Figure 1) and this presumably correlates with their different motor properties. A large number of SF1 helicases are also documented in viral genomes, mainly positive-strand RNA viruses (7). Finally, recent structural characterisation of the AddAB and RecBCD helicase-nucleases unveiled an unorthodox class of inactivated SF1 helicase; proteins that share the same architecture as a UvrD/Rep-like enzyme, but which do not possess the expected motifs (16-17).

4.1. UvrD/Rep-like helicases

Much of our mechanistic insight into helicases has arisen from studies of bacterial UvrD-like enzymes, of which there are typically four in each species (18). A protein equivalent to *E. coli* UvrD (the prototype for this family) appears to be almost ubiquitous in bacteria although, for historical reasons, the equivalent helicase in many Gram-positive organisms (including *Bacillus subtilis*) has been annotated PcrA (19). Comparison of the known cellular functions, biochemical properties and (most significantly) the primary structures of UvrD and PcrA suggest that they might reasonably be considered to be the same protein. In *E. coli*, UvrD plays roles in DNA repair as part of the nucleotide excision and mismatch repair pathways, and in the replication of plasmid DNA via a rolling circle mechanism (20-22). Other studies have identified a role for UvrD in the suppression of illegitimate recombination via the disruption of RecA nucleoprotein filaments, an activity that does not involve DNA unwinding (23). Most recently, UvrD was shown to assist the passage of the replication fork through highly transcribed regions of the genome (24-25). The enzyme appears to be directed to these diverse pathways via an array of protein:protein interactions, at least some of which map to a short disordered C-terminal extension (26-28). Interestingly, gamma-proteobacteria contain a second helicase called Rep, which is generally very similar to UvrD/PcrA at the level of primary and tertiary structure but which displays a distinctive C-terminal extension. This C-terminal region was shown to direct Rep to the replisome where it helps resolve conflicts with the transcription apparatus (25, 29). Rep also plays a role in supporting the replication of phage DNA in the rolling circle mode (30).

UvrD-like enzymes are also found as components of helicase-nuclease complexes involved in the initiation of double-stranded DNA break repair (17, 31-32). For example, the *B. subtilis* AddAB complex is powered by a single UvrD-like helicase domain in the AddA subunit and an equivalent motor is found in the RecB subunit of the functionally analogous RecBCD complex (33-34). Interestingly, in RecBCD, the RecB helicase is complemented by a second SF1 helicase that is a member of the Pif1-family (Section 4.2) (35-36). The AdnAB complex, which promotes DNA end resection in mycobacteria, provides an example of a protein complex that may contain two UvrD-like helicases (one in each of the AdnA and AdnB subunits) (37). Mycobacteria contain other distinctive UvrD homologues that have been annotated UvrD1 and UvrD2. UvrD1 is implicated in nucleotide excision repair, double-stranded DNA break repair and anti-recombination pathways (38-40). The UvrD2 helicase was shown to be essential, but its function is unknown (41). Finally, there are many examples of bacterial enzymes that remain relatively poorly characterised, such as *E. coli* HelD which is involved in the RecFOR pathway of recombination (42).

Eukaryotes typically contain fewer UvrD/Rep-like enzymes than bacteria. Perhaps the best studied is the yeast Srs2 protein which, like UvrD, is implicated in the suppression of homologous recombination via Rad51 filament disruption (43). Yeast also contains the Hmi1p protein, which is localized to mitochondria and critical for maintenance of mitochondrial DNA stability (44-45). In human cells, the Fbh1 protein appears to be a functional analogue of Srs2 (46). Also, Trank1 (TPR and ankyrin repeat-containing protein 1) is a very large and poorly characterised protein with partially conserved UvrD/Rep-like helicase motifs.

4.2. Pif1-like helicases

Probably the most intensively studied member of this family, certainly from a mechanistic viewpoint, is the phage T4 Dda protein. Dda is involved in the initiation and maintenance of phage replication and has been used as a model system for interrogating the mechanism of SF1B helicases (47). As mentioned above, the RecD component of the RecBCD complex is a Pif1-like family member, and this enzyme provides a 5′-3′ ssDNA motor activity to complement the RecB helicase, driving movement of RecBCD along DNA using a bipolar, dual-motor mechanism (36, 48). In some bacteria, a RecD-like enzyme that is not a component of the RecBCD complex has been identified and termed RecD2. This seems to be required for growth in extreme environments and for the repair of some classes of DNA damage (49). An interesting and distinctive member of this family is the TraI protein, encoded by the F plasmid, which contains both transesterase and SF1B helicase domains (50-51). This protein is responsible for both the nicking of the F plasmid at a specific site and subsequent unwinding to produce the T-strand for transfer to the donor bacterium. This process shares some mechanistic parallels with the role of UvrD/Rep proteins in rolling circle replication, which involves non-covalent interactions between the helicase and a replication initiator protein with transesterase activity (51).

The eponymous member of the family, Pif1, is a ubiquitous eukaryotic protein that fulfils a wide variety of roles in maintaining nuclear and mitochondrial DNA, including G-quadruplex resolution, telomerase inhibition at DNA breaks and Okazaki fragment maturation (52). Yeast contains a second Pif1-like helicase Rrm3, which is a component of the replisome and helps to promote replication fork progression through a variety of protein roadblocks, including highly transcribed regions of the genome (53). This activity is analogous to that of the bacterial Rep helicase discussed earlier. Another human Pif1-like enzyme is Dna helicase B (HDHB), which is localized to damage-induced nuclear foci in G1 phase and proposed to be required for the repair of damage prior to onset of S phase (54).

