# DNA HELICASES

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In all organisms, genetic information is locked within a double helix formed by the two antiparallel deoxyribonucleic acid (DNA) strands. Although double-stranded DNA (dsDNA) is suitable for secure information storage, hydrogen bonds formed between complementary bases impair readout of this information by the cellular machineries that frequently require single-stranded DNA (ssDNA) as the template. The unwinding of dsDNA into ssDNA, a function critical for virtually every aspect of cellular DNA metabolism including replication, recombination and repair, is catalyzed by a ubiquitous class of enzymes called DNA helicases. First identified in the 1970s, DNA helicases are motor proteins that convert chemical energy into mechanical work. Chemical energy is derived from the hydrolysis of adenosine triphosphate (ATP) or other nucleoside triphosphates, and is coupled to mechanical work during at least two important steps within the helicase reaction cycle (Fig. 1): (1) the unidirectional translocation of the enzyme along the DNA molecule, and (2) the separation of the DNA duplex.

## Classifications

On the basis of amino acid sequence comparisons, helicases are divided into the three large superfamilies and two smaller families that are illustrated schematically in

Fig. 2. Moreover, these analyses identified several protein signature motifs that are indicative of nucleic acid strand-separation activity and/or the capacity to translocate along DNA. Biochemical and structural data show that helicases can function as monomers, dimers, and multimers (predominantly hexamers) and that they can be further classified on the basis of their requirement: dsDNA, dsRNA, or DNA-RNA hybrid.

Superfamilies 1 (SF1) and 2 (SF2) include thousands of DNA and RNA helicases. SF1 and SF2 enzymes are primarily monomeric and dimeric helicases that unwind DNA with either 3 $\rightarrow$5' or 5' $\rightarrow$ 3' polarity (see below). Domains 1A and 2B in SF1 helicases and domains 1 and 2 in SF2 helicases contain similar sets of seven helicase signature motifs that comprise the basic structural core of these enzymes (Fig. 2). This core is responsible for both nucleotide binding and hydrolysis, and it includes a repeat of either of two structural domains: the RecA-fold in SF1 helicases, or the related adenylate kinase-fold in SF2 helicases. Variation at the N- and C-termini, as well as the insertions between the core domains, give rise to differences in the substrate specificity or to additional enzyme activities. Motifs I and II (Walker A {GxGKT} and B {DExo/H} motifs, respectively) are the most conserved among all helicases. Superfamily 3 (SF3) helicases are found in small DNA and RNA viruses. In contrast to SF1 and SF2 helicases, each subunit of these enzymes comprises a single *vAAA*$^{+}$- fold that is responsible for nucleotide binding and hydrolysis. The smaller superfamilies 4 and 5 consist primarily of 5' $\rightarrow$ 3' hexameric helicases related either to the *E. coli* DnaB helicase (SF4), or to the transcription termination factor Rho (SF5). The nucleotide binding and hydrolysis core of these enzymes possesses the highest degree of similarity to that of $F^1$ ATPase.

**Directional translocation**

Helicases are further classified by their polarity of unwinding as either 3' → 5' or 5' → 3' enzymes. This polarity refers to an experimentally defined requirement for a region of ssDNA of the given polarity adjacent to the dsDNA to be unwound to act as a loading site for the helicase. It is now widely accepted that the unwinding polarity relates to a directional bias in translocation on ssDNA. For example, the enzyme depicted in Fig. 1 is a 3' → 5' helicase. Upon binding to the ssDNA, it starts moving toward the 5' end of the loading strand, which brings the enzyme to the ssDNA-dsDNA junction; subsequent translocation along the ssDNA, coupled to either physical or electrostatic destabilization of the dsDNA, results in strand separation in the duplex portion of the substrate. Conversely, a 5’ → 3’ helicase translocates toward the 3’ end of the loading strand and, therefore, requires a 5’-terminated region of ssDNA to unwind a DNA duplex.

