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Conformational selection and induced fit as a useful framework for molecular motor mechanisms

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Abstract

In this review, we explore mappings between mechanisms of ligand-binding, molecular motor translocation, and helicase unwinding of nucleic acids. The linkage between macromolecular binding and conformational change that is ubiquitous in biological molecules can be understood in the context of the of conformational selection and induced fit. The mechanism of biased motion exhibited by molecular motors is typically described as either a thermal ratchet or a power-stroke. Lastly, helicases are characterized by either active or passive unwinding mechanisms. We posit that both Brownian ratchet translocation and passive unwinding are examples of conformational selection and that both power-stroke translocation and active unwinding are examples of induced fit. In ligand-binding reactions, both conformational selection and induced fit may exist in parallel and the flux through either mechanistic path depends on ligand concentration. Given these mappings, we propose, in analogy with ligand-binding mechanisms, that motors may be able to function via parallel ratchet and stroke mechanisms and that helicases may be able to function via parallel active and passive mechanisms.

Introduction

Biology is built upon the ability of macromolecules to transduce information by changing their shape or conformation. However, this alone is not sufficient. These conformations must be linked to environmental conditions (i.e. temperature, salt concentration, pH, or the concentration of other molecules) and different conformations must lead to different molecular activities. With these three conditions satisfied, an environmental variable may now produce a change in activity via the modulation of the conformation of a macromolecule. This molecular signaling is ubiquitous in biology and allows for cells to adapt to a changing environment or to enact developmental programs. Evidence for the importance of each of these requirements can be found in the fact that much of the research of biochemistry and biophysics can be described as the investigation of how molecular conformations are linked to environmental conditions and function.

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Switches in macromolecular conformation often reveal a dependency on ligand binding in that the distribution of conformers changes in the presence of bound ligand. In this case, one may further inquire as to the *mechanism* that underlies this linkage: How does ligand binding produce a shift in conformation? For example, does the association of the ligand to a ground state conformation create new conformations that were not previously populated or does the ligand select from pre-existing conformations excited by the thermal bath. That is, whether the preferred conformation in a ligand-bound complex is *induced by* ligand association or is *selected from* a pre-existing ensemble. These two extreme possibilities are known as induced fit and conformational selection respectively. The concept of induced fit was introduced by Daniel Koshland and was first used to describe the formation of the catalytically competent conformation of an enzyme active site upon ligand binding [1]. The concept of conformational selection was introduced by Monod, Wyman, and Changeux in their studies of allosteric interactions between subunits of hemoglobin [2]. These limiting cases can be restated as a kinetic problem of which event happens first. In conformational selection, conformational change is followed by ligand binding. In induced fit, ligand binding occurs concomitantly with or is followed by conformational change (Figure 1).

While distinguishing between conformational selection and induced fit is facile conceptually, the experimental determination of mechanism can be challenging. Historically, the question has been addressed experimentally through the quantification of relaxation kinetics under different molecular concentration regimes [3], via flux-based studies most commonly performed with NMR methodologies [4], or by structurally characterizing intermediate states along either of the two pathways (e.g. the observation of an apo-protein conformation similar to that of the bound complex or a ligand bound complex that has a structure not present in the unbound ensemble) [5]. Recently, a renaissance in this field is rediscovering and redefining how observables acquired from different experimental techniques may be used to eliminate or support either conformational selection or induced fit mechanisms [4,6–9]. While these concepts are regularly discussed for binding reactions that lead to enzymatic catalysis or signaling, they are not often used to describe the mechanisms of a unique class of proteins for which ligand-coupled cycles of conformational change produce directed motion.

Motor molecules are defined by their ability to convert chemical energy (i.e. the hydrolysis of ATP) into mechanical work (i.e. the movement of RNA polymerase along the DNA during transcription). In the field of molecular motors, the concepts of *Brownian ratchet* and *power stroke* mechanisms are routinely used to divide motors into two classes [10–13]. In the more specific sub-field of nucleic acid helicase motors, the additional concepts of *active* and *passive* helicases are used to distinguish between different modes of nucleic acid unwinding [14,15]. In each case, dual classifications present limiting mechanistic frameworks. While molecular motor conformations do not transduce information in the sense of a signaling pathway, their function is dependent on links between conformational states and ligand states (i.e. binding, hydrolysis, and dissociation). Therefore, these motor classifications should have mappings onto the concepts of conformational selection and induced fit. Here, we directly map the classes of molecular motor function onto the fundamental mechanisms of ligand binding to highlight the capacity of conformational selection and induced fit to broadly describe molecular mechanisms.