4.3. Upf1-like helicases

Upf1-like helicases are relatively abundant in eukaryotic cells, probably reflecting their roles in various RNA processing events. Upf1 itself is a widely conserved eukaryotic RNA helicase functioning in nonsense mediated decay, a mechanism for mRNA quality control and the regulation of transcript stability (55). Intron binding protein 160 (IBP160) also plays a key role in mRNA processing by acting to recruit components of the Exon Junction Complex to the splicesosome (56). The yeast Sen1 protein is implicated in transcription termination and the removal of R-loops which might otherwise provoke recombination and genomic instability (57). Mutations in its human homologue, Senataxin, are associated with amyotrophic lateral sclerosis and Ataxia with oculomotor apraxia type 2. The Dna2 protein is a conserved eukaryotic helicase-nuclease complex originally shown to be involved in Okazaki fragment processing (58). Recent studies have also implicated Dna2 in the initiation of double-stranded DNA break repair as a component of the Dna2-BLM-RPA-MRN complex, which is functionally analogous to the bacterial AddAB and RecBCD complexes discussed above (59-60). Finally, the human HelZ protein is an example of a zinc-finger containing RNA helicase which forms a complex with RNA polymerase II, but is otherwise poorly characterised (61).

4.4. Viral Superfamily 1 helicases

Positive strand RNA viruses including alphaviruses, rubiviruses, hepatitis E viruses, arteriviruses, coronaviruses and many plant virus families contain SF1 helicases that are required for the replication and transcription of the viral genome (62). Examples include the coronavirus nsp13 and arterivirus nsp10 proteins, which are SF1B enzymes with activity on both RNA and DNA duplexes (62). The replication fork of Herpes simplex virus contains a helicase-primase complex of which the well-studied SF1 helicase UL5 is a component, and a further example of an SF1B class enzyme (63).

4.5. Inactivated Superfamily 1 helicases

A final, non-conventional, class of SF1 helicase-like proteins is suggested by the presence of catalytically inactivated UvrD-like helicase structures as components of bacterial DNA break processing complexes. These proteins do not translocate along or unwind DNA, but instead appear to have co-opted the ssDNA motor design of a SF1 helicase for use as a scanner for specific recombination hotspot sequences (16-17). These "inactivated SF1 helicases" have presumably evolved this activity via the modification of a conventional SF1A ssDNA motor. Interestingly, certain parasite Pif1 homologues involved in kinetoplast maintenance also appear to have lost most of the helicase signature motifs (64).

5. GENERAL STRUCTURE AND BIOCHEMICAL PROPERTIES OF SUPERFAMILY 1 HELICASES

5.1. Domain architecture

Several structures of SF1 enzymes in various liganded states have been solved including examples of each of the three families (Figure 1) (16, 65-71). All SF1 DNA helicases share a common core structure comprising two tandem RecA-fold domains that contain all of the conserved signature motifs and which constitutes the DNA motor. These are denoted N-core and C-core and are colored red and blue respectively in the top panel of Figure 1. The NTP binding pocket is at the interface of the core domains and the ssDNA binding site is formed across the top surface of both core domains. SF1 enzymes commonly contain "accessory" domains either flanking, or formed as inserts within, the core domains. The position and primary structures of these accessory domains are highly variable between different enzymes. Furthermore, accessory domains may adopt different conformations with functional relevance. For example, in both the PcrA and Rep helicases the 2B accessory domain can undergo a large rotation relative to the rest of the structure (66, 69). These "open" and "closed" conformations of 2B appear to play important roles in the unwinding activity and its regulation (Sections 7.2 and 8.1). Understanding the role of accessory domains may be key to understanding the diversity of function in SF1 helicases, because they play general roles in targeting helicases to specific substrates or cellular locations, modulating helicase activity, or adding catalytic functionality. They may also act as sites for interactions with partner proteins that stimulate or modify the helicase activity (Section 8.2).

5.2. Nucleoside triphosphate hydrolysis

The binding site for the nucleotide is formed by motifs I, II, III, IV and VI which cluster at the interface of the N- and C-core domains (68). The precise structure of this binding pocket is highly conserved across SF1, with only subtle differences observed in the available crystal structures (Figure 1). Some specific roles have been elucidated for the amino acids in the binding pocket. The triphosphate tail of the nucleotide is cradled by interactions with a conserved lysine in motif I, and the base and phosphates interact with residues in motif IV. In many SF1 (and SF2) helicases an additional motif (the Q-motif) positions a glutamine residue near the NTP where specific hydrogen bonds to adenine result in selectivity for ATP over other NTPs (72). Binding of NTP causes the core domains to close around the nucleotide

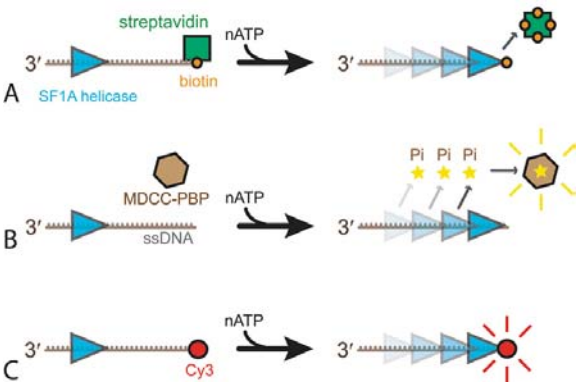

Figure 2. Assays for single-stranded DNA translocation. For simplicity, only 3′-5′ polarity ssDNA translocation by a SF1A helicase is shown. The gradual fading of the helicase shown in the right panels is intended to indicate its movement in the 3′-5′ direction. (A) Streptavidin displacement assay. Streptavidin is pre-bound to an oligonucletoide labelled with biotin at the 5′-end. Movement of the helicase towards the end of the DNA molecule generates force against the biotin-streptavidin interaction and increases the rate of streptavidin dissociation. The released streptavidin is bound by free biotin. By comparing displacement of streptavidin from an oligonucleotide with a biotin moiety at the 3′-end, this assay provides a simple test for translocation polarity. (B) Phosphate release assay. The kinetics of ATP hydrolysis associated with ssDNA translocation can be monitored with MDCC-PBP; a fluorescent probe for inorganic phosphate (P_i). The hydrolysis of multiple ATP molecules drives unidirectional translocation along the DNA and sequentially releases P_i, which are rapidly bound by MDCC-PBP leading to a fluorescence change. The kinetics and amplitude of P_i release provide information on the translocation rate and ATP coupling efficiency. (C) Direct ssDNA translocation assay. An oligonucleotide is labelled with a fluorophore (eg Cy3) at the 5′-end. Arrival of the helicase near the fluorophore results in a fluorescence change that provides direct information on the kinetics of ssDNA translocation.

