Mechanisms of nucleic acid translocases: lessons from structural biology and single-molecule biophysics

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Enzymes that translocate nucleic acids using ATP hydrolysis include DNA and RNA helicases, viral genome packaging motors and chromatin remodeling ATPases. Recent structural analysis, in conjunction with single-molecule studies, has revealed a wealth of new insights into how these enzymes use ATP-driven conformational changes to move on nucleic acids.

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Introduction

The ability of proteins to move directionally on nucleic acids is important to many biological processes. Perhaps best known, DNA and RNA helicases typically use ATP-hydrolysis-dependent translocation on nucleic acids to separate two strands of the duplex. Other motor proteins translocate double-stranded (ds)DNA to slide nucleosomes, move DNA Holliday junctions, or transport nucleic acids into or out of viral capsids. These enzymes are structurally and functionally very diverse, and are grouped into at least three superfamilies (SFs) [1]. SFI and SFII helicases and translocases are typically single subunit. The translocation module is formed by a single polypeptide chain and consists of two RecA/F1 fold domains. By contrast, translocation by SFIII or other multimeric helicases (e.g. *Escherichia coli* DnaB or bacteriophage T7 gene 4 protein) requires oligomerization of AAA+ domains or related RecA/F1 fold domains, respectively. Oligomeric helicases typically form hexamers. Despite this structural and functional diversity, enzymes from all three families share a mechanistic concept for directed translocation: ATP-driven conformational changes between RecA/F1 fold domains translocate nucleic acids by repositioning nucleic-acid-binding sites on the protein surface.

The precise mechanism of nucleic acid translocation by ATP-driven conformational changes has been difficult to unveil. It is necessary to obtain crystal structures of nucleic acid complexes that capture intermediates during translocation, and to quantitatively correlate ATP binding and hydrolysis cycles with mechanical steps of the enzyme on the nucleic acid substrate. Although many key features have been revealed, bulk biochemistry approaches are often not well suited to capturing the details of the underlying dynamics during processive translocation. Over the past few years, several single-molecule studies have begun to directly address the properties of individual nucleic-acid-associated motor proteins (Table 1). Single-molecule techniques enable high spatial and temporal resolution studies of a single enzyme (see e.g. [2,3] for recent reviews). Different approaches using distance and force measurements have visualized the dynamics, major and minor step sizes, and associated force of translocases on their nucleic acid substrates. In particular, helicases and DNA translocases have been studied successfully using optical tweezers [4,5•,6••,7], magnetic tweezers [8–11], stretching in flow [12,13], tethered bead assays [14], or single-molecule fluorescence and fluorescence resonance energy transfer [15] (Figure 1). Several surprising properties of translocases have been uncovered that can now be reconciled within emerging structural frameworks. Together, the combination of structural studies and single-molecule biochemistry provides a powerful means to mechanistically dissect complex molecular machines such as nucleic acid motor proteins. In this review, we highlight recent advances in our structural and functional understanding of nucleic acid translocases, using a selected set of model cases.

Hexameric helicases and translocases: closing iris, coordinated escort or rotary inchworm?

Hexameric helicases and translocases form RecA/F1 fold based oligomers or belong to the related AAA+ (ATPases associated with various cellular activities) family of multimeric ATPases [16] (Figure 2). These ring structures are often associated with highly processive reactions, such as replicative unwinding and viral genome packaging. Structural results suggest that these ring helicases and translocases encircle nucleic acids [17•,18••,19–22]. ATP-binding sites are situated at the interface of adjacent protomers [23,24], suggesting that ATP binding and

Table 1

Selected single-molecule studies on nucleic acid translocases.