Thermally activated molecular transitions in covalent and non-covalent reactions

On the length and energy scales relevant for biological molecules, the energy contained in the thermal bath is sufficient to activate transitions between states. The probability of these transitions is inversely related to the highest free energy state (i.e. the transition state) on the lowest free-energy path connecting two states [16,17]. In general, the diffusion of a macromolecule on a multi-dimensional energy landscape determines which conformational states are populated under given conditions. States that are connected by relatively low energy barriers will be populated while those cordoned off by high barriers will be less accessible.

The chemical reactions performed by biological enzymes are subject to high energy barriers that largely prevents the spontaneous reaction from occurring in solution and leads to a *requirement* for enzymatic catalysis [18]. The active sites of these enzymes provide a micro-environment in which activation barriers are reduced to levels in which thermal energy is now sufficient to produce appreciable flux through the transition state.

For non-covalent reactions, such as protein conformational changes, the situation is different. Whereas enzymes specifically stabilize the transition state of a reaction to increase reaction flux [18], ligand binding can theoretically affect the relative stabilities of two protein conformations as well as the energy of the transition state barriers between different conformations. Thus, there are multiple mechanisms through which ligand binding may modulate the energy landscape. In general, there will be protein conformations that are readily accessible via thermal energy in the absence of ligand and there may be another set of conformations that are only accessible when energy barriers are lowered (i.e. catalysis) by the presence of other factors. In one mechanism, a ligand may bind to an already accessible protein conformation *selectively* and reduce its free energy. (Figure 1A). Alternatively, in analogy with an enzyme-substrate system, the ligand may associate to a readily accessible conformation and *induce* the transition to an alternative conformer by lowering the activation barrier to that conformation. In this case, one may say that the ligand has catalyzed the formation of the new conformation. Thus, ligand binding may stabilize and catalyze the formation of specific conformations.

Conformational selection and induced fit

Part of the mechanistic dissection of macromolecular processes is a delineation of the order of events that lead to an activity. As described above for binding reactions, at least two paths exist (Figure 1). In a *conformational selection* mechanism, a conformational change (i.e. $A \rightarrow A^*$) occurs prior to the binding of a ligand ($A^* \rightarrow A^*L$). This pathway or mechanism describes the binding to, and stabilization of, a preferred conformation (i.e. A^*). In an *induced fit* mechanism, the events are reversed and binding of the ligand ($A \rightarrow AL$) occurs first and catalyzes the formation of A^*L ($AL \rightarrow A^*L$).

Importantly, the mechanism of binding need not be one or the other exclusively. In the most general mechanism, both conformational selection and induced fit pathways are viable and

the preference for one of the other will depend on the underlying energy landscapes and thus the values of the rate constants connecting the four states. This combination of the two paths in parallel creates a thermodynamic cycle subject to detailed balance in which the two pathways compete with one another. In this case, the flux of molecules through each path is controlled by the ligand concentration such that at lower concentrations of ligand, the conformational selection pathway dominates the flux and at higher concentrations of ligand, the induced fit pathway dominates [Hammes:2009im; 9].

To gain experimental insight into the paths utilized by a system of interest, kinetic information is required. By studying how observed relaxation rates depend on ligand concentration one may gain evidence for the existence of one or both mechanisms [9]. Examples of this manner of investigation include studies of trypsin [19], the interplay of the transcriptional regulator CarD with RNAP initiation complexes [20], and the binding of ssDNA to single-stranded binding protein [21]. In addition, measurements of equilibrium flux via dynamic NMR-based methods which allow for the quantitation of both rates and conformational populations provides a unique experimental avenue for testing mechanistic models [4]. Perhaps the most powerful experimental technique for addressing questions of conformational selection and induced fit are single-molecule in nature. These techniques allow for straightforward direct measurements of the effect of a ligand on the dwell-times of different conformations [22]. For example, using single-molecule fluorescence-based assays, magnesium was shown to shift the mechanism of the preQ₁ riboswitch aptamer from induced fit to conformational selection [23].