partly because conserved residues from motif III (N-core domain) and VI (C-core domain) move closer together to co-ordinate the gamma–phosphate (69). These nucleotide-induced conformational changes are propagated to the ssDNA site where they modify the interactions of the helicase with the nucleobases (Section 6.2). NTP hydrolysis probably proceeds by an associative mechanism involving nucleophilic attack by an activated water molecule and the formation of a trigonal-bypyrimidal pentavalent phosphate as the transition state intermediate (67). Catalysis is promoted by a divalent cation which is co-ordinated against the beta– and gamma–phosphates by conserved threonine and aspartate molecules in motifs I and II respectively (69). Another critical residue is a conserved glutamate residue in motif II which, based on its position relative to the gamma-phosphate, is thought to act as the general base to activate a H_2O molecule for in-line attack at the gamma–phosphate (73).

5.3. Nucleic acid binding

Residues from motifs Ia, Ib, III, IVa and V all contribute to the bipartite single-stranded nucleic acid binding site formed over the two core domains (8, 66, 69). Regardless of the polarity of the helicase, the single-stranded nucleic acid is always seen to bind across the core domains with the 3′-end associated with the N-core domain and the 5′-end bound to the C-core (16, 71). Structural data is available for the interactions of ssDNA with four UvrD/Rep-like helicases (16, 66-67, 69). In each case the interactions are similar, but not identical, and involve stacking interactions that bind the ssDNA with the nucleobases in pockets across the top surface of the core domains. In contrast, the Pif1-like enzyme RecD2 makes greater use of backbone phosphate interactions and the ssDNA is held in a conformation more akin to a B-form (i.e. like one strand of DNA within a B-form duplex, with base stacking maintained) (71). In this respect, the RecD2 interactions are somewhat more reminiscent of the interaction mode associated with SF2 helicases (74). Interestingly, some of the deoxyribose moieties in the ssDNA form hydrophobic stacking interactions with histidine residues in motifs Ia and V that would be sterically excluded for an RNA substrate. As noted previously (71), these residues are conserved in SF1 DNA helicases but not in the SF1 RNA helicase Upf1 (Figure 1). In the inactivated SF1 helicases related to the UvrD/Rep-like family, this ssDNA site appears to have evolved a new function as a recognition site for the specific ssDNA sequence Chi. However, because there is currently no structure available for a Chi-recognition domain bound to a recombination hotspot sequence, the details of this interaction remain to be unveiled. Given that different AddAB or RecBCD enzymes recognise different Chi sequences, these systems may provide valuable lessons on fundamental aspects of ssDNA:protein molecular recognition.

SF1 helicases have also been shown to possess binding sites for duplex DNA associated with accessory domains. In the PcrA and UvrD helicases, accessory domains 1B and 2B bind to the DNA duplex "ahead" of the ssDNA motor (Figure 1). Although originally proposed to act as a mechanism for the distortion or wrenching of the duplex to assist translocation into the duplex (67, 69), it has also been suggested that this interaction might fulfil a regulatory function by inhibiting the enzyme and/or be involved in the re-setting of ssDNA translocation during repetitive shuttling events (Section 6.1) (75-76). Recent studies also show that this binding site might actually bind duplex DNA most favourably *behind* the core motor (75). In the RecBCD complex, the duplex is bound by an unusual "arm" structure, entirely formed by accessory domain 1B, that reaches ahead of the translocating enzyme and may play a role as a non-conventional motor domain (77). In the RecD2 protein the diminuitive 1B domain, just a short loop of protein emanating from the 1A core domain, is thought to bind the ss-ds junction and act as a pin to separate the strands during translocation (70). Structures such as these, which prise apart the strands of duplex DNA, have also been described as "wedges" and are a common feature of SF1 and SF2 helicases (5). Several SF1 helicases also appear to contain flanking Zn^{2+} binding domains which may act as specific or non-specific duplex DNA binding sites

5.4. Energy transduction

SF1 enzymes typically display a low basal ATPase activity that is strongly stimulated by binding to single-stranded nucleic acids. Evidence from the PcrA helicase suggests that ssDNA binding may lead to a remodelling at the nucleotide binding pocket that allows the co-ordination of Mg^{2+} ions that are required for hydrolysis (73). This view is supported by kinetic analyses which suggest that the rate limiting step for ATP hydrolysis in the absence of DNA is chemical cleavage as opposed to ATP binding or product release (78-80). As would be expected, there is a reciprocal allosteric effect between ATP and ssDNA binding and ternary structures of UvrD, PcrA and RecD2 bound to different nucleotide analogues and ssDNA have shown clearly that the ssDNA binding site is remodelled in response to cycles of ATP binding and hydrolysis (67, 69, 71). These observations have led to detailed proposals of mechanisms for directional ATP-dependent translocation using an inchworm mechanism (Section 6). Site directed mutagenesis studies in PcrA, UvrD and UvrD1 have implicated helicase motifs III, V and VI in coupling ATP hydrolysis to ssDNA translocation and helicase activity (73, 81-83). This makes good sense, because these motifs extend from the nucleotide binding pocket to the ssDNA binding site, and contain conserved residues that interact with both ligands (Figure 1).

6. SUPERFAMILY 1 HELICASES AS SINGLE-STRANDED DNA MOTOR PROTEINS

There is clearly an intimate allosteric communication between the ATP binding and ssDNA binding sites but a major challenge has been to understand exactly how the free energy associated with ATP binding and hydrolysis is transduced to directional motion along DNA, and this is a matter of ongoing research.

