Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Structural Biology

Stepwise translocation of nucleic acid motors

Sua Myong[1,3] and Taekjip Ha[2,3,4]

Recent single molecule studies have made a significant contribution to the understanding of the molecular mechanism involved in the movement of motor proteins which process DNA and RNA. Measurement of stepsize in two disparate motors, NS3 helicase and ribosome both revealed 3-bp steps, which consist of three hidden substeps. Combined with previous structural studies, NS3 is likely taking a single nucleotide step of translocation coupled to one ATP binding event and this mode may be conserved in multitude of helicases. Such a stepwise translocation movement appears to occur through main contacts with the phosphate backbone. Double stranded RNA and DNA motor, RIG-I and ϕ29, respectively, showed translocation on a duplex while tracking exclusively a single strand of RNA or DNA in a directional manner, 5′–3′ in both cases. Spontaneous dynamics displayed by ribosome ratcheting and SSB (single stranded DNA binding protein) diffusing on DNA were rectified by interacting cofactors and proteins, EF-G and RecA, respectively.

Addresses
[1] Department of Bioengineering, University of Illinois at Urbana-Champaign, USA
[2] Department of Physics, University of Illinois at Urbana-Champaign, USA
[3] Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA
[4] Howard Hughes Medical Institute, Urbana, IL 61801, USA

Corresponding author: Ha, Taekjip (tjha@illinois.edu)

Current Opinion in Structural Biology 2010, **20**:121–127

This review comes from a themed issue on
Protein–nuclei acid interactions
Edited by Simon Phillips and Karolin Luger

Available online 12th January 2010

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2009.12.008

Introduction

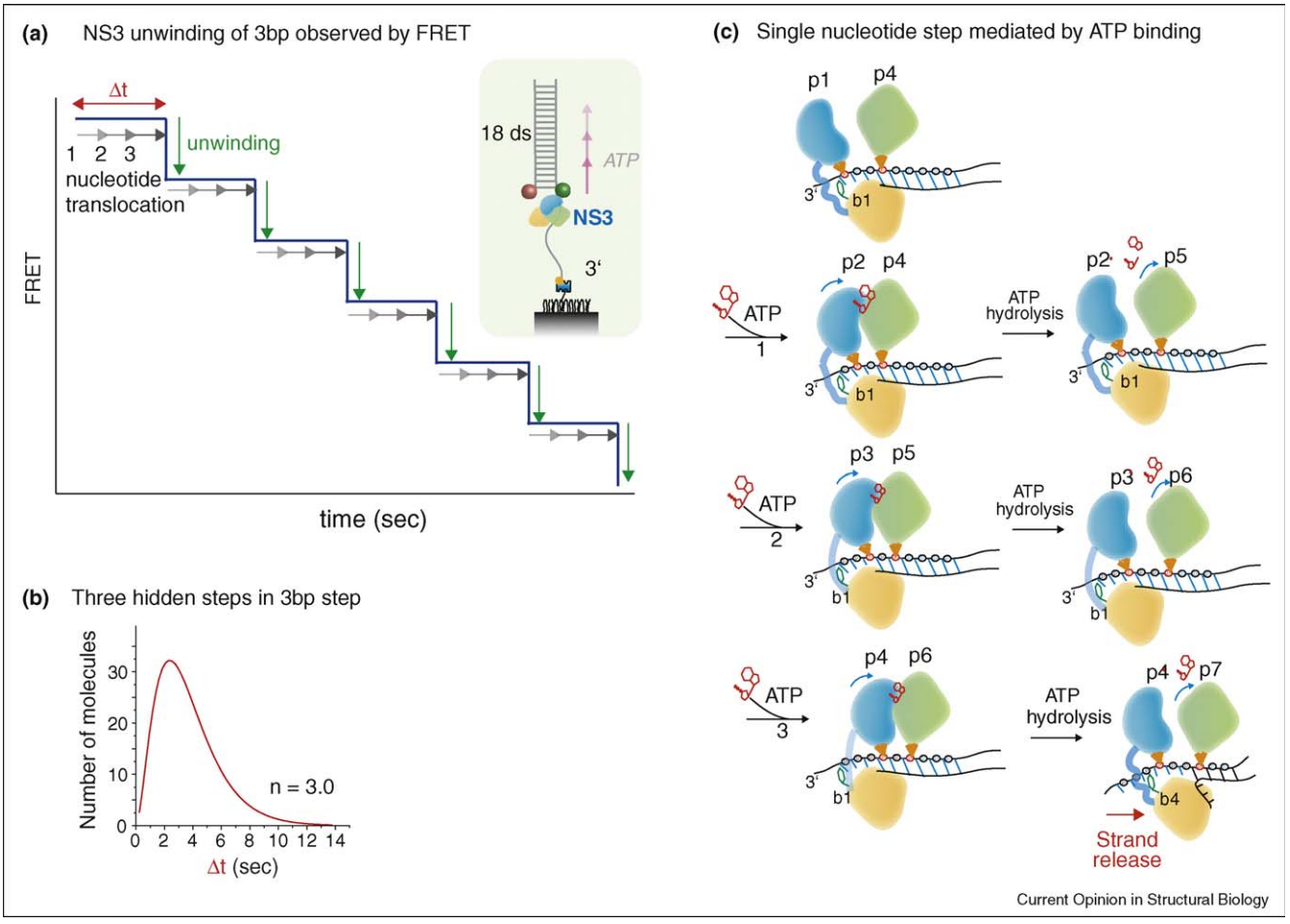
Molecular motors are much like cars driving on a highway. They are often directional with variable speed and their processivity is determined by how long they stay on track before trailing off to an exit. Many motors consume energy to translocate on their track and thus slow down or stop when lacking sufficient amount of fuel as in the case of motor vehicles. Such behaviors have been examined through the glasses of highly sensitive single molecular binoculars and structural eye pieces to reveal hidden steps and mechanistic details involved in the movement of motor proteins. The new insights from these studies imply a mechanism similar to the gears controlling a mechanical clock where the movement of the minute and hour hands are under the control of the pendulum, which converts the swinging motion into rotational movement of the corresponding wheels for the two hands. Similarly, the mechanical translocation of a motor protein is governed by ATP hydrolysis which modulates their conformation, thereby converting chemical energy into mechanical movement in a stepwise manner. This review attempts to articulate some key findings reported in the past two years by single molecule studies on proteins which process nucleic acids in various contexts. The proteins under review include helicases, a viral nucleic acid packaging motor, reverse transcriptase, single stranded DNA binding proteins, and the ribosome, all of which are well known proteins whose motion on the DNA or RNA lattice are important for the processing of nucleic acids.