	Function	Organism	Velocity	Processivity	Force dependence	Helix tracking (a)	Direction reversal	References	Structure
FtsK	Chromosome segregation	*E. coli*	7000 bp/s	6000 bp	Processivity velocity (weak)	No	Yes	[6••,8,30••,31]	[17•]
UvrD	Helicase/repair	*E. coli*	250 bp/s	240 bp	Not observed	Yes	Yes	[48]	[33,49] (b)
*Eco*R124I	Restriction modification	*E. coli*	550 bp/s	2.5 kbp	Processivity	Yes	Yes	[9,47••]	(c)
RuvAB	Branch migration	*E. coli*	50 bp/s	>15 kbp	Velocity (weak)	Yes	Yes	[14,50,51]	[52,53]
RecBCD	Helicase/repair	*E. coli*	1000 bp/s	30 kbp	Backsliding	NA	No	[54–58]	[59]
Rad54	DNA translocase, homologous recombination	*S. cerevisiae*	300 bp/s	11.5 kbp	Not observed	?	Yes	[13]	[38•,39]
RSC	Nucleosome remodeling	*S. cerevisiae*	300 bp/s	380 bp	Processivity	No	Yes	[10]	(d)
Rep (ss translocase)	Helicase	*E. coli*	60 b/s	>180 b	Not observed	NA	Snapback	[15]	[49]
Rep (unwinding)	Helicase	*E. coli*	45 bp/s	30 bp	Not observed	Yes	No	[60]	[49]
T7 gene 4 protein	Helicase/primase	T7	160 bp/s	17 kbp (with polymerase)	Not observed	?	No	[12]	[23]
ϕ29 portal motor	DNA packaging	ϕ29	100 bp/s	Whole genome	Velocity	?	No	[5•]	[61] (e)
NS3 (unwinding)	RNA helicase	HCV	51 bp/s	18 bp	Processivity	NA	No	[4]	[37]

(a) Question marks are used when it is not known whether a DNA translocase tracks the helix. NA, not applicable as the enzyme functions as a helicase. (b) Structure of homologous SFI helicases. (c) Predicted to share homology with Swi2/Snf2 enzymes such as Rad54. (d) ATPase domain homologous to Rad54. (e) Structure of connector ring, does not include ATPase part.

hydrolysis are influenced by the relative orientation of protomers (Figure 2).

Two extreme principal modes of ATP-induced conformational changes can be envisioned. In a concerted mechanism, all six subunits act simultaneously, preserving symmetry throughout the enzymatic process. Alternatively, in a sequential mechanism, adjacent protomers are in different conformational states and these states 'rotate' around the ring. This sequential mode implies substantial asymmetry within the hexamer, similar to that observed in the F_1-ATPase ring during ATP hydrolysis [25]. Nucleotide-binding-induced asymmetry was first observed in the structure of the gene 4 hexamer [23], the replicative helicase of phage T7, consistent with the asymmetry seen in T7 gene 4 protein, DnaB and MCM using biochemical and electron microscopy techniques [26–28]. However, it was long unclear how DNA is bound and transported by these asymmetric ring structures. A conceptual breakthrough for the sequential helicase mechanism is now provided by the crystal structure of papillomavirus E1 protein bound to ADP and a single-stranded (ss) deoxyoligonucleotide [18••]. ssDNA is bound as a tight spiral staircase in the central hole of the hexamer (Figures 2 and 3a). The protomers are progressively tilted and bind to the backbone of adjacent deoxynucleotides. One of the surprising implications of this structure is the idea that protein loops remain bound to the DNA backbone during translocation and 'escort' the DNA (Figure 3b). This 'coordinated escort' model is different to a previous model based on structural and biochemical studies of T7 gene 4 protein, whereby DNA is handed off between protomers. However, the crystal structures of gene 4 and E1 do not completely represent a snapshot of translocation, because only one type of nucleotide is present. In a sequential mechanism, different protomers differ in their nucleotide state. Nevertheless, in the case of the E1 structure, some of the ADP-bound active sites contained a chloride ion at the expected position of the ATP γ-phosphate and could mimic the ATP-bound state [18••].

Based on these new structural observations and recent biochemical data [27], the major conformational change is triggered either by ADP release or by ATP binding, during which a new protomer is loaded onto the upstream DNA. In this regard, a good understanding of how different nucleotide states differ in function may be gathered through single-molecule experiments on the viral dsDNA packaging motor of bacteriophage ϕ29 [5•]. The translocation kinetics were measured under different opposing forces and nucleotide concentrations to infer the translocation state and states that are weakly or strongly bound to DNA. Interestingly, the experiments show that product release and not ATP binding leads to translocation. The motor domain that binds the incoming ATP is at that moment not engaged with the DNA and engagement happens only after ATP binding.