An Arginine Finger Regulates the Sequential Action of Asymmetrical Hexameric ATPase in the Double-Stranded DNA Translocation Motor

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Biological motors are ubiquitous in living systems. Currently, how the motor components coordinate the unidirectional motion is elusive in most cases. Here, we report that the sequential action of the ATPase ring in the DNA packaging motor of bacteriophage ϕ29 is regulated by an arginine finger that extends from one ATPase subunit to the adjacent unit to promote noncovalent dimer formation. Mutation of the arginine finger resulted in the interruption of ATPase oligomerization, ATP binding/hydrolysis, and DNA translocation. Dimer formation reappeared when arginine mutants were mixed with other ATPase subunits that can offer the arginine to promote their interaction. Ultracentrifugation and virion assembly assays indicated that the ATPase was presenting as monomers and dimer mixtures. The isolated dimer alone was inactive in DNA translocation, but the addition of monomer could restore the activity, suggesting that the hexameric ATPase ring contained both dimer and monomers. Moreover, ATP binding or hydrolysis resulted in conformation and entropy changes of the ATPase with high or low DNA affinity. Taking these observations together, we concluded that the arginine finger regulates sequential action of the motor ATPase subunit by promoting the formation of the dimer inside the hexamer. The finding of asymmetrical hexameric organization is supported by structural evidence of many other ATPase systems showing the presence of one noncovalent dimer and four monomer subunits. All of these provide clues for why the asymmetrical hexameric ATPase gp16 of ϕ29 was previously reported as a penta-meric configuration by cryo-electron microscopy (cryo-EM) since the contact by the arginine finger renders two adjacent ATPase subunits closer than other subunits. Thus, the asymmetrical hexamer would appear as a pentamer by cryo-EM, a technology that acquires the average of many images.

The ASCE (additional strand catalytic E) superfamily, including the AAA+ (ATPases associated with various cellular activities) superfamily, is a broad class of proteins among which are several nano-biological molecular motors, or nano-motors. Nanomotors facilitate a wide range of functions (1–5), many of which are involved in DNA replication, repair, recombination, chromosome segregation, protein degradation, membrane fusion, microtubule severing, peroxisome biogenesis, gene regulation, DNA/RNA transportation, bacterial division, and many other processes (6–10).

Despite their functional diversity, ring-shaped P-loop NTPases share two conserved modules with Walker A and Walker B motifs (11), exerting their activity through the ATP-dependent remodeling for translocation of macromolecules. The Walker A motif is responsible for ATP binding, while the Walker B is responsible for ATP hydrolysis (12, 13). This energy transition can result in either a gain or loss of substrate affinity, therefore generating a mechanical force exerted on the substrate to produce a mechanical motion. This motion will lead to a contact with or a separation from the substrate molecule, resulting in molecule folding/unfolding, complex assembly/disassembly, or translocation of DNA, RNA, protein, or other substrates (2–4, 14).

Both the revolving mechanism and the sequential reaction mechanism adapted by biological systems through evolution are efficient methods of unidirectional translocation of lengthy double-stranded DNA (dsDNA) genomes, with minimum consumption of energy and without tangling or coiling (15–19). However, both the revolving mechanism and/or the sequential reaction mechanism for DNA translocation require signal communication from one component to another in the motor complex. It has been reported that ASCE ATPases contain one arginine finger motif along with the Walker A and Walker B motifs (20–30). In the active ATPase ring, the arginine residue is located in proximity to the gamma-phosphate of the bound ATP in the adjacent ATPase subunit (22, 25–27). An arginine finger has been confirmed to associate with the formation of the ATP binding pocket (24, 27–30). To understand how the motor component coordinates its motion necessary for unidirectional DNA translocation activity and sequential action of the ATPase ring, we analyzed the role of the arginine finger motif in the ATPase core of the dsDNA translocation motor. It was found that this motif controls the formation of the coordinating dimer inside the hexamer of the motor ATPase. The dimer, however, is not static but shifts and alters with time in a sequential manner, and this sequential reaction mechanism is regulated by the arginine finger.