6.1. Monitoring processive and unidirectional movement along ssDNA

Monitoring directional motion on ssDNA is challenging (because the reaction does not have a product as such) but several methods have now been developed (Figure 2). The streptavidin displacement assay can be used to infer directional motion from the ability of the helicase to displace streptavidin from a biotin moiety placed at either end of a synthetic oligonucleotide (84). This method benefits from ease of implementation, but its discontinuous and indirect nature limits the mechanistic insight that can be obtained. Directional

translocation does result in ATP turnover, and the kinetics and amplitude of ATP hydrolysis can provide information on the translocation process. For example, a fluorescent probe for inorganic phosphate (MDCC-PBP) has been used both to infer unidirectional translocation and to determine the rate and apparent efficiency of this process (85). A direct and continuous assay for protein motion on ssDNA was developed by placing fluorescent labels at either the 3′- or 5′-end of ssDNA. Arrival and accumulation of the translocating enzyme at one or the other end of a synthetic oligonucletide is monitored as a protein-induced change in fluorescence of the base analogue 2-aminopurine (86) or, alternatively, a Cy3 or fluorescein molecule (87). Finally, the triplex displacement assay, although originally developed as a tool for monitoring DNA translocase activity (ie translocation without unwinding), is equally adept at monitoring (indirectly) the movement of SF1 helicases, albeit in the context of translocation into a DNA duplex (88-90). The kinetics of DNA translocation (monitored either by phosphate release, arrival at the terminal DNA end or via triplex displacement) are interpreted in terms of "n-step sequential" models for the stepwise movement of a motor protein along a DNA lattice (91-93). These analysis methods allow the extraction of the polarity, rate, and processivity of translocation and can equally well be (and were in fact originally) applied in the context of DNA translocation and unwinding (Section 7.1). An additional outcome of developing rigorous tools for the quantitative analysis of stepping kinetics was the emergence of a fourth parameter: the "kinetic step size". If each step along the nucleic acid is considered to be kinetically identical and essentially irreversible then it is possible to extract a measurement of the average translocation distance associated with each rate limiting step in the overall process. In the simplest scenario, this value relates directly to a repeated physical movement along the DNA and could, in principle be equivalent to the step associated with each ATP hydrolysis event. However, it is now apparent that the kinetic step size is a very complex term, which may be affected by any source of heterogeneity in the population, unaccounted-for effects associated with the arrival at a DNA end, or the presence of multiple rate limiting steps of a similar order in each cycle of translocation (76, 92, 94-95). The use of these techniques for the PcrA, UvrD and RecD2 proteins showed that they moved directionally on ssDNA with 3′-5′, 3′-5′ and 5′-3′ polarities respectively (71, 85-87, 96). In all three cases, the use of MDCC-PBP suggested an apparent motor efficiency of 1 ATP per base. For UvrD, side-by-side analysis of the data for translocation to a DNA end suggests a non-uniform stepping mechanism (96). In addition to the single base steps driven by each ATP turnover, there is a kinetic step size of 4-5 ntds. Recent work on the RecBC subcomplex of RecBCD showed that, on the basis of the direct translocation assay, this enzyme contained both a 3′-5′ and a 5′-3′ tracking activity, and both of these activities were dependent on the RecB ATPase (77). The RecB protein is, like its close relatives in the UvrD/Rep-like family, classified as a SF1A enzyme and so the observed 5′-3′ tracking activity is highly unexpected. It was suggested that this activity might arise from the action of the 1B "arm" domain, ratcheting forward the duplex DNA independently of the conventional ssDNA motor formed by the core helicase domains.

Recent years have seen the application of many single molecule detection methods to the study of DNA motor proteins, including single molecule TIRF/FRET microscopy, fluoresecence microscopy of flow-stretched DNA, and optical and magnetic tweezers (97). Studies on SF1 helicases have been prominent, and have provided new insights into the translocation mechanism including the observation of static disorder (i.e. different translocation rates associated with individual enzymes), stochastic pausing, backsliding, strand switching and spontaneous changes in the translocation rate (98-103). For the RecBCD complex, a change in the translocation rate occurs when the enzyme encounters the recombination hotspot sequence Chi in the DNA track, because the lead RecD motor in the complex is switched to RecB (104-105). Of special interest is the observation that the Rep and PcrA helicases continually track and re-track along the same section of ssDNA, a phenomenon termed repetitive shuttling (76, 106). This behaviour is a good example of information that would be difficult to obtain using bulk methods, and may help these enzymes remove proteins (such as RecA) from single-stranded regions of DNA at stalled replication forks. For PcrA, analysis of the distribution of the durations of these repeated tracking events suggests that a rate limiting step is associated with translocation of a single base, in agreement with the ATP coupling ratio of 1 base per ATP (76). Interestingly, treatment of the data as a whole for multiple PcrA molecules showed a wider distribution of tracking times due to static disorder, and may provide an alternative explanation for the larger kinetic step sizes observed in conventional bulk studies.

6.2. The structural basis for translocation and translocation polarity

Although some of the finer details of the ssDNA interactions differ for PcrA and UvrD, work on both systems showed that the changes to the ssDNA binding site brought about by ATP binding and hydrolysis are fairly subtle (4-5, 67, 69). Conformational changes between the core domains and of residues in contact with the ssDNA nucleobases cause movements of parts of the ssDNA chain in single base steps. The effect is to alter the relative grip of the N- and C-core domains on the ssDNA. Together with changes in the separation between each half of the ssDNA binding site, due to movement of the core domains first together (in the ATP-bound state) and then apart (in the post hydrolysis state), these conformational movements could support unidirectional translocation along ssDNA in single base steps. The movement is similar to that of an inchworm, where the C-core domain acts as the head and the N-core domain as the tail. For both PcrA and UvrD, this structural mechanism is consistent with the directionality and ATP coupling ratio of ssDNA tracking measured using the assays described above, and is also supported in principle by computational studies (107-108). In the SF1B model helicase RecD2, a similar translocation model was proposed except, in this case, the core domains track in the opposite direction relative to the ssDNA polarity with the N-core domain at the front of the enzyme (71). The different directionality was assigned to differences in the interactions between the ssDNA and helicase motifs Ia and III. Accordingly, it is exactly these motifs (as well as motif IVa) that appear to distinguish SF1A and SF1B enzymes at the level of primary structure (Figure 1).