Interestingly, high-resolution structural data suggest that the helicase signature motifs are not essential for the duplex DNA separation *per se*, but for the ATP-dependent unidirectional motion of the helicases on the DNA lattice. Consequently, it is proposed that the helicase signature motifs define a modular structure for a DNA motor, while the additional domains, which may vary from one protein to another, are responsible for either modulating DNA unwinding, mediating protein-protein interactions, or determining structure specificity. Although the classical substrate for a DNA helicase depicted in Fig. 1 is duplex DNA adjacent to a region of ssDNA, some enzymes preferentially unwind more complicated structures, such as flaps, forks, bubbles, or three- and four-way junctions.

DNA translocation can also be characterized in terms of processivity, which is defined as the probability of forward translocation at each base pair opening cycle. For a highly processive enzyme, stepping forward is much more likely than dissociation. Consequently, an enzyme that never dissociates after each step has a processivity equal to one (which is a physically unrealistic situation where the enzyme is infinitely processive), whereas an enzyme that dissociates after each step has a processivity equal to zero (and is referred to as being dispersive or distributive). The average number of base pairs unwound (or translocated) by an enzyme in a single binding event (N) relates to the processivity (P) as $N = 1/(1-P)$. For example, UvrD helicase, whose processivity is 0.98, unwinds on average 50 base pairs. On the other hand, the highly processive RecBCD enzyme ($P = 0.99997$) unwinds on average 30,000 base pairs before dissociation.

**Step size**

Though seemingly simple, one of the more poorly defined parameters for DNA helicase behavior is the "step size". The step size has been defined in at least three different ways: (1) the number of base pairs that the enzyme unwinds upon hydrolyzing one ATP molecule under its most efficient reaction conditions; (2) as a physical step size, which is a measure of the helicase's ability to "step over" a discontinuity in the DNA; and (3) as a distance associated with a kinetically rate-limiting step. The parameters obtained using these three methods are not necessarily identical. For example, RecBCD enzyme hydrolyses about 2 molecules of ATP per base pair unwound, but has a kinetic step size of six base pairs. The physical step size of the related RecBC enzyme was determined to be 23 base pairs.

**Accessory factors**

Once a helicase has separated the strands of dsDNA , spontaneous reannealing of the duplex can be avoided if the nascent ssDNA strands are trapped by a single-stranded DNA binding protein or by other factors that pass the unwound nucleic acid intermediate to the next step of a reaction pathway (Fig. 1). While ssDNA binding proteins have frequently been shown to stimulate helicase activity *in vitro*, helicase activity is also stimulated by other accessory factors that increase the rate or processivity of unwinding (such as processivity factors, clamp loaders, polymerases, *etc*.). The primary replicative helicase of *Escherichia coli*, DnaB, is a good example of a helicase that acts poorly in isolation but, as part of the replisome (the DNA synthesis machinery of the cell), DnaB efficiently separates the DNA strands at the replication fork. The rate of movement of the replication machinery at the fork is coordinated by an interaction between DnaB and DNA polymerase (the enzyme that synthesizes the complementary strand of DNA) that enhances its translocation rate by almost 30-fold to 1000 base pairs per second.

**Single-molecule analysis of DNA helicases**

Until recently, virtually all biochemical data on helicases were derived from conventional bulk-phase techniques, which observe the population-averaged properties of molecular ensembles. Direct observation of dsDNA unwinding by a single helicase enzyme has the potential to uncover valuable information on protein and DNA dynamics that may be obscured in bulk-phase assays. Two of these techniques were used to visualize translocation of a single molecule of RecBCD, a multifunctional enzyme involved in the repair of dsDNA breaks by homologous recombination. RecBCD is an

exceptionally fast and processive helicase that can unwind, on average, 30,000 bp of dsDNA per binding event, at a rate of 1000 bp/s, while simultaneously degrading the ssDNA products of its helicase activity.

In one approach, a device called an optical trap is used to manipulate individual, fluorescently labeled DNA molecules and to visualize their unwinding and degradation by RecBCD enzyme. In the presence of ATP, RecBCD enzyme unwinds the dsDNA, which is observed as a progressive shortening of the fluorescently labeled DNA molecule. Recently (2003), this technique was used to demonstrate that RecBCD enzyme pauses for several seconds at a specific DNA sequence called Chi, which is a hotspot for DNA recombination and then continues but at a reduced translocation speed. This finding nicely illustrates the ability of single molecule studies to discover new behavior, as this phenomenon had not been detected in conventional bulk-phase kinetic analyses.