Brownian ratchet and power stroke motor mechanisms

A molecular motor converts the energy stored in chemical bonds into directional motion and mechanical work. In principle, this may occur via two limiting mechanisms. In one form of *Brownian ratchet* mechanism, the motor experiences thermally activated displacements along its track [13,24–27]. Directionality is established by an NTP-hydrolysis cycle that preferentially takes place in forward displaced motor positions. For example, a motor may experience spontaneous isomerizations between position N and position N+1 along a track in the absence of NTP. If the next NTP cycle can only occur in the post-translocated state (i.e. position N+1), ATP hydrolysis will result in directional motion. In contrast, in a *power stroke* mechanism, motor displacement is directly induced by the NTP chemical cycle [11,28,29]. Here, the motor is unable to reach position N+1 via thermal activation alone. Instead NTP binding in position N leads to events (i.e. hydrolysis and dissociation of products) that result in the arrival of the motor at position N+1.

Perhaps the best examples of a Brownian ratchet can be found in the translocation of multi-subunit cellular RNA polymerases [25]. This motor templates the formation of an RNA transcript from a DNA strand which also serves as the track for translocation. Evidence from DNA foot-printing experiments directly suggests that in the absence of NTP-binding, the polymerase experiences a thermally excited equilibrium between pre-translocated and post-translocated states [30,31]. Further backwards motion past the pre-translocated state is possible and can be stimulated by a hindering applied force, but is slowed by the energetics imposed by protein structural elements and hairpin structures that form in the transcribed

strand [25,32–34]. Further forwards motion past the post-translocated state is possible, but is slowed considerably by the energetic penalty stemming from the shortening of the RNA-DNA hybrid [35,36]. Only the post-translocated state is capable of binding NTP in the proper orientation in the active site for hydrolysis and condensation onto the growing RNA chain. After a complete NTP hydrolysis cycle, the motor has moved a single base pair forward and now experiences an equilibrium between N+1 and N+2 positions. Thus, one may say that NTP binding *selects* the post-translocated state suggesting a link between ratchet models and conformational selection mechanisms.

In contrast, both T7 RNAP [37,38], myosins [39,40], and kinesins [41] are thought to use a conformational change directly coupled to different nucleotide binding states (e.g. pyrophosphate or ADP release) to produce motion and force. Here, there are no pre-NTP-binding conformational equilibria. Instead, the incoming nucleotide binds to the active site first, hydrolysis occurs, and upon the release of pyrophosphate, a new conformation is produced: namely, the forward translocated position of the enzyme on the DNA template. Here, one may say that pyrophosphate dissociation *induces* a previously unpopulated conformation (i.e. the N+1 state) suggesting a link between power stroke models and induced fit mechanisms.

A mapping between the concepts of motor mechanisms and binding mechanisms

Motor proteins have at least two separate activities. They perform NTP hydrolysis and they move along the track. If we ignore the translocation activity and focus simply on NTP hydrolysis cycle, the binding of nucleotide may occur either by conformational selection or induced fit in the traditional way. However, if instead of considering the conformations that underlie the mechanism of NTP binding, we consider pre- and post-translocated positions along the track as the pertinent conformations, we can use the language of conformational selection and induced fit to describe the mechanism of translocation itself. Specifically, we can map the Brownian ratchet mechanism onto conformational selection and power stroke mechanisms onto induced fit (Figure 2).

The mapping assigns the pre-binding conformational equilibrium ($A \leftrightarrow A^*$) to the thermally activated translocations of a Brownian ratchet ($N \leftrightarrow N+1$). Instead of selecting a pre-formed protein conformation, the ligand selects a pre-formed translocation state. In contrast, if no pre-binding equilibrium exists, the binding of NTPs occurs in state N and activates the forward movement to N+1 analogously to the induction of A^* in the bound state of an induced fit mechanism.

As mentioned above in the context of ligand binding, the concepts of conformational selection and induced fit are limiting cases. In general, both mechanisms may exist and operate in parallel depending on environmental conditions such as ligand concentration. In this case, a thermodynamic cycle is formed by combining the two pathways [4,9]. By analogy, this raises the possibility that the stepping of molecular motors need not behave via a single mechanism. Depending on the initial equilibrium between states N and N+1 and the affinity of NTP to both states, mixed mechanisms may exist where the dominant mechanism

depends, for example, on NTP concentration. We stress that the parallel mechanism model is distinct from cases where a certain *fraction* of a step is attributed to a stroke-like induced conformational change and the remaining fraction is attributed to a ratchet-like selection of extended conformations as in the case of myosin 5 [42,43] and kinesin [44]. This parallel mechanism is also different from intermediate mechanisms defined by the position of the transition state along the a one-dimensional reaction coordinate [10]. Instead, the analogy described here suggests that for each occurrence of a step, motion may take place at different times relative to the chemical/conformational transitions; especially if the rates of these two processes happen to be similar.