There is a reasonable consensus from structural analysis as well as bulk and single molecule kinetics, that SF1 helicases can function as ssDNA motor proteins in a monomeric form, that movement is unidirectional (or at least biased in one direction), and that translocation is driven by an underlying "ATP step size" of 1 base per ATP. However, as we shall see below, the picture for helicase activity (i.e. strand separation) is rather more complex and contentious.

7. COUPLING OF TRANSLOCATION AND STRAND SEPARATION

Given that SF1 enzymes move directionally along ssDNA in an ATP-dependent fashion, it is intuitive to imagine that helicase activity would arise simply as a result of the displacement of the non-translocated strand at the ss-dsDNA junction. However, unwinding requires not only the ssDNA motor formed by the core domains and the classical protein motifs, but also a range of other structural elements that promote the separation of the duplex. Furthermore, a surprising finding has been that the pre-requisites for DNA unwinding may differ from those for ssDNA translocation. Unwinding often requires functional co-operativity between helicase protomers, the presence of protein co-factors (such as single-stranded DNA binding proteins) and can be stimulated strongly by the binding of partner proteins that load or activate the helicase. A final complexity to consider is that, despite their ability to unwind DNA duplexes *in vitro*, some aspects of the cellular functions of SF1 helicases do not require unwinding, for example the disruption of RecA/Rad51 filaments by UvrD/PcrA and Srs2.

7.1. Assays for monitoring unwinding

Several assays have been developed for monitoring the separation of nucleic acid duplexes (Figure 3). Early methods relied on coupling helicase activity to product degradation by ssDNA-specific exonucleases, and assaying for acid soluble nucleic acid fragments (109). These were superseded by strand displacement assays, in which the displacement of radio- or fluorescently-labelled oligonucleotides are detected by gel electrophoresis (110-111). Although relatively straightforward to perform, strand displacement assays are discontinuous and time consuming.

Nevertheless, in combination with a quenched-flow single-turnover approach (112), this powerful assay has been used in many key mechanistic studies of SF1 helicases. Modified versions of strand displacement assays, for instance using FRET or fluorophore/quencher pairs, allow continuous measurement of unwinding (113-114). For SF1 enzymes, there is typically a requirement for a ssDNA tail flanking the

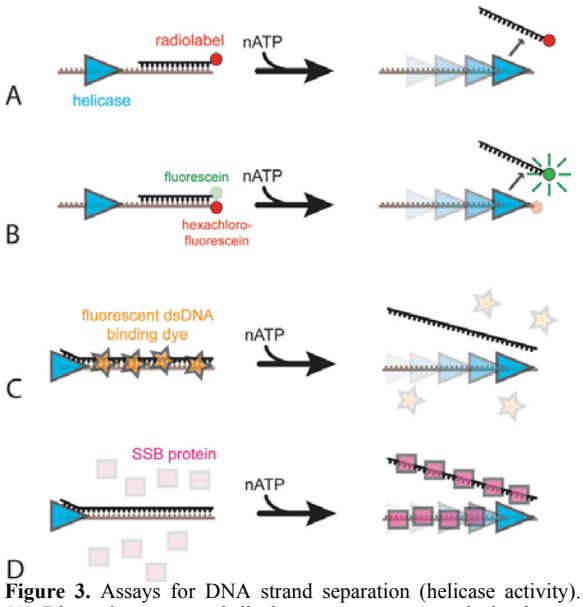

Figure 3. Assays for DNA strand separation (helicase activity). (A) Discontinuous strand displacement assay. A typical substrate contains a ssDNA overhang of appropriate polarity flanking a duplex region and the shorter strand is 5′-radiolabelled. Helicase activity results in displacement of the labelled strand into free solution, which can be detected by gel electrophoresis. (B) Continuous strand displacement assay. The substrate is similar to that used in the discontinuous assay except that the two strands are now labelled with a FRET pair to allow a continuous readout of strand displacement. Removal of the shorter strand to free solution decreases energy transfer between the two dyes. (C) Dye displacement assay. A fluorescent dsDNA binding dye such as Hoechst 33258 is pre-bound to the DNA. Translocation and unwinding of DNA results in dye displacement, which is followed in real time as a change in the fluorescence of the dye. This assay is suitable for relatively long range unwinding events so that the dye binding to the substrate DNA is pseudo-homogenous. (D) SSB binding assay. DNA unwinding occurs in the presence of SSB protein, which binds very rapidly and tightly to the ssDNA products. The binding can either be followed by a change in the internal tryptophan fluorescence of SSB or, with higher signal to noise ratio, by an environmentally sensitive fluorophore conjugated to the SSB protein. This assay is suitable for relatively long range unwinding events because the ssDNA binding site size of SSB protein is fairly large.

duplex for strand displacement (111). In that case, there is generally a strong preference for a particular polarity of the ssDNA overhang and this may be diagnostic of the polarity of the ssDNA motor (Figure 4). It is on this basis, rather than by direct measurement of ssDNA translocation polarity, that most SF1 helicases have been assigned a polarity. For example, Pif1- and Upf1-like enzymes which are members of the SF1Bα class require a 5′-terminated overhang. Conversely, Rep/UvrD-like enzymes generally show a strong preference for unwinding DNA duplexes with 3′-terminated ssDNA overhangs consistent with their classification as SF1Aα helicases. An exception is *D. radiodurans* helicase IV, which is similar to *B. subtilis* YvgS (considered a UvrD/Rep-like enzyme) and which displays 5′-3′ unwinding polarity (115). The RecBCD complex contains two helicase subunits, one of each polarity, and moves along DNA using a bipolar translocation mechanism in which each motor engages with one strand of the anti-parallel duplex. Some PcrA homologues have also been described as "bipolar", based on their ability to unwind DNA duplexes flanked by both 3′- and 5′-ssDNA overhangs (116). It should be noted however, that the observed unwinding polarity measured with strand displacement assays reflects both the loading of the helicase and the polarity of ssDNA translocation. It is perfectly possible for a SF1A helicase to unwind 5′-terminated and blunt end duplexes providing it can load onto the substrate in a productive manner (Figure 4). Indeed, this was shown to be the case for the *B. stearothermophilus* PcrA helicase (33, 117), which is strictly a unidirectional ssDNA motor. The assignment of motor polarity based on observed unwinding polarity is potentially equivocal and, ideally, polarity should be determined using a combination of unwinding and ssDNA translocation assays.