An alternative single-molecule approach uses light microscopy to follow translocation of a biotin-tagged RecBCD enzyme bound to a streptavidin-coated polystyrene bead in the tethered particle motion experiment. The two single-molecule experiments are different yet complementary: the tethered particle motion experiment directly measures translocation, whereas the optical trap method measures dsDNA unwinding. Together, these studies provide additional powerful evidence for the coupling of DNA strand separation with movement of the helicase protein on its substrate lattice. Both single-molecule visualization methods show that RecBCD translocates unidirectionally and processively on dsDNA, with each molecule moving at a constant rate (within the limit of experimental detection). Although the average translocation rate is similar to that derived from bulk measurements, considerable variation is observed in

the translocation rate of individual RecBCD enzymes. This surprising observation is another example of the kind of information that is accessible only by single-molecule studies.

To study helicases that have a low processivity, still other single-molecule strategies were developed . In one such strategy, fluorescence resonance energy transfer (FRET) is used to detect the separation of strands of individual base-pairs in a DNA molecule. By following the temporal fluctuations of the FRET signal from a single molecule, the kinetics of complex biochemical reaction that are unsynchronized can be interpreted more clearly that when performing bulk-phase FRET experiments.

Yet another technique for detecting helicase action at the single-molecule level measures changes in the extension of a DNA molecule as a consequence of unwinding by the UvrD helicase. At forces above 5 pN, ssDNA is longer than dsDNA, and so unwinding results in an increase in the DNA length. This technique allows determination of the individual helicase rate ($\cong$ 40 bp $s^{-1}$, which is 3-fold higher than bulk-phase estimates), the average number of base pairs unwound per binding event (240 bps), and the step size (6 bp per enzymatic cycle). Surprisingly, in some cases UvrD helicase is observed to switch strands and translocate on the opposite strand resulting in slow "re-zipping" of the unwound DNA.

**Conclusion**

Although considerable progress has been made in our understanding of the molecular mechanisms of DNA helicases, many questions remain to be answered. The new techniques of single molecule detection will play an important role in this endeavor.

Perhaps the next big challenges in this field are to understand how these DNA motors are incorporated into, and function within, the context of macromolecular machines to orchestrate complex DNA processing events.

See also: Adenosine triphosphate (ATP); Deoxyribonucleic acid (DNA); Enzyme; Molecular biology; Nucleoprotein

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*Additional Reading*

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A movie of translocation/unwinding by RecBCD helicase along fluorescently labeled DNA molecule can be viewed at

*http://microbiology.ucdavis.edu/sklab/kowalczykowskilab.htm*

Brownian motion of DNA-tethered beads can be viewed at

*http://www.bio.brandeis.edu/~gelles/movies.html*

**Figure legends**

Fig. 1 **Schematic representation of DNA helicase action**. The helicase enzyme translocates along the DNA molecule and separates the strands. Energy for this unfavorable reaction is coupled to the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate ($P_i$). In the presence of a single-stranded DNA binding protein, reannealing of the DNA duplex is prevented. The helicase depicted here displays a 3' $\rightarrow$ 5' polarity, tracking unidirectionally along the lower of the two DNA strands in the duplex (the loading strand).

Fig. 2. **Five major superfamilies of DNA helicases.** The conserved signature motifs are shown relative to the modular domains (shown as solid blocks) of helicases that belong to 5 major families. The motifs that are characteristic of all helicases within a particular superfamily are shown in black, while the motifs characteristic only to subsets of the SF2 helicases are represented by a hashed block. The areas containing large insertions that define additional enzymatic activities are indicated by dashed lines. The oligomeric state and translocation polarity for each superfamily is indicated on the right of the block diagram.