Stepsize as small as a single nucleotide

The stepsize of a motor protein can be defined as the physical distance moved by the protein on the track during a biochemical cycle in which chemical energy consumption is coupled to a mechanical cycle of advancing the protein. Previous biochemical studies have revealed variable stepsizes from DNA motors ranging from a single nucleotide (nt) to 23 nt [1–4], indicating that the intrinsic stepsize of such motors can be as tiny as a single base of DNA or RNA. Proteins involved in replication and transcription are likely to process nts as a chemical entity one at a time to ensure an accurate information transfer [5,6]. In contrast, proteins such as a virus packaging motor may treat more than 1 nt as a physical unit, thus process it with less rigorous measures [7••].

A recent study demonstrated that a single nt is likely the smallest step possible for proteins that participate in genome processing. Using single molecule FRET, the unwinding steps of NS3, a helicase found in Hepatitis C virus, were detected. NS3 unwound DNA in discrete steps of about 3 bp. Interestingly, the dwell time in each plateau was not exponential, as would be expected if there is a single rate-limiting step. Instead, the dwell time histogram showed a rising phase and a decaying phase, most consistent with three irreversible hidden steps before each step of 3 bp unwinding [8••]. Combining with the structural data on NS3 without ATP [9] and related RNA helicases with ATP [10,11], it was proposed that 3 bp are unwound in a burst after accumulation of elastic energy in the protein–DNA complex during three substeps, 1 nt per ATP, of NS3 translocation on the DNA lattice (Figure 1). The identical set of the structural arrangement was also present in other

Figure 1



(a) NS3 unwinding was observed by single molecule FRET measurement where high FRET from two dyes placed at the duplex junction decreased stepwise during unwinding. Six steps obtained from 18 bp unwinding reveals 3 bp stepsize. **(b)** Dwell time analysis revealed a nonexponential decay, which indicates three hidden substeps within 3 bp unwinding steps. **(c)** NS3 takes three steps of single nucleotide translocation coupled to ATP binding, which builds strain on the DNA–protein interface. The strain is released about every 3 bp, leading to a burst of 3 bp unwinding observed in (a).