MATERIALS AND METHODS

Cloning, mutagenesis, and protein purification. The engineering of enhanced green fluorescent protein (eGFP)-gp16 and the purification of the...
gp16 fusion protein have been reported previously (31). Construction of eGFP-gp16 mutants, including arginine finger mutant R146A, Walker A mutant G27D, and Walker B mutant E119A as well as mCherry-gp16 mutant R146A was accomplished by introducing mutations in the gp16 gene by Keyclone Technologies.

Glycerol gradient ultracentrifugation. Fifty microliters of eGFP-gp16 (500 μg/ml) was dropped on the top of 5 ml of linear 15 to 35% glycerol gradients in TMS buffer (50 mM Tris–HCl, pH 8.0, 100 mM NaCl, 10 mM MgCl₂). After centrifugation at 35,000 rpm in an SW55 rotor at 4°C for 22 h, the gradients were collected into 31 fractions from bottom to top and measured using a plate reader under 488-nm excitation wavelengths for GFP, mCherry, and Cy5, respectively.

The proposed mechanism of ATPase coordination with a series of conformational changes during DNA binding and ATP hydrolysis that are regulated by the arginine finger (RF, red).

**FIG 1** The proposed mechanism of ATPase coordination with a series of conformational changes during DNA binding and ATP hydrolysis that are regulated by the arginine finger (RF, red).
that are organized in a sequential manner and that this sequential mechanism is coordinated by the arginine finger (Fig. 1), in accordance with the supporting data described below.

**Identification of arginine finger motifs in gp16 ATPase.**

gp16 shares the common ATP binding domain typical of all ASCE family members, including AAA + proteins (2, 38). This domain contains very well conserved motifs responsible for ATP binding and ATP hydrolysis (12), which have been previously identified as Walker A (11) and Walker B motifs (17), respectively. However, detailed information about the arginine finger motif of gp16 has remained elusive. Sequence alignment was subsequently performed with similar ASCE family proteins to identify this motif (Fig. 2A). From the alignment, we identified the position of the arginine fingers (residue 146) localized after the position of the beta-4 strand, as seen in other ATPases, which correlates well with the known structural information and consensus sequences for this motif found in other proteins (27, 34–41) (Fig. 2A). The single mutant R146A gp16 was produced and examined for its ATPase activity. As expected, the arginine finger mutant was severely impaired in both ATP hydrolysis activity (Fig. 2B) and DNA binding in the presence of S-ATP (Fig. 2C), possibly due to the impaired affinity for S-ATP, which is similar to that of the Walker A mutant (17). In contrast, the Walker B mutants retained their binding affinity for ATPγS.
DNA in the presence of γ-S-ATP and were also sufficient in binding DNA in the presence of ATP, although they could not hydrolyze ATP (16, 17).

The arginine finger extends to the upstream adjacent ATPase subunit to serve as a bridge for the formation of a dimeric subcomplex and regulates the sequential action of the subunits in the hexameric ATPase ring. The arginine finger has been reported to have various functions, including a major role in subunit communications by pivoting upon ATP hydrolysis to trigger the conformational changes of the subunits of ATPase (23, 42–46). The formation of the dimeric complex of gp16 in the absence of ATP was demonstrated by different approaches: glycerol gradient ultracentrifugation (Fig. 3), electrophoretic mobility shift assay (EMSA) (Fig. 4A to C), size exclusion chromatography, and native gel electrophoresis (17). These assays were based on the previous finding that fusion of the GFP protein to the N terminus of gp16 did not interfere with activity of the ATPase gp16 in DNA packaging (31, 47, 48). It was found that mutation of the arginine finger abolished dimer formation within the ATPase (Fig. 3). Although the arginine mutants alone could not form dimers, interactions were observed when the arginine mutants were mixed with either the wild type or mutants that contained an intact arginine finger, which can provide an arginine residue for dimer formation (Fig. 3). The disruptive effect of the arginine finger mutation on assembly ability was also reflected in protein activity since it was observed that one single inactive subunit of an arginine finger mutant was able to completely inactivate the whole ATPase ring in an assembly inhibition assay (Fig. 4D and E). This supports the idea that in the ATPase ring, one adjacent wild-type ATPase provided an arginine finger to interact with the arginine mutant and that the lack of one arginine in the entire ring completely abolished the activity of the whole ring.