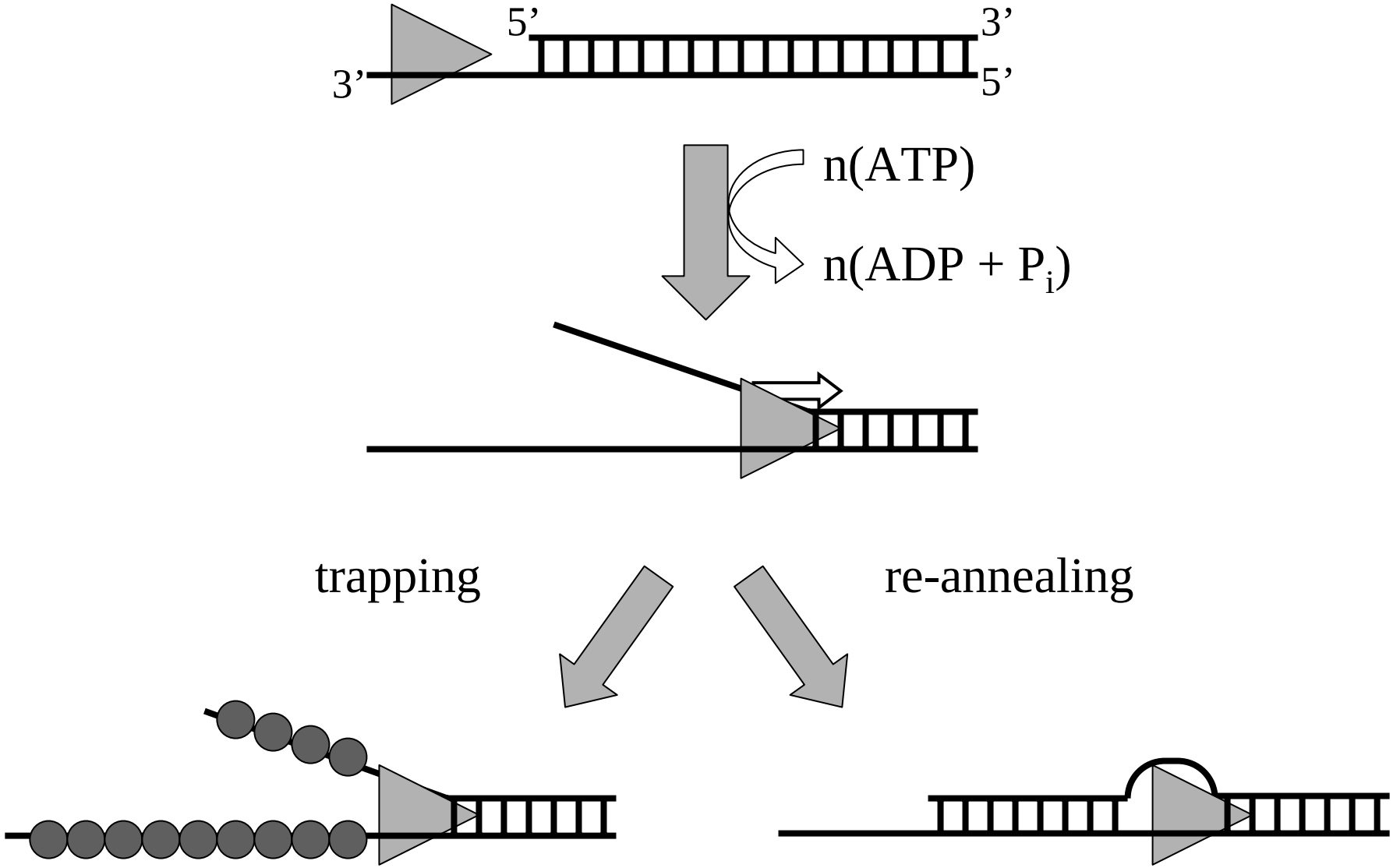


**Fig. 1.**

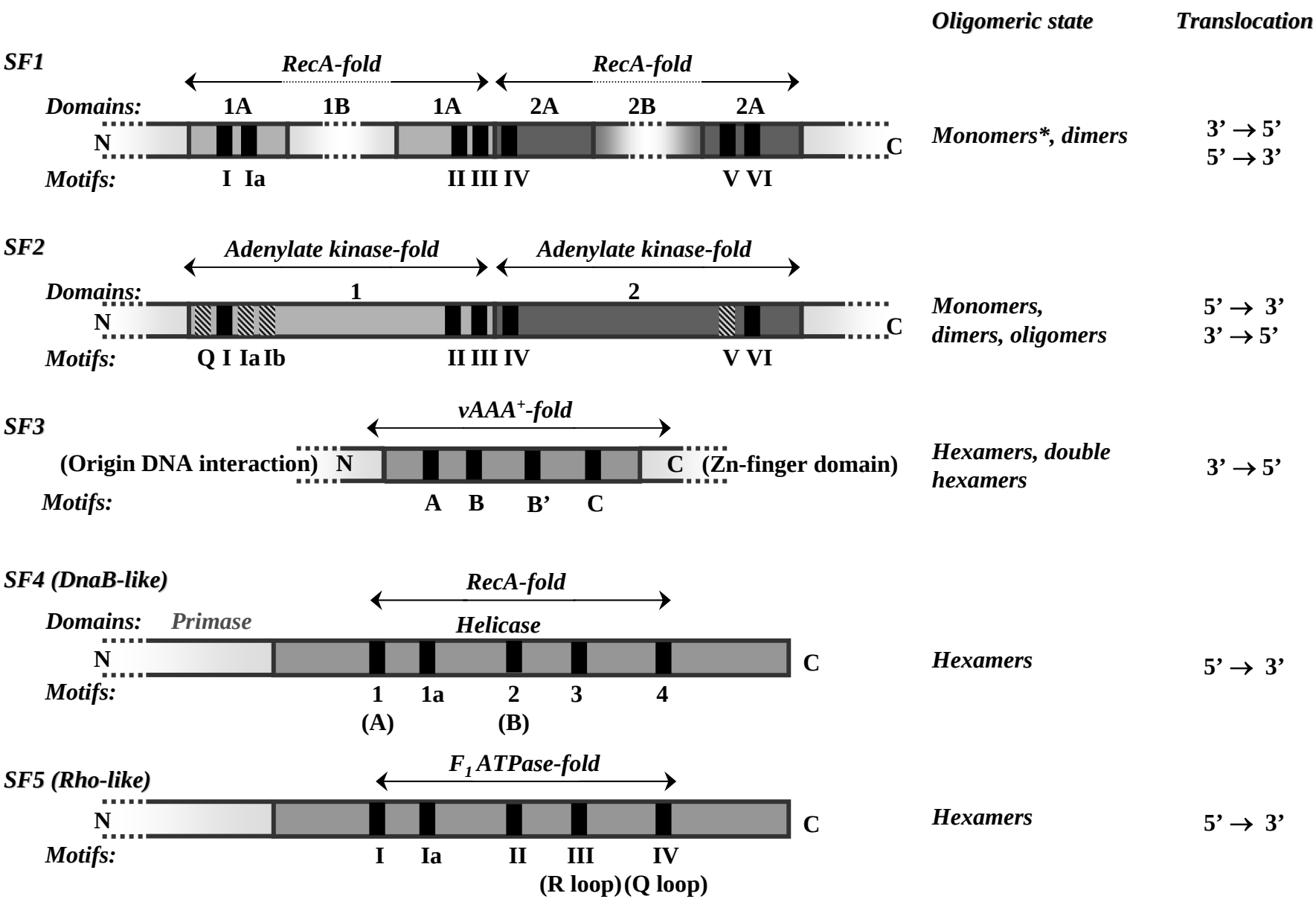
Oligomeric state
Translocation
SF1
RecA-fold
RecA-fold
Domains:
1A
1B
1A
2A
2B
2A
N
C
Motifs:
I Ia
II III IV
V VI
Monomers*, dimers
3' → 5'
5' → 3'
SF2
Adenylate kinase-fold
Adenylate kinase-fold
Domains:
1
2
N
C
Motifs:
Q I Ia Ib
II III IV
V VI
Monomers,
dimers, oligomers
5' → 3'
3' → 5'
SF3
vAAA+-fold
(Origin DNA interaction)
N
C
(Zn-finger domain)
Motifs:
A
B
B'
C
Hexamers, double
hexamers
3' → 5'
SF4 (DnaB-like)
RecA-fold
Domains:
Primase
Helicase
N
C
Motifs:
1
1a
2
3
4
(A)
(B)
Hexamers
5' → 3'
SF5 (Rho-like)
F1 ATPase-fold
N
C
Motifs:
I
Ia
II
III
IV
(R loop)
(Q loop)
Hexamers
5' → 3'

**Fig. 2.**