Active and passive nucleic acid duplex unwinding

Helicases are a special class of motors that move along nucleic acids catalyzing the separation of duplex nucleic acids into the single strand intermediates often required in nucleic acid metabolism [45,46]. While not all ssDNA translocases are helicases (e.g. UvrD, PcrA, and Rep monomers [47–49]) and not all helicases require canonical protein translocation (e.g. RecBCD [50]), for helicases that translocate on ssDNA and unwind dsDNA, two limiting cases were proposed to provide a general framework for understanding the mechanism of strand separation [14]. Specifically, *active* helicases directly destabilize the downstream base-pair to catalyze the formation of ssDNA template prior to translocation while *passive* helicases do not. As this distinction involves a coupling between the conformational state of the nucleic acid duplex and the position of the helicase, one should be able to apply the concepts of induced fit and conformational selection to the unwinding process. Before continuing, we stress that helicases possess both translocase and DNA unwinding activities. The underlying mechanism governing translocation need not be related to the unwinding mechanism: both passive and active helicases may translocate along single-stranded templates with power stroke (induced fit) or ratchet (conformational selection) mechanisms. Therefore, a full description of a helicase motor should include both aspects of its activity (i.e. an active ratchet).

Helicases that use a passive mechanism interact with ssDNA and rely on the conformational fluctuations in the nucleic acid duplex near the duplex termini. The duplex termini undergo thermally induced fraying, transiently exposing more single-stranded nucleic acid. The helicase then translocates onto this newly available template, prevents reannealing, and results in a stably unwound duplex. Since passive helicases select out the single-stranded conformation (as opposed to the double-stranded conformation), one may say that they utilize a conformational selection mechanism. In contrast, helicases that use an active mechanism interact with dsDNA and increase the rate of DNA unwinding, consistent with an induced fit mechanism (Figure 3).

For the experimental assessment of whether an unwinding mechanism is active or passive, one may use the ratio of the unwinding rate and single-stranded translocation rate [15]. The theory behind this comparison is that a passive helicase will be slowed by the need to wait for spontaneous duplex unwinding while an active helicase may unwind at rates close to or above its ssDNA translocation rate. In this context, a helicase is defined as active if its unwinding rate is greater than or equal to 0.25 time its ssDNA translocation rate and passive

if the ratio is less than 0.25 [15]. This cutoff was chosen based on the predicted ratio for a helicase that reduces the free energy of base-pairing by half while taking single base-pair steps with no slipping [51]. Interestingly, the cutoff seems convenient based on the experimental distribution of observed ratios in that most helicases exhibit ratios significantly above or below 0.25. As such helicases have been classified as either active (Dda [52], RecQ [15], UvrD dimers [15], RecBC [53], and RecBCD [54]) or passive (T4 gp41 and Rep B) [15].

Another criterion used to differentiate active and passive mechanism involves the quantification of sequence-dependent effects on the unwinding rate. As passive helicases must wait for spontaneous base-pair fraying, one may quantitatively predict how much slower the enzyme should unwind GC-rich duplex. Assuming a rapid equilibrium between base-paired and unpaired states, the observation of a relative lack of sequence-dependence can then be taken as evidence of an active mechanism [15]. Lastly, single-molecule manipulation experiments may be used to measure the force-dependence of hairpin unwinding to judge helicase mechanisms [15]. In these experiments, a force-dependent unwinding reaction suggests a passive mechanism while a constant unwinding rate as a function of force suggests an active one.

Within the four-state cycle, active mechanisms may be represented as an induced-fit-like path wherein the presence of the helicase (binding) stimulates unwinding (conformational change) while passive mechanisms are accounted for by the conformational selection path wherein the ssDNA state is selected via the act of translocation (Figure 3). This mapping is consistent with the original definitions, but points out that in the most general case, and in analogy to ligand binding, both selective (passive) and inductive (active) paths may exist in parallel. If so, the concepts of pathway flux may be useful to quantitate the relative use of each mechanism [4]. For example, given a kinetic mechanism for unwinding that includes rates for DNA fluctuations in the presence and absence of the helicase, what is the fraction of helicase steps that occur via the capture of ssDNA made available by breathing compared to the fraction of steps occurring via helicase-induced (i.e. accelerated) unwinding. Furthermore, as is the case in ligand-concentration dependent mechanisms of conformational change, a certain mechanism may dominate under different conditions. For example, a passive (conformational selection) mechanism may be favored at low NTP concentrations (slow translocation) while the active pathway (induced fit) would be preferred at high NTP concentrations (fast translocation).