To get a better understanding of the structural role of the arginine finger, we modeled a gp16 hexameric ring using I-TASSER (33) and Phyre2 software (81). The gp16 sequence aligned well with the crystal structure of the hexameric FtsK DNA translocase of Escherichia coli (Fig. 5). Using this model, we observed that the position of the arginine finger of one subunit of gp16 extends to the active site of a neighboring subunit. The predicted structure showed that the arginine finger was part of the ATP binding pocket (Fig. 5). The structural model provides an explanation for the observed cooperative behavior in the hexameric ring of gp16. Not surprisingly given the importance in the formation of the active site, mutations in arginine fingers greatly impaired the ability of gp16 to...
bind to ATP, to hydrolyze ATP (Fig. 2B), to bind to DNA (Fig. 2C), and consequently to package the DNA (Fig. 4E).

Both dimer and monomer forms were present in gp16 hexamer. As demonstrated in the above sections, the arginine finger serves as a bridge between two independent subunits, thus forming a transient dimeric subunit. In wild-type gp16, it was observed that both dimer and monomer forms were present in solution, as revealed by glycerol gradient centrifugation experiments. The mo-
molecular masses relative to such fractions were confirmed by protein marker calibrations in the same assay (the 66-kDa bovine serum albumin [BSA] localized around fraction 23, the 140-kDa alcohol dehydrogenase localized around fraction 18, and the 200-kDa beta-amylase localized at around fraction 15). We thus proceeded to test the packaging activity of the different fractions of the gp16 ATPase recovered from the gradient. Interestingly, it has been observed that DNA packaging activity was retained with the fractions containing monomers, while fractions containing only dimers displayed no DNA packaging activity (Fig. 3C). These results agree with the finding that the addition of fresh gp16 monomer to the DNA packaging intermediates is required for reinitiating motor DNA packaging activity and the conversion of the intermediates into infectious viruses (49).

ATP binding resulted in the change of conformation and entropic landscape of gp16. ASCE family proteins undergo a cycle of conformational changes during ATP binding and hydrolysis with basically two major states: high or low affinity for the DNA substrate. In recent publications (16, 17, 32, 50), we proposed a similar model for gp16, in which binding to ATP exerted an effect on the conformational state of the protein that predisposed it to binding to DNA (high affinity). Conversely, ADP would promote another conformational state in which DNA binding is not favorable. This notion, together with the observation that the arginine finger has a role in regulating both the conformational state of gp16 and its interaction with the adjacent subunit, prompted us to question whether the effect of ATP binding on gp16 was able to modify not only the conformation of the DNA binding portion of the protein but also the structural characteristics of gp16 altogether. We thus tested if ATP binding was able to alter the shape of gp16 by partial proteolysis treatment and by intrinsic tryptophan fluorescence assay (Fig. 6A and B). Interestingly, both assays indicated a conformational change in the gp16-ATP complex. Moreover, as visible from the partial proteolysis test, protection from proteolysis is indicative of a larger population of gp16 with a constrained conformation before ATP binding.

An electrophoretic mobility shift assay was also employed to study the interaction between ATPase and dsDNA in the presence of γ-S-ATP, a nonhydrolysable ATP analog. Stronger binding of gp16 to dsDNA was observed when gp16 was incubated with γ-S-ATP (Fig. 6C), suggesting that the gp16/dsDNA complex is stabilized through addition of the nonhydrolysable ATP substrate. Hydrolysis of ATP transformed the ATPase into a second conformation with low affinity for dsDNA, thus pushing the dsDNA toward an adjacent ATPase subunit. Consequent to the first structural change, it was also observed that the binding of the ATP/gp16 complex to DNA resulted in ATP hydrolysis and also the passage to a second conformational change with a low-
DNA-affinity configuration (11, 16, 36, 51). This explains the finding in 1987 that the φ29 DNA packaging protein gp16 is a DNA-dependent ATPase (11). Such a state resulted in the release of dsDNA for its concomitant transfer to the adjacent subunit. The conclusion was also supported by the finding that the addition of normal ATP promoted the release of dsDNA from the gp16–γ-S-ATP–dsDNA complex (Fig. 6D).