Unwinding measured by the strand displacement assay has been described as "all or none" because partially unwound intermediates do not score in the assay. Therefore, the single turnover unwinding kinetics measured by strand displacement typically display a lag before ssDNA release. The kinetics can be analysed using n-step sequential models to obtain quantitative information on the rate, processivity and step size of unwinding (92). The Rep, UvrD, RecBCD, and Dda proteins have been studied extensively using these methods (95, 112, 114, 118-126) and a kinetic step size of unwinding of about 3-4 bp is typically observed. These methods are subject to the same caveats as when applied to ssDNA tracking (Section 6.1). Furthermore, in applying these techniques, it is important to appreciate that failure of the helicase to completely release the non-translocated strand can be caused by phenomena other than insufficient speed or processivity to reach the distal end at a given timepoint. For example, the terminal end of the substrate may be unusually difficult to unwind as there is no duplex "ahead" of the translocating enzyme (67) or the nascent ssDNA strands may have begun to re-anneal behind the translocating helicase before the enzyme has reached the distal end (see Section 8) (95). This latter concern has been addressed by repositioning the FRET pairs to the front of the duplex region of the substrate (114).

Several assays are specifically useful for measuring highly processive unwinding activity (ie of kilobase pairs of DNA). Various dsDNA-specific binding dyes produce a signal when displaced by a translocating helicase, and can be used to study DNA unwinding in real time in multiple or single turnover assays (48, 127). This assay was modified through the use of an optical trap to hold flow-stretched DNA and directly visualise translocation and unwinding by a single RecBCD complex (101). The intrinsic fluorescence of SSB protein, which changes upon the very rapid, tight and specific binding of SSB to nascent ssDNA, has also been exploited to monitor DNA unwinding (128). This method is made considerably more sensitive by placing an extrinsic fluorophore on the SSB protein (129). A variant of this fluorescent SSB protein was developed to monitor helicase activity at the single molecule level by TIRF microscopy (130). Most recently, probes for DNA unwinding have been developed by fusing SSB with fluorescent proteins (131).

7.2. Structural models for helicase activity

Crystal structures of PcrA and UvrD monomers show that accessory domains 1B and 2B are involved in binding duplex DNA ahead of the core translocation motor. Based on these structures, the 2B domain was suggested to either assist in the melting of the duplex, or in the wrenching of the duplex into the core motor domains (67, 69). These models are consistent with an "active" mechanism for DNA unwinding in which the free energy associated with ATP binding and hydrolysis is used to directly destabilise the duplex, assisting translocation along nascent ssDNA. This role for the 2B domain, in which it makes a positive contribution to DNA unwinding, is supported by site directed mutagenesis (67, 132). Steady state DNA unwinding by PcrA and UvrD is reduced as a result of point mutations in residues which contact duplex DNA based on the crystal structures. Paradoxically however, studies on the Rep helicase showed that removal of the 2B domain actually activates DNA unwinding (133) and mutations designed to destabilise the conformation of 2B observed in the UvrD crystal structure prevent duplex DNA binding but do not reduce helicase activity (67). Furthermore, monomers of Rep, UvrD and PcrA cannot unwind DNA *in vitro* (Section 7.3), and so it has been suggested that the crystal structures might represent auto-inhibited (i.e. inactive) forms of the enzyme (3, 134). Resolving the differences between structural models for helicase activity and these biochemical observations is an important area for future work. A generic structural element in SF1 and SF2 helicases is a "pin": a region of the structure which forms a steric wedge at the ss-dsDNA junction and helps to prise the strands apart during translocation (5). In many UvrD/Rep-like helicases the pin emanates from the C-core motor domain at the front of the enzyme. In the Pif1-like enzyme RecD2, which translocates in the opposite direction, the pin is a small loop inserted into the N-core domain (70). The RecBCD complex also contains a pin, but this is not associated with either the RecB or RecD motor protein, but is instead located in the third subunit RecC, in the expected position at the junction of single- and double-stranded DNA (16).

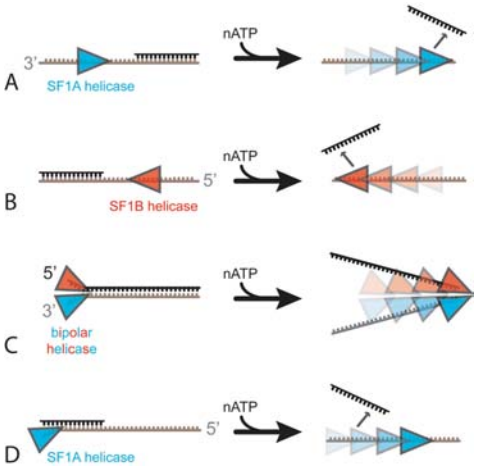

Figure 4. Unwinding polarity of Superfamily 1 helicases. (A) SF1A helicases (blue) translocate along ssDNA in the 3′-5′ direction and typically require a 3′-terminated ssDNA overhang as a loading site to unwind a flanking duplex. (B) Conversely, a SF1B helicase (red) translocates in the 5′-3′ direction to unwind DNA with a 5′-terminated ssDNA tail flanking the duplex. (C) A bipolar helicase (eg RecBCD) has both SF1A and SF1B motor activities. By engaging with either strand of the anti-parallel duplex, these co-operate to drive translocation and unwinding in the same overall direction. (D) A SF1A helicase *can* unwind a DNA substrate with a 5′-terminated tail, providing it is able to load onto the substrate in a productive manner. An equivalent reaction is possible for a SF1B helicase on a substrate with a 3′ssDNA tail.