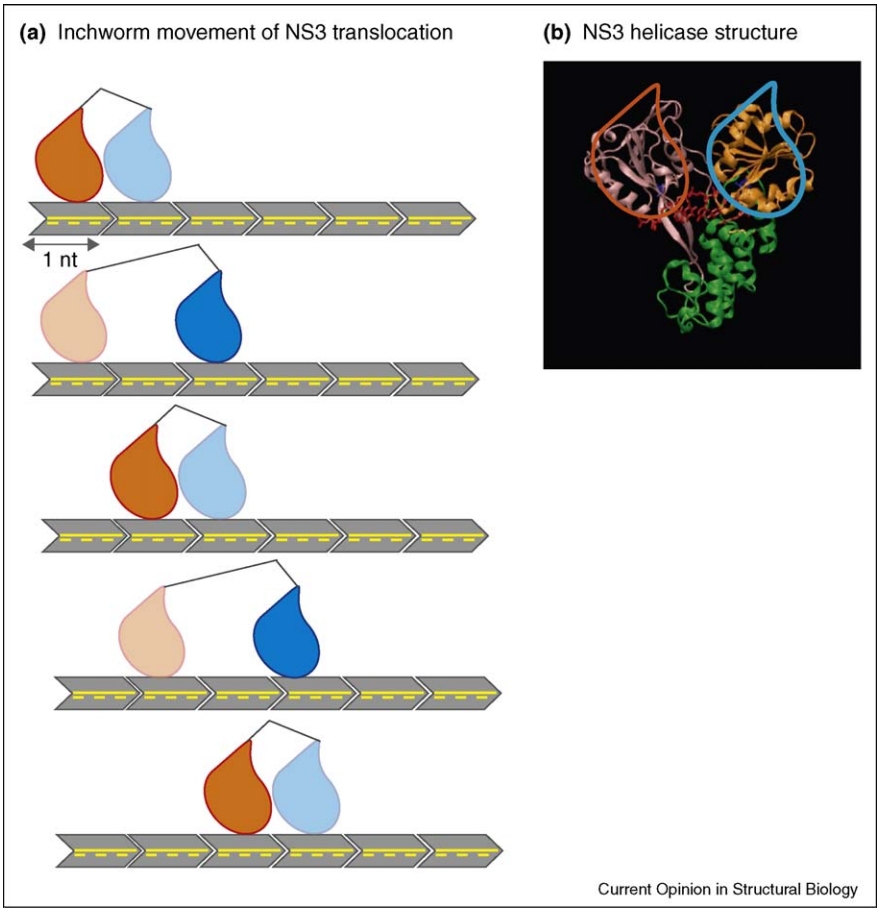
helicases including Rep, PcrA, UvrD, and Hel308, strongly suggesting that a single nt step inchworm-like movement may be a universal mechanism shared among numerous helicases (Figure 2).

A strikingly similar observation was made for the ribosome during translation of mRNA, visualized by an optical tweezers technique [12••]. The mRNA formed a hairpin, and ribosome motion on the RNA codon-by-codon was captured upon unwinding of the hairpin in 3 bp increments. The dwell time histogram was again best explained by three hidden steps occurring before ribosome translocation over a single codon. Presently, the origin of these hidden steps is unclear but it will be interesting to test if similar kinetics can be observed even when the ribosome does not need to unwind duplexes for translocation (Figure 3).

Phosphate backbone is the main contact

Most DNA and RNA motor proteins do not bind in a sequence specific manner and the structure of double stranded DNA (dsDNA) and RNA reveals an apparent difficulty for a protein to reach into the grooves for approaching bases. It is perhaps for this reason that a multitude of proteins including helicases appear to translocate primarily on the phosphate backbone. The aforementioned single nt stepping of NS3 was based on the structural data which presented two phosphate contacting threonine (Thr) residues on two domains of NS3 which are highly conserved and indispensible for the ATPase and unwinding activity of NS3 [9]. These Thr residues at the elbows formed by a beta strand and an alpha helix each are 3 nt apart in the absence of ATP, but shows 2 nt distance in ATP bound structure of its close homologs, Vasa, and eIF4AIII [10,11] (Figure 4). This single nt

Figure 2



(a) NS3 and related helicases use two RecA-like domains to translocate on the nucleic acid track, 1 nt at a time in an inchworm like manner. The two domains 'walk' on the nucleic acid track by alternating affinities, mediated by ATP binding and ADP release. Dark and fair colored units represent tight and lose binding of two RecA-like domains respectively. **(b)** Two RecA-like domains are depicted in the NS3 crystal structure (PDB: 1A1V) [9] with the same color designation as in (a).