Although not considered in detail here, it would be interesting to see if a mapping exists between the continuous scale of the ratio of the unwinding and translocation rates and the fractional flux through the two sides of the four-state model. Here ratios above 0.75 may represent a “mostly active” helicase that always takes the induced fit pathway of the model. A ratio of 0.5 may indicate a “partially active” helicase that exhibits equal flux through the two mechanisms. Lastly, ratios less than 0.25 may indicate a “mostly passive” helicase where the fractional flux is dominated by the conformational selection pathway.

Summary

We have described an explicit mapping of mechanisms of molecular motors and nucleic acid helicases onto the more general concepts of conformational selection and induced fit. As the function of all macromolecules ultimately involves the coupling between binding events and conformational changes, this kind of mapping is likely always possible. Aside from the academic exercise of unifying the vocabulary used in different classes of macromolecules, the mapping points out the potential coexistence of mechanistic paths which are typically considered all-or-none. Whether these kinds of parallel mechanistic paths are available to molecular motors in the same way they are available to ligand binding systems remains to be seen. If they are, one further expects, in analogy to ligand binding, that the favored pathway may depend on the conditions under which the motor is functioning including NTP concentration, force, or the presence of other protein factors.

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Bibliography

1. Koshland DE. Application of a Theory of Enzyme Specificity to Protein Synthesis. *Proc Natl Acad Sci USA*. 1958; 44:98–104. [PubMed: 16590179]
2. MONOD J, WYMAN J, CHANGEUX JP. ON THE NATURE OF ALLOSTERIC TRANSITIONS: A PLAUSIBLE MODEL. *J Mol Biol*. 1965; 12:88–118. [PubMed: 14343300]
3. Bernasconi, CF. Relaxation Kinetics. Academic Press; San Diego: 1976.
4. Hammes GG, Chang YC, Oas TG. Conformational selection or induced fit: a flux description of reaction mechanism. *Proceedings of the National Academy of Sciences*. 2009; 106:13737–13741. DOI: 10.1073/pnas.0907195106
5. Chakrabarti KS, Agafonov RV, Pontiggia F, Otten R, Higgins MK, Schertler GFX, et al. Conformational Selection in a Protein-Protein Interaction Revealed by Dynamic Pathway Analysis. *Cell Reports*. 2016; 14:32–42. DOI: 10.1016/j.celrep.2015.12.010 [PubMed: 26725117]
6. Galletto R, Jezewska MJ, Bujalowski W. Kinetics of allosteric conformational transition of a macromolecule prior to ligand binding: analysis of stopped-flow kinetic experiments. *Cell Biochem Biophys*. 2005; 42:121–144. DOI: 10.1385/CBB:42:2:121 [PubMed: 15858229]
7. Vogt AD, Di Cera E. Conformational selection or induced fit? A critical appraisal of the kinetic mechanism. *Biochemistry*. 2012; 51:5894–5902. DOI: 10.1021/bi3006913 [PubMed: 22775458]
8. Gianni S, Dogan J, Jemth P. Distinguishing induced fit from conformational selection. *Biophys Chem*. 2014; 189:33–39. DOI: 10.1016/j.bpc.2014.03.003 [PubMed: 24747333]
9. Galburt EA, Rammohan J. A kinetic signature for parallel pathways: conformational selection and induced fit. Links and disconnects between observed relaxation rates and *Biochemistry*. 2016; doi: 10.1021/acs.biochem.6b00914
10. Wagoner JA, Dill KA. Molecular Motors: Power Strokes Outperform Brownian Ratchets. *The Journal of Physical Chemistry B*. 2016; 120:6327–6336. DOI: 10.1021/acs.jpcc.6b02776 [PubMed: 27136319]
11. Howard J. Protein power strokes. *Current Biology*. 2006; 16:R517–9. DOI: 10.1016/j.cub.2006.06.045 [PubMed: 16860722]
12. Oster, G., Wang, H. How Protein Motors Convert Chemical Energy into Mechanical Work. Wiley-VCH Verlag GmbH & Co; KGaA, Weinheim, FRG: 2003.
13. Wang H, Oster G. Ratchets, power strokes, and molecular motors. *Applied Physics a: Materials Science & Processing*. 2002; 75:315–323.