**DISCUSSION**

φ29 genomic DNA packaging involves multiple components, including a 12-subunit connector, a hexameric prohead RNA (pRNA) ring (52, 53), and an ASCE ATPase gp16 hexamer. Great interest has arisen about this packaging system for its intriguing mechanism of action and for its useful applications in nanotechnology (54–60). It has been demonstrated that pRNA works as a point of connection between ATPase and the connector (61) and that the hexameric ATPase (16, 17) provides the pushing force for the packaging of genomic DNA, acting in coordination with the connector that acts as a one-way valve (50, 62, 63).

Nanobiomotors have been previously classified into two main categories: linear and rotational motors. These two categories have been clearly documented in single-molecule imaging and X-ray crystallography (64–69). Recently, it has been discovered that the φ29 dsDNA packaging motor uses a revolving mechanism that does not require rotation or coiling of the dsDNA (15–17, 70). The discovery of a revolving mechanism establishes a third class of biomotors. This finding resolves many puzzles and debates that have arisen throughout the history of painstaking studies on the motor (19, 70).

The ATPase hexameric ring exerts a force, pushing the dsDNA in a sequential manner to advance through the dodecamer channel, which acts as a one-way valve as reported for the phi29 motor (5, 19, 62, 70, 71). The interest in the sequential revolving mechanism lies in the fact that it elegantly integrates all the known functional and structural information about the packaging core (the ATPase, pRNA, and connector). Moreover, it offers solutions for many questions that arise from investigations of the DNA packaging phenomenon (i.e., coordination between energy consumption and DNA packaging and the ability to translocate a long strain of dsDNA without coiling or tangling). However, in order to have a sequential mechanism (which has been proposed for many proteins belonging to the AAA+/ASCE family) (30, 72, 73), several conditions need to be fulfilled. The most important are the following: (i) only one or two subunits of the oligomer are able to bind the substrate with the same affinity exhibited in the entire hexamer; (ii) both the ATPase activity and translocation activity need to demonstrate negative cooperativity when one subunit is able to bind ATP and is not able to hydrolyze the nucleotide (as in the case of the Walker B mutation); (iii) only the ATP-bound state of the protein is the unique state that efficiently binds to DNA.

We demonstrated that, indeed, this is the case for the φ29 motor ATPase (16, 32). One important question that then arises with the demonstration of the sequential mechanism is how the different subunits of the ATPase can sense the ATP binding/DNA binding state of others. In the present work, we addressed this question by identifying the arginine finger motifs of the ATPase gp16 by sequence alignment and proved that the arginine finger is an essential motif that participates in the formation of the ATP binding pocket by examining the behavior of gp16 mutants with the arginine finger removed. The gp16 mutated in the arginine finger was unable to package DNA, to hydrolyze ATP, or to bind to DNA. The profile of gp16 in ultracentrifugation indicated the presence of a mixture of monomeric and dimeric forms. Mutation of the arginine finger eliminated the capacity of gp16 to assemble into dimeric forms. Arginine finger motifs were thus shown to link two subunits to each other since the arginine motif of one subunit participates in the formation of the ATP binding site of the next subunit (Fig. 7). The importance of the dimer, moreover, is evident, as shown by the DNA packaging assay, in which a reconstituted hexamer of gp16 can efficiently pack DNA inside the procapsid only when ultracentrifuged fractions containing both dimeric and monomeric gp16 are mixed together (data not shown) (49).