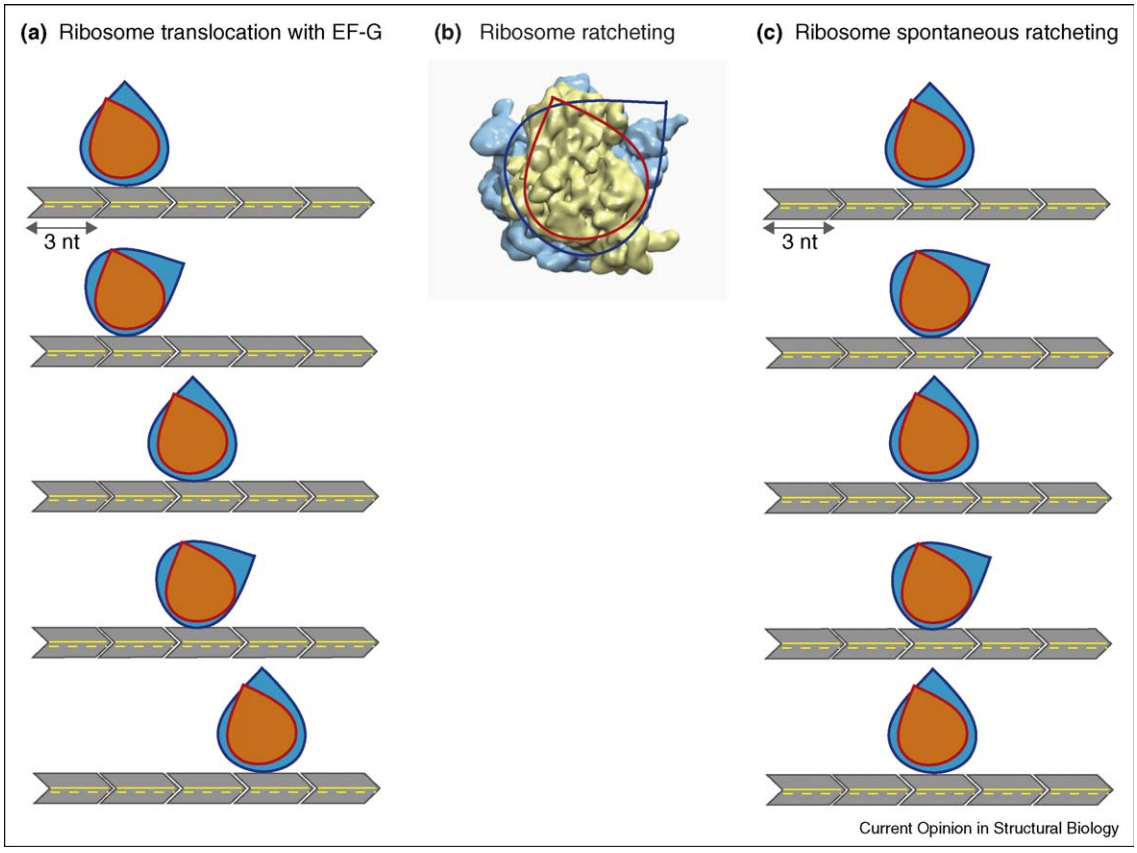
distance change mediated by two Thr-phosphate contacts is conserved in various helicases as discussed above and may represent a unifying mechanism for motor proteins to walk on the phosphate backbone as a primary translocating track (Figure 1).

The significance of the phosphate contact was also demonstrated by Aathavan *et al.* who investigated the interaction between a viral packaging motor from ϕ29 and its substrate, dsDNA using the optical tweezers technique [13••]. The directional movement of dsDNA through the ring-shaped ATPase was greatly hindered when methylphosphonate modified bases were interjected in its tracking strand. Methylphosphonate replacement of phosphate moiety removes negative charges from the backbone, hence the phosphate contacts with the neighboring amino acid of the packaging motor may lose their grip on the phosphates, leading to stalling.

Single strand tracking by double stranded DNA and RNA motors

The concept of directionality becomes uncertain when it comes to motor proteins which translocate on dsDNA or RNA. In most cases, they are multimeric complexes composed of two or more units of the proteins, which make contact with both strands by encompassing the entire double strand. Recently, we discovered that RIG-I, an antiviral sensor and an ATPase is a robust translocase on double stranded RNA (dsRNA) [14••]. RIG-I recognizes and binds dsRNA of viruses as a pathogenic signature and elicits an antiviral signaling cascade. When subjected to various substrates, RIG-I displayed a strong preference to bind dsRNA as a dimer whereas it did not bind or translocate on dsDNA. On RNA/DNA heteroduplexes, however, RIG-I showed translocation activity comparable to dsRNA, indicating that single strand of RNA is sufficient to serve as a tracking strand

Figure 3



(a) In the presence of EF-G elongation factor, the two rotating subunits of the ribosome translocate on mRNA taking 3 nt steps corresponding to the size of one codon. **(b)** The 30S and 50S subunits are represented on the ribosome structure. **(c)** The ribosome undergoes a spontaneous ratcheting motion in the absence of EF-G.