14. Lohman TM. Escherichia coli DNA helicases: mechanisms of DNA unwinding. *Mol Microbiol.* 1992; 6:5–14. [PubMed: 1310794]
15. Manosas M, Xi XG, Bensimon D, Croquette V. Active and passive mechanisms of helicases. *Nucleic Acids Res.* 2010; 38:5518–5526. DOI: 10.1093/nar/gkq273 [PubMed: 20423906]
16. Jones WW, Eyring H. The absolute rate of reactions in condensed phases. *The Journal of Chemical Physics.* 1935; doi: 10.1063/1.1749713
17. Evans MG, Polanyi M. Some applications of the transition state method to the calculation of reaction velocities, especially in solution. *Trans Faraday Soc.* 1935; doi: 10.1039/TF9353100875
18. Lienhard GE. Enzymatic Catalysis and Transition-State Theory. *Science.* 1973; 180:149–154. DOI: 10.1126/science.180.4082.149 [PubMed: 4632837]
19. Pozzi N, Vogt AD, Gohara DW, Di Cera E. Conformational selection in trypsin-like proteases. *Current Opinion in Structural Biology.* 2012; 22:421–431. DOI: 10.1016/j.sbi.2012.05.006 [PubMed: 22664096]
20. Rammohan J, Ruiz-Manzano A, Garner AL, Stallings CL, Galburt EA. CarD stabilizes mycobacterial open complexes via a two-tiered kinetic mechanism. *Nucleic Acids Res.* 2015; doi: 10.1093/nar/gkv078
21. Kozlov AG, Lohman TM. Kinetic mechanism of direct transfer of Escherichia coli SSB tetramers between single-stranded DNA molecules. *Biochemistry.* 2002; doi: 10.1021/bi020361m
22. Hatzakis NS. Single molecule insights on conformational selection and induced fit mechanism. *Biophys Chem.* 2014; 186:46–54. DOI: 10.1016/j.bpc.2013.11.003 [PubMed: 24342874]
23. Suddala KC, Wang J, Hou Q, Walter NG. Mg²⁺ Shifts Ligand-Mediated Folding of a Riboswitch from Induced-Fit to Conformational Selection. *Journal of the American* 2015; 137:14075–14083. DOI: 10.1021/jacs.5b09740
24. Morin JA, Cao FJ, Lázaro JM, Arias-Gonzalez JR, Valpuesta JM, Carrascosa JL, et al. Mechanochemical kinetics of DNA replication: identification of the translocation step of a replicative DNA polymerase. *Nucleic Acids Res.* 2015; 43:3643–3652. DOI: 10.1093/nar/gkv204 [PubMed: 25800740]
25. Dangkulwanich M, Ishibashi T, Liu S, Kireeva ML, Lubkowska L, Kashlev M, et al. Complete dissection of transcription elongation reveals slow translocation of RNA polymerase II in a linear ratchet mechanism. *Elife.* 2013; 2:e00971.doi: 10.7554/eLife.00971 [PubMed: 24066225]
26. Córdova NJ, Ermentrout B, Oster GFB. Dynamics of single-motor molecules: the thermal ratchet model. *Proc Natl Acad Sci USA.* 1992; 89:339–343. [PubMed: 1530889]
27. HUXLEY AF. Muscle structure and theories of contraction. *Prog Biophys Biophys Chem.* 1957; 7:255–318. [PubMed: 13485191]
28. Craig EM, Linke H. Mechanochemical model for myosin V. *Proceedings of the National Academy of Sciences.* 2009; 106:18261–18266. DOI: 10.1073/pnas.0908192106
29. De La Cruz EM, Ostap EM. Relating biochemistry and function in the myosin superfamily. *Current Opinion in Cell Biology.* 2004; 16:61–67. DOI: 10.1016/j.ceb.2003.11.011 [PubMed: 15037306]
30. Kireeva M, Kashlev M. Mechanism of sequence-specific pausing of bacterial RNA polymerase. *Proc Natl Acad Sci USA.* 2009; doi: 10.1073/pnas.0900407106
31. Bar-Nahum G, Epshtein V, Ruckenstein AE, Rafikov R, Mustaev A, Nudler E. A ratchet mechanism of transcription elongation and its control. *Cell.* 2005; 120:183–193. DOI: 10.1016/j.cell.2004.11.045 [PubMed: 15680325]
32. Maoiléidigh DO, Tadiogola VR, Nudler E, Ruckenstein AE. A unified model of transcription elongation: what have we learned from single-molecule experiments? *Biophysical Journal.* 2011; 100:1157–1166. DOI: 10.1016/j.bpj.2010.12.3734 [PubMed: 21354388]
33. Galburt EA, Grill SW, Wiedmann A, Lubkowska L, Choy J, Nogales E, et al. Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner. *Nature.* 2007; 446:820–823. DOI: 10.1038/nature05701 [PubMed: 17361130]
34. Komissarova N, Kashlev M. Transcriptional arrest: Escherichia coli RNA polymerase translocates backward, leaving the 3' end of the RNA intact and extruded. *Proc Natl Acad Sci USA.* 1997; 94:1755–1760. [PubMed: 9050851]