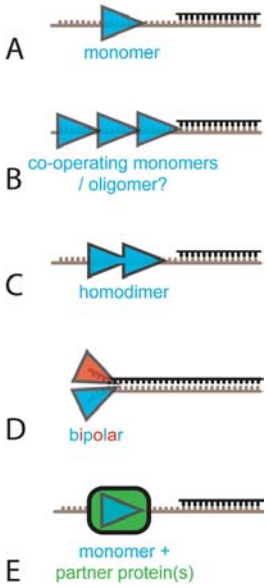

Figure 5. Unwinding modes for Superfamily I helicases. There has been extensive debate over the functional oligomeric state of SF1 helicases and it appears that several different mechanisms may exist to promote DNA unwinding (see the text for details). In all cases, the underlying motor activity is essentially the same (ie ATP-dependent directional translocation on ssDNA in a monomeric unit) but the unwinding mode differs. (A) A SF1 helicase monomer can unwind DNA. (B) Multiple helicases can co-operate to unwind a DNA molecule. It is not clear if this activity requires physical interactions between the monomers. (C) A dimer may be the active form of the helicase. (D) Studies on the RecBCD helicase-nuclease have established that monomeric units of opposite polarity can co-operate to drive bipolar translocation. (E) Many helicases are stimulated to unwind DNA by interactions with accessory proteins.

7.3. The functional oligomeric state of Superfamily 1 helicases

Proposals for unwinding mechanisms based on the crystal structures of PcrA and UvrD have been criticised based on a lack of experimental validation that monomers can catalyse unwinding (3). Indeed, several single-turnover and single molecule DNA unwinding experiments actually show clearly that (in the absence of single-stranded DNA binding proteins or partner proteins) monomers of PcrA, Rep and UvrD are unable to catalyse stable DNA strand separation *in vitro*, even though they are efficient ssDNA motor proteins (100, 114, 118, 123, 134-136). Instead, this body of work suggests that either a dimer or higher order oligomeric form is required. In support of this general view, studies on the Dda helicase and nsp13 show enhanced unwinding by multimeric forms of the helicase (124-125, 137). Nevertheless, studies of Dda, TraI, and a deletion mutant of Rep show that a monomer is also capable of processive DNA unwinding (50, 126, 133). Critically, this means that a minimal structure for unwinding needs only a single set of SF1 helicase motifs, and therefore a single DNA motor domain. Characterisation of the AddAB and RecBCD complexes support this view because, although multimeric, these complexes only require the activity of a single motor domain to promote processive unwinding (33, 35-36, 48). Thus, the requirement for a dimeric or oligomeric form of UvrD, PcrA, or Rep seems to be specific for those systems rather than being a general mechanistic feature of SF1 helicases. Furthermore, it is not actually clear that the activation of helicase activity observed in dimeric or oligomeric forms of these enzymes requires physical interactions between protomers because the putative protein:protein interaction interfaces are not well defined. Given that the helicase activities of PcrA, UvrD and Rep are all known to be dramatically affected by interactions with partner proteins (Section 8.2), it may be most relevant to characterise the structure of these helicases in the context of those interactions and/or when acting on more physiological DNA substrates. Taken together, the available data for several different SF1 helicases suggests that DNA unwinding may occur in a variety of different mechanistic modes *in vitro* (Figure 5).

7.4. SSB proteins and the suppression of re-annealing

Inside cells, the ssDNA products of helicase activity are either immediately "handed-off" to other enzymes (e.g. a replicative polymerase on the leading strand in the replisome) or are rapidly bound by single-stranded DNA binding proteins, such as bacterial SSB protein (138). SSB proteins act to stabilise DNA in the single-stranded form and to protect it from nucleolytic degradation. Moreover, they are important mediators of downstream ssDNA transactions such as strand exchange catalysed by RecA/Rad51. Given the obvious propensity of unwound DNA duplexes to spontaneously re-anneal, it is perhaps surprising that the activity of most helicases has only been assessed in the complete absence of SSB proteins. One complication associated with using SSB proteins in unwinding assays relates to their potential to competitively inhibit the binding of the helicase to the substrate, especially where a ssDNA tail is essential for unwinding. Nevertheless, there are examples of SF1 helicases where the presence of SSB greatly enhances, or is even required for, stable DNA unwinding (90, 139-140). A recent study on the AddAB helicase-nuclease showed that, in the absence of SSB, this enzyme was almost completely unable to stably separate DNA in a single turnover, even though it was able to translocate efficiently along the duplex (90). SSB is required to prevent the nascent ssDNA strands from *immediately* re-annealing behind the translocating AddAB complex. Interestingly, this re-annealing was also suppressed by the activity of multiple AddAB complexes on one DNA molecule or by the recognition of recombination hotspot sequences, which alters the manner in which the nascent ssDNA strands exit the enzyme. Finally, the ability of several helicases to interact directly with SSB proteins (138) implies that some helicases may recruit the co-factor to assist in unwinding, and reinforces the idea that SSB proteins play a critical role in supporting strand separation. In our view, the importance of SSB proteins as co-factors in promoting helicase activity *in vitro* has been underappreciated.

8. SUPERFAMILY 1 HELICASES AS MODULAR COMPONENTS OF NUCLEIC ACID PROCESSING MACHINES

SF1 helicases are involved in a diverse range of nucleic acid and nucleoprotein complex processing pathways, yet they share a highly conserved core motor domain with relatively little variability in the basic motor properties. It follows that functional specificity arises from the presence of distinctive accessory domains, or via interactions with partner proteins that stimulate, target, or catalytically modify the translocation activity associated with the core domains.