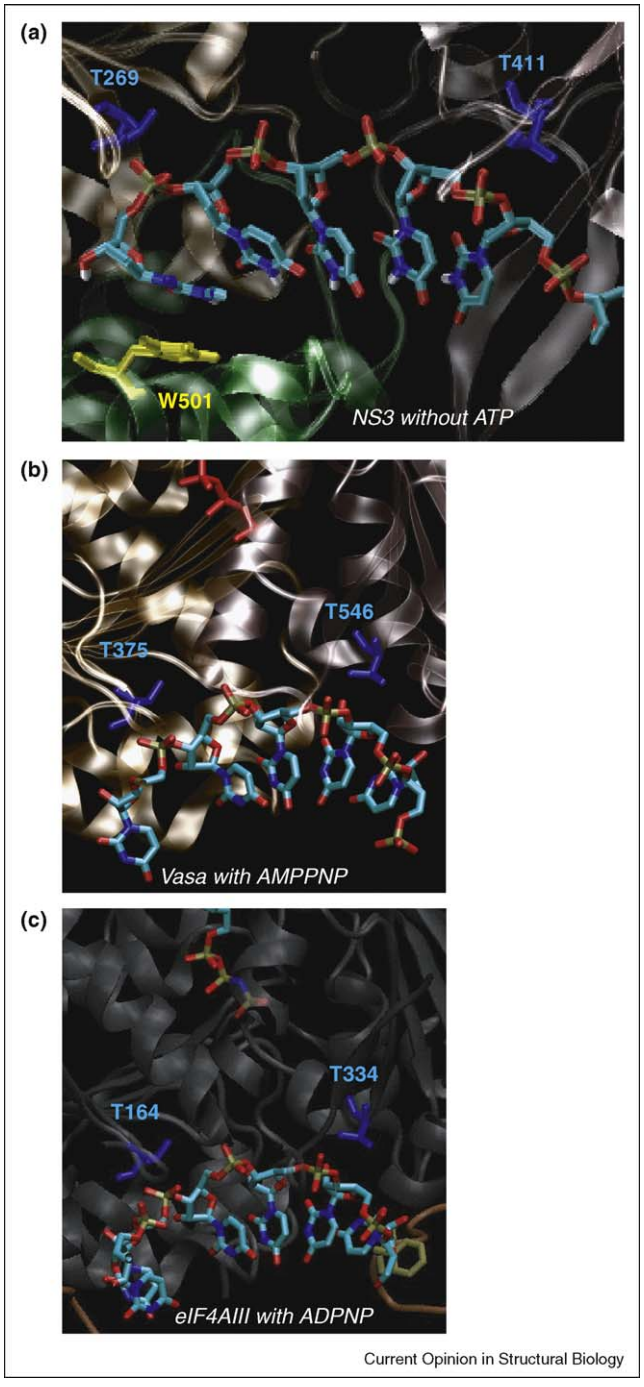
and to support RIG-I translocation. Directionality of RIG-I translocation was obtained by observing the translocation on RNA:DNA heteroduplex of a truncation mutant of RIG-I without the N-terminal signaling domain. The fluorescence signal changes were consistent with 5′–3′ directional translocation on the RNA strand. A similar bias to track one strand of a duplex DNA was shown in the study of the viral packaging motor, ϕ29. The translocation of dsDNA through a pentameric ring of ATPases was not disrupted when the methylphosphonate modification was placed on 3′–5′ directional strand. The same degree of modification (10 nt), however perturbed the translocation dramatically on the counter strand in 5′–3′ in the direction of viral packaging, suggesting a primary contact with the single strand DNA in the 5′–3′ direction [13••].

DNA versus RNA motors

Most motor proteins are highly selective to either DNA or RNA as a preferred substrate although there are exceptions such as NS3 helicase, which unwinds both RNA and DNA [15].