35. Nedialkov YA, Nudler E, Burton ZF. RNA polymerase stalls in a post-translocated register and can hyper-translocate. *Transcr.* 2012; 3doi: 10.4161/trans.22037
36. Larson MH, Greenleaf WJ, Landick R, Block SM. Applied force reveals mechanistic and energetic details of transcription termination. *Cell.* 2008; 132:971–982. DOI: 10.1016/j.cell.2008.01.027 [PubMed: 18358810]
37. Yin YW, Steitz TA. The structural mechanism of translocation and helicase activity in T7 RNA polymerase. *Cell.* 2004; 116:393–404. [PubMed: 15016374]
38. Steitz TA. Visualizing polynucleotide polymerase machines at work. *Embo J.* 2006; 25:3458–3468. DOI: 10.1038/sj.emboj.7601211 [PubMed: 16900098]
39. Huxley HE. The mechanism of muscular contraction. *Science.* 1969
40. Spudich JA. The myosin swinging cross-bridge model. *Nat Rev Mol Cell Biol.* 2001; 2:387–392. DOI: 10.1038/35073086 [PubMed: 11331913]
41. Hancock WO. The Kinesin-1 Chemomechanical Cycle: Stepping Toward a Consensus. *Biophysical Journal.* 2016; 110:1216–1225. DOI: 10.1016/j.bpj.2016.02.025 [PubMed: 27028632]
42. Andrecka J, Ortega Arroyo J, Takagi Y, de Wit G, Fineberg A, MacKinnon L, et al. Structural dynamics of myosin 5 during processive motion revealed by interferometric scattering microscopy. *Elife.* 2015; 4:e05413.doi: 10.7554/eLife.05413
43. Veigel C, Wang F, Bartoo ML, Sellers JR, Molloy JE. The gated gait of the processive molecular motor, myosin V. *Nat Cell Biol.* 2002; 4:59–65. DOI: 10.1038/ncb732 [PubMed: 11740494]
44. Isojima H, Iino R, Niitani Y, Noji H, Tomishige M. Direct observation of intermediate states during the stepping motion of kinesin-1. *Nat Chem Biol.* 2016; 12:290–297. DOI: 10.1038/nchembio.2028 [PubMed: 26928936]
45. Lohman TM, Tomko EJ, Wu CG. Non-hexameric DNA helicases and translocases: mechanisms and regulation. *Nat Rev Mol Cell Biol.* 2008; 9:391–401. DOI: 10.1038/nrm2394 [PubMed: 18414490]
46. Pyle AM. Translocation and unwinding mechanisms of RNA and DNA helicases. *Annual Review of Biophysics.* 2008; 37:317–336. DOI: 10.1146/annurev.biophys.37.032807.125908
47. Maluf NK, Fischer CJ, Lohman TM. A Dimer of *Escherichia coli* UvrD is the active form of the helicase in vitro. *J Mol Biol.* 2003; 325:913–935. [PubMed: 12527299]
48. Niedziela-Majka A, Chesnik MA, Tomko EJ, Lohman TM. *Bacillus stearothermophilus* PcrA monomer is a single-stranded DNA translocase but not a processive helicase in vitro. *J Biol Chem.* 2007; 282:27076–27085. DOI: 10.1074/jbc.M704399200 [PubMed: 17631491]
49. Brendza KM, Cheng W, Fischer CJ, Chesnik MA, Niedziela-Majka A, Lohman TM. Autoinhibition of *Escherichia coli* Rep monomer helicase activity by its 2B subdomain. *Proc Natl Acad Sci USA.* 2005; 102:10076–10081. DOI: 10.1073/pnas.0502886102 [PubMed: 16009938]
50. Simon MJ, Sokoloski JE, Hao L, Weiland E, Lohman TM. Processive DNA Unwinding by RecBCD Helicase in the Absence of Canonical Motor Translocation. *J Mol Biol.* 2016; 428:2997–3012. DOI: 10.1016/j.jmb.2016.07.002 [PubMed: 27422010]
51. Betterton MD, Jülicher F. Opening of nucleic-acid double strands by helicases: active versus passive opening. *Physical Review E, Statistical, Nonlinear, and Soft Matter Physics.* 2005; 71:011904.
52. Byrd AK, Matlock DL, Bagchi D, Aarattuthodiyil S, Harrison D, Croquette V, et al. Dda helicase tightly couples translocation on single-stranded DNA to unwinding of duplex DNA: Dda is an optimally active helicase. *J Mol Biol.* 2012; 420:141–154. DOI: 10.1016/j.jmb.2012.04.007 [PubMed: 22504228]
53. Wu CG, Xie F, Lohman TM. The primary and secondary translocase activities within *E. coli* RecBC helicase are tightly coupled to ATP hydrolysis by the RecB motor. *J Mol Biol.* 2012; 423:303–314. DOI: 10.1016/j.jmb.2012.07.009 [PubMed: 22820092]
54. Xie F, Wu CG, Weiland E, Lohman TM. Asymmetric Regulation of Bipolar Single-stranded DNA Translocation by the Two Motors within *Escherichia coli* RecBCD Helicase. *J Biol Chem.* 2013; 288:1055–1064. DOI: 10.1074/jbc.M112.423384 [PubMed: 23192341]