8.1. Targeting and modification of Superfamily 1 helicases via accessory domains

An emerging role for accessory domains is in the *inhibition* of helicase activity. It has been shown that removal of the 2B subdomain of Rep activates the DNA unwinding activity: whereas monomers of wild type Rep cannot unwind duplex DNA, monomers of a domain 2B deletion mutant are proficient, albeit poorly processive, helicases (133). Consequently, domain 2B provides a regulatory mechanism for Rep as an auto-inhibitory domain, and it was suggested that this inhibition could be relieved by interactions with additional Rep molecules or other partner proteins (3). A similar auto-inhibition phenomenon appears to be at play in the Upf1 protein (141). Unwinding is inhibited *in cis* by the CH domain, and this is relieved by sequestration of the CH domain by the interacting partner Upf2. This phenomenon may operate more generally in helicase-like proteins. For example, the SF2 translocase Mfd acts to displace RNA polymerase that is stalled at sites of damage, and is activated by interaction with RNA polymerase via a specific accessory domain (142). Accessory domains are also important for targeting helicases to nucleic acids. Interactions with duplex DNA may be non-specific, as is the case for the helix-hairpin-helix motif in the 2B domain of PcrA (143) which directs the enzyme to a ss-dsDNA junction. Targeting to nucleoprotein complexes is exemplified by the interaction of Srs2 with Rad51 via a C-terminal extension, which is required for Rad51 filament disruption (144). A further role for accessory domains is in adding novel catalytic functionality to a DNA motor, with fusions between SF1 helicases and nuclease domains, as in bacterial helicase-nuclease complexes (17), are quite common. The TraI protein provides another example: a helicase is covalently attached to the transesterase activity required to produce an initiating nick for unwinding (51).

8.2. Functional programming of Superfamily 1 helicases by partner proteins

Although targeting and modifications of helicase activity can be achieved by accessory domains, the use of partner proteins to fulfil this role offers more flexibility: the functional programming of the helicase is potentially reversible and the motor can be recruited by different proteins to fulfil other cellular roles. This principle is well-illustrated by bacterial UvrD/Rep-like enzymes (18). For all of these enzymes that are well characterised, there are examples of protein:protein interactions which modulate their function. Moreover, there are cases in which multiple different interaction partners appear to direct the helicase to a variety of different pathways. *E. coli* UvrD is involved in both mismatch repair and nucleotide excision repair and interacts physically with components of each pathway (MutL and UvrB) (27-28, 145-146, 151). Furthermore, by analogy with the PcrA helicase, its recruitment as a replicative helicase for rolling circle plasmids likely reflects a physical interaction with a plasmid-encoded initiator protein (22). Interestingly, a mycobacterial UvrD homologue is implicated in non-homologous end joining via interaction with the DNA end bridging Ku protein (39). There is an emerging body of evidence to suggest that SF1 helicases act at the interface of key DNA processing pathways and some are recruited to replication forks by interactions with the replicative helicase or the sliding clamp. In the case of Rep and Rrm3, these accessory helicases have been shown to be important for avoiding conflicts with the transcription apparatus. There are also reports of SF1 helicases interacting directly with RNA polymerase, although the function(s) of these interactions are poorly characterised (61, 147-148). Although many interaction partners have been shown to stimulate or modify the unwinding activity of SF1 helicases, we know relatively little in detail about their role in tweaking the ssDNA motor for a specialised activity. Some of the best insights into how SF1 helicases are integrated with other protein machinery come from the crystal structure of the RecBCD helicase-nuclease complex (16). The RecB motor is bound to RecC via an unusual protein:protein interaction in which the entire 2B accessory domain is bound within a hole in the RecC polypeptide. The RecD helicase also interacts with RecC, this time via its N-terminal flanking accessory domain. The machine-like architecture of the complex positions the motor domains to deliver the nascent ssDNA strands to a nuclease domain at the rear of the complex for degradation.

9. SUMMARY AND FUTURE DIRECTIONS

We now have an extensive and more or less complete portfolio of SF1 helicase structures. Indeed, at the time of submission of this manuscript, the first crystal structure of a SF1 helicase in complex with RNA was published (141), adding one of the final pieces to the puzzle of SF1 structure. Comparison of this new Upf1 structure with RecD2 is certain to provide important insights into the structural basis for specificity for either an RNA or DNA track. We have detailed models for how the core domains act as a directional inchworm motor for nucleic acids in either direction and these are, in principle, similar for all members of the SF1 class and well-supported by the available biochemical data. The precise details of this stepping mechanism will continue to be an area of interest and will be best addressed through single molecule and computational methods. The mechanism that harnesses the DNA motor activity to promote DNA unwinding is less well understood. Surprisingly, the current data suggest that a range of different unwinding modes might operate in different systems. Some enzymes can clearly function as monomeric units but unwinding may be promoted by the co-operative activity of multiple monomers. Some enzymes require dimerisation or oligomerisation for activity, and many are strongly stimulated by accessory proteins. Experiments to probe helicase mechanism have often been performed using a highly reductionist approach in which individual helicase polypeptides are purified and tested for their *in vitro* biochemical properties. Such methods will remain important for the basic characterisation of helicases. Indeed, due to their abundance, there remain a surprisingly large number of uncharacterised systems from bacteria right through to humans. However, oligonucleotide based substrates are not necessarily good models for physiological substrates, and key partner proteins or protein co-factors may well be missing. Therefore, for model systems such as the UvrD/Rep-like proteins, the challenge for the future will be to work in a more physiological context, to explore the effect of protein partners and *bona fide* substrates on nucleic acid translocation and unwinding, and to better understand the diverse cellular functions of these remarkably adaptable enzymes. Ultimately, this will require the real-time study of helicases in action in live cells, and this will be dependent on the development and uptake of new technologies. In the meantime, given that the *in vivo* targets for many enzymes are still poorly defined, reverse genetics and other methods such as ChIP-sequencing will play a key role in defining the biochemistry that needs to be performed. Proteomics approaches will play a supporting role by defining the network of interaction partners for each helicase. Finally, structural information for helicases in complex with these partners will eventually allow us to decode how the motor is targeted and modified for specific DNA processing events.

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