Liu *et al.* presented an interesting case of a motor protein, reverse transcriptase (RT) from HIV, which transcribes DNA from viral ssRNA template. When given a RNA:DNA heteroduplex, RT showed a random sliding behavior which is stabilized by the presence of a cognate dNTP, promoting the positioning of RT at the 3′ termini of the DNA where the dNTP needs to be incorporated. Using a fluorescently labeled RT, they also showed that RT undergoes a rapid conformational change to engage into a state where it can carry out polymerization. When subjected to a dsDNA construct, the same sliding behavior and the dNTP driven positioning occurred, indicating that the RT sliding is allowed in both types of duplex compositions, consistent with its *in vivo* functions of reverse transcription and plus-strand DNA synthesis. Hence, the substrate selectivity of RT is toward RNA:DNA and DNA:DNA, yet the polymerization mode ensues only at the trailing end of 3′ DNA thereby achieving its catalytic specificity [16••]. In terms of a primary contact, RT is likely to engage more tightly to ssDNA strand in search for the 3′ terminal polymerization site while loosely gripping the complementary ssRNA. On

Figure 4



(a) NS3 crystallized without ATP shows two well conserved threonine residues (T269, T411 in blue) contacting the backbone phosphate 3 nt apart (PDB: 1A1V) [9]. W501 (yellow), also known as 'gatekeeper' is base stacked at the 3′ end of the bound nucleotide. **(b,c)** Both Drosophilla Vasa (PDB: 2DB3) [11] and eIF4AIII (PDB: 2HYI) [10], two other SF2 helicases were cocrystallized with ATP analogs, AMPPNP and ADPNP, respectively. The structurally equivalent threonine residues (T375, T546 in Vasa, T164, T334 in eIF4IIIA) show phosphate contacts in 2 nt distance, suggesting 1 nt movement coupled to one ATP binding in between domain 1 and 2, bringing the two RecA-like domains together.

the basis of its bidirectional movement, it would be of interest to compare the ssDNA contact surface of RT and other directional ssDNA translocases to understand the molecular basis of directional motors.

A similar substrate specificity is also revealed in the RIG-I study mentioned above. RIG-I forms a dimer [17] and recognizes dsRNA, a molecular pattern present in viruses [18]. While RIG-I did not bind or translocate on dsDNA, it bound and translocated on RNA:DNA heteroduplexes as well as on dsRNA although the binding affinity was lower [14••]. The activity of RIG-I on heteroduplex construct indicates that RIG-I tracks primarily one ssRNA even in the context of dsRNA, hence ssRNA is the minimum requirement of RIG-I translocation. The ability of RIG-I to track ssRNA, yet not unwind the duplex may be an intriguing attribute exclusive to this family of proteins. Structural studies of RIG-I ATPase domain bound to RNA substrate should illuminate the distinct features that allow this translocation mode.

Spontaneous conformational dynamics utilized for function

While catalytic reactions such as unwinding by a helicase are strictly coupled to ATP (or NTP) hydrolysis, some motor proteins exhibit dynamic movements even in the absence of an energy input. Often these movements are programmed to maximize its activity upon encountering an ideal active site or a correct substrate. The Ribosome is a universal translational machinery which consists of 30S and 50S subunits. Although there has been much prediction about the intersubunit rotations, also known as 'ratcheting', correlated with the translation cycle, in particular the translocation of the ribosome on the mRNA lattice [19–24] the recent report by Cornish *et al.* marks the first real-time observation of this phenomenon [25••]. The two ribosome subunits rotate spontaneously between the two conformational states referred to as classical and hybrid states (Figure 3). The two subunits modulated their bias to either state depending on the applied reaction conditions, confirming the two states as the intermediate steps involved in translation. Remarkably, removing the amino acid from the tRNA bound to the so-called 'P-site' led to the 100-fold acceleration of the ratcheting dynamics, showing that a single amino acid change could lubricate the 2.6 MDa machine! It is likely that this ratcheting dynamics is equivalent to the inchworming motion of helicases (Figure 2). Just like ATP binding and hydrolysis modulate the opening and closing of two RecA-like domains on the helicases to cause a directional movement, binding and GTP hydrolysis of an elongation factor known as EF-G may rectify the spontaneous ribosome dynamics to enable it to translocate on the mRNA track in a directional motor. In this sense, we may view the ribosome as an inchworming Brownian ratchet (Figure 3).