Highlights

- The mechanisms of ligand binding can be mapped onto the mechanisms of molecular translocation and helicase unwinding.
- Conformational selection can be used to describe Brownian ratchet mechanisms and passive helicase mechanisms.
- Induced fit can be used to describe power stroke mechanisms and active helicase mechanisms.

Figure 1. Parallel pathways of ligand binding: conformational selection and induced fit

The conformational change of a macromolecule ($A \leftrightarrow A^*$) is linked to ligand binding ($A \leftrightarrow AL$ and $A^* \leftrightarrow A^*L$). An additional transition is shown as a gray diagonal and is meant to represent a single reaction coordinate in which ligand binding must occur concomitantly with a change in conformation. Conformational selection is represented by the $A \rightarrow A^* \rightarrow A^*L$ pathway. In the pure conformational selection model, there is no AL state and ligand simply reduces the free energy of the A^* conformation. Induced fit is represented by the $A \rightarrow AL \rightarrow A^*L$ pathway. In the pure induced fit model, there is no A^* state and ligand binding to A results in the destabilization of A and/or the stabilization of the transition state on the path to A^*L . The free energy diagrams in the absence (black) and presence (red) of ligand are shown to illustrate each individual mechanism.

Figure 2. Pathways of molecular motor translocation: Brownian ratchet and power stroke

In analogy to Figure 1, the two motor mechanisms are represented as parallel paths in a four-state cycle. A motor (triangle) exists in either a pre-translocated state (N) or post-translocated state (N+1). These states are analogous to conformations of the macromolecule (i.e. A and A*) in Figure 1. ATP binding is analogous to ligand binding and is represented by the dark gray pyrimidine rings (NL and (N+1)L). A Brownian ratchet follows the path: $N \rightarrow N+1 \rightarrow (N+1)L$, while a power stroke follows the path: $N \rightarrow NL \rightarrow (N+1)L$. The concerted diagonal path represents a mechanism where ATP binding itself is inseparable from the motion from N to N+1.

Figure 3. Pathways of helicase mechanisms: active and passive

In analogy with Figure 1, a four-state cycle is used to illustrate the pathways of nucleic acid unwinding. A helicase (triangle) is shown in both a pre- and post-translocation state (N and N+1 respectively) and the state of the nucleic acid is represented as either paired or unpaired. A passive mechanism is represented by the pathway: N(paired) $\rightarrow$ N(unpaired) $\rightarrow$ N+1(unpaired). An active mechanism is represented by the pathway: N(paired) $\rightarrow$ N+1(paired) $\rightarrow$ N+1(unpaired). The diagonal transition represents a concerted mechanism where the motion of the helicase is inseparable from the state of the nucleic acid.

Figure 4.