Another example of spontaneous dynamics was manifested by SSB (Single Stranded DNA Binding protein). It was shown that that *E. coli* SSB diffuses on ssDNA with an estimated diffusion coefficient of $\sim$300 $(nt)^2/s$ and with an average stepsize of about 3 nt [26••]. The diffusion occurred at least of 65 nt and could be used to resolve secondary structures dynamically, and to enhance RecA filament growth. This first example of any protein diffusing on single stranded DNA provides a new model with which many proteins that bind to single stranded DNA or RNA via multiple binding interactions may redistribute themselves after initial binding and coordinate with other proteins for access to the DNA. Similar to the Browinian inchworm model for the ribosome movement, the spontaneous motion of SSB protein on the DNA can be rectified via the directional 5′–3′ extension of the RecA filament. Of note, RecA filament grows and shrinks in units of single monomers [27] and the binding site size of RecA monomer is 3 nt [28], which matches the estimated diffusion stepsize of SSB. Because recent single molecule analysis suggested that the eukaryotic homolog Rad51 also extends and shrinks its filament in single monomer units [29,30], it is plausible that the mechanism discovered from the studies of *E. coli* SSB and RecA is also applicable to the eukaryotic systems. The intersubunit rotation of the ribosome and the diffusion of SSB on single stranded DNA are two different modes of spontaneous dynamics, yet both motions are likely to offer an advantage for their function.

Conclusion

DNA and RNA are much like molecular highways on which motor proteins run. They have a distinct polarity with different chemistry, which attracts specific motors to latch on and travel. The motor proteins can be classified into two categories for the purpose of our discussion. The first class is the genome processing proteins including helicases, ribosomes, and reverse transcriptases, which partake in gene transfer reactions. Second class is the proteins that process RNA and DNA in a nongenomic manner such as the viral packaging motor and the antiviral receptor, RIG-I. Single molecule approaches have been successfully employed to extract the fundamental mechanisms underlying the function of both types of proteins. As the first class proteins, NS3 and ribosome revealed highly regular stepsize of 3 bp, each of which are composed of three substeps representing single nt steps of translocation. For the second class proteins, RIG-I and the viral packaging motor, ϕ29 are both dsRNA and DNA translocases, respectively, yet both translocate on one of the double strands in a directional manner. Other characteristics such as phosphate contact and DNA versus RNA selectivity are attributed to both classes of proteins. Future studies would reveal more exciting details about the motor protein mechanism, which will bring about further understanding of these proteins.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- •• of outstanding interest

1. Dillingham MS, Wigley DB, Webb MR: **Demonstration of unidirectional single-stranded DNA translocation by PcrA helicase: measurement of step size and translocation speed**. *Biochemistry* 2000, **39**:205-212.

2. Bianco PR, Kowalczykowski SC: **Translocation step size and mechanism of the RecBC DNA helicase**. *Nature* 2000, **405**:368-372.

3. Serebrov V, Pyle AM: **Periodic cycles of RNA unwinding and pausing by hepatitis C virus NS3 helicase**. *Nature* 2004, **430**:476-480.

4. Ali JA, Lohman TM: **Kinetic measurement of the step size of DNA unwinding by Escherichia coli UvrD helicase**. *Science* 1997, **275**:377-380.

5. Christian TD, Romano LJ, Rueda D: **Single-molecule measurements of synthesis by DNA polymerase with base-pair resolution**. *Proc Natl Acad Sci U S A* 2009, **106**:21109-21114.

6. Greenleaf WJ, Block SM: **Single-molecule, motion-based DNA sequencing using RNA polymerase**. *Science* 2006, **313**:801.

7. •• Moffitt JR, Chemla YR, Aathavan K, Grimes S, Jardine PJ, Anderson DL, Bustamante C: **Intersubunit coordination in a homomeric ring ATPase**. *Nature* 2009, **457**:446-450.
Observation of 10 basepair stepsize of a viral packaging motor, ϕ29 using an optical tweezers measurement.

8. •• Myong S, Bruno MM, Pyle AM, Ha T: **Spring-loaded mechanism of DNA unwinding by hepatitis C virus NS3 helicase**. *Science* 2007, **317**:513-516.
Observation of three base pair unwinding step of NS3 helicase which is composed of three single nucleotide substeps, using single molecule FRET assay.

9. Kim JL, Morgenstern KA, Griffith JP, Dwyer MD, Thomson JA, Murcko MA, Lin C, Caron PR: **Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding**. *Structure* 1998, **6**:89-100.

10. Andersen CB, Ballut L, Johansen JS, Chamieh H, Nielsen KH, Oliveira CL, Pedersen JS, Seraphin B, Le Hir H, Andersen GR: **Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA**. *Science* 2006, **313**:1968-1972.

11. Sengoku T, Nureki O, Nakamura A, Kobayashi S, Yokoyama S: **Structural basis for RNA unwinding by the DEAD-box protein Drosophila Vasa**. *Cell* 2006, **125**:287-300.

12. •• Wen JD, Lancaster L, Hodges C, Zeri AC, Yoshimura SH, Noller HF, Bustamante C, Tinoco I: **Following translation by single ribosomes one codon at a time**. *Nature* 2008, **452**:598-603.
Observation of three nucleotide step (one codon) of a ribosome which consists of three substeps monitored by optical tweezers technique.

13. •• Aathavan K, Politzer AT, Kaplan A, Moffitt JR, Chemla YR, Grimes S, Jardine PJ, Anderson DL, Bustamante C: **Substrate interactions and promiscuity in a viral DNA packaging motor**. *Nature* 2009, **461**:669-673.
Report on the significance of phosphate backbone of DNA for directional translocation of ϕ29 using an optical tweezers measurement.

14. •• Myong S, Cui S, Cornish PV, Kirchhofer A, Gack MU, Jung JU, Hopfner KP, Ha T: **Cytosolic viral sensor RIG-I is a 5′-triphosphate-dependent translocase on double-stranded RNA**. *Science* 2009, **323**:1070-1074.
Discovery of a robust translocational activity of an antiviral receptor, RIG-I on double stranded RNA using single molecule protein induced fluorescence enhancement (PIFE) assay.

15. Pang PS, Jankowsky E, Planet PJ, Pyle AM: **The hepatitis C viral NS3 protein is a processive DNA helicase with cofactor enhanced RNA unwinding**. *EMBO J* 2002, **21**:1168-1176.

16. •• Liu S, Abbondanzieri EA, Rausch JW, Le Grice SF, Zhuang X: **Slide into action: dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates**. *Science* 2008, **322**:1092-1097.
Observation of spontaneous sliding and dynamic shuttling of reverse transcriptase of HIV using single molecule FRET measurement.

17. Cui S, Eisenacher K, Kirchhofer A, Brzozka K, Lammens A, Lammens K, Fujita T, Conzelmann KK, Krug A, Hopfner KP: **The C-terminal regulatory domain is the RNA 5'-triphosphate sensor of RIG-I**. *Mol Cell* 2008, **29**:169-179.

18. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M *et al.*: **5′-Triphosphate RNA is the ligand for RIG-I**. *Science* 2006, **314**:994-997.

19. Cukras AR, Southworth DR, Brunelle JL, Culver GM, Green R: **Ribosomal proteins S12 and S13 function as control elements for translocation of the mRNA:tRNA complex**. *Mol Cell* 2003, **12**:321-328.

20. Fredrick K, Noller HF: **Catalysis of ribosomal translocation by sparsomycin**. *Science* 2003, **300**:1159-1162.

21. Frank J, Agrawal RK: **A ratchet-like inter-subunit reorganization of the ribosome during translocation**. *Nature* 2000, **406**:318-322.

22. Munro JB, Altman RB, O'Connor N, Blanchard SC: **Identification of two distinct hybrid state intermediates on the ribosome**. *Mol Cell* 2007, **25**:505-517.

23. Kim HD, Puglisi JD, Chu S: **Fluctuations of transfer RNAs between classical and hybrid states**. *Biophys J* 2007, **93**:3575-3582.

24. Fei J, Kosuri P, MacDougall DD, Gonzalez RL Jr: **Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation**. *Mol Cell* 2008, **30**:348-359.

25. •• Cornish PV, Ermolenko DN, Noller HF, Ha T: **Spontaneous intersubunit rotation in single ribosomes**. *Mol Cell* 2008, **30**:578-588.
Observation of spontaneous intersubunit rotation of a ribosome using single molecule FRET measurement.

26. •• Roy R, Kozlov AG, Lohman TM, Ha T: **SSB protein diffusion on single-stranded DNA stimulates RecA filament formation**. *Nature* 2009, **461**:1092-1097.
Discovery of diffusional movement of single stranded DNA binding protein (SSB) by single molecule FRET visualization.

27. Joo C, McKinney SA, Nakamura M, Rasnik I, Myong S, Ha T: **Real-time observation of RecA filament dynamics with single monomer resolution**. *Cell* 2006, **126**:515-527.

28. Chen Z, Yang H, Pavletich NP: **Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures**. *Nature* 2008, **453**:489-494.

29. Arata H, Dupont A, Mine-Hattab J, Disseau L, Renodon-Corniere A, Takahashi M, Viovy JL, Cappello G: **Direct observation of twisting steps during Rad51 polymerization on DNA**. *Proc Natl Acad Sci U S A* 2009, **106**:19239-19244.

30. van Mameren J, Modesti M, Kanaar R, Wyman C, Peterman EJ, Wuite GJ: **Counting RAD51 proteins disassembling from nucleoprotein filaments under tension**. *Nature* 2009, **457**:745-748.