In the sequential action of gp16, we proposed that one subunit

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**FIG 7** Asymmetrical structure of various ATPase hexamer models. Structure illustrations of V1-ATPase (adapted from reference 75 with permission of the publisher), TRIP13 (adapted and modified from reference 76 with permission of the publisher), ClpX (adapted and modified from reference 77 with permission of the publisher), and F1-ATPase (80) are shown as representatives of asymmetrical hexamers. PDB accession numbers are as follows: V1-ATPase, 3VRS; TRIP13, 4XGU; F1-ATPase, 1BMF; ClpX, 4B18. The EM reconstruction of the MCM helicase is deposited in the EMDDataBank under accession number EMD-5429.
of the hexamer binds to the DNA, subsequently hydrolyzing ATP to perform a translocation of a certain number of base pairs of DNA (11, 74). The DNA is then passed to the subsequent subunit, and the process is repeated. It is intriguing to notice that the position and function of ATPase offer the possibility of carrying the information of ATP/DNA binding from one ATPase subunit to another, with the cooperative behavior of gp16 seen in the case of other mutants (Walker B mutations) (16).

The sequential action mechanism of the φ29 ATPase is essential for optimal translocation efficiency. This mechanism integrates well with our overall model of the revolving motor and a “push through one-way valve” model (16, 50). Without coordination during the energy production of gp16, the cycles of binding and release of DNA would create futile cycles of ATP hydrolysis, inhibiting the unidirectional translocation process (15, 16, 32). Arginine fingers thus act as integrators of information for the entire process of DNA packaging. Years of evolution have created an efficient biomotor, one that can be used in the future for applications in nanotechnology (54–60).

Furthermore, the conclusion that coordination is provided by an asymmetrical hexamer was supported by structural computation, X-ray diffraction, and cryo-electron microscopy (cryo-EM) imaging of other hexameric ATPase systems (Fig. 7) (71, 75–80). These results could provide some clues as to why the asymmetrical hexameric ATPase of gp16 of φ29 and gp17 of T4 was previously interpreted as a pentameric configuration by cryo-EM. Since the two adjacent subunits of the ATPase could interact with each other and form a closer dimer configuration, this dimer will appear as a monomeric subunit different from the others, and the hexameric ring will be asymmetrical (Fig. 7).

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REFERENCES

1. Zhang X, Wigley DB. 2008. “The glutamate switch” provides a link between ATPase activity and ligand binding in AAA+ proteins. Nat Struct Mol Biol 15:1223–1227. http://dx.doi.org/10.1038/nsmb.1501.
2. Guo P, Noji H, Yengo CM, Zhao Z, Grainge I. 2016. Biological nanomotors with a revolution, linear, or rotation motion mechanism. Microbiol Mol Biol Rev 80:161–186. http://dx.doi.org/10.1128/MMBR.00056-15.
3. Snider J, Thibault G, Houry WA. 2008. The AAA+ superfamily of functionally diverse proteins. Genome Biol 9:216. http://dx.doi.org/10.1186/gb-2008-9-4-216.
4. Ammelburg M, Frickey T, Lupas AN. 2006. Classification of AAA+ proteins. J Struct Biol 156:62–113. http://dx.doi.org/10.1016/j.jsb.2006.05.002.
5. Chen C, Guo P. 1997. Sequential action of six virus-encoded DNA-packaging RNAs during phage φ29 genomic DNA translocation. J Virol 71:3864–3871.
6. Martin A, Baker TA, Sauer RT. 2005. Rebuilt AAA + motors reveal operating principles for ATP-fueled machines. Nature 437:1115–1120. http://dx.doi.org/10.1038/nature04031.
7. Guo P, Grainge I, Zhao Z, Vieweger M. 2014. Two classes of Nucleic acid translocation motors: rotation and revolution without rotation. Cell Bio-Sci 4:54. http://dx.doi.org/10.1186/2045-3701-4-54.
8. Soong RK, Bachand GD, Neves HP, Olkhowets AG, Craighead HG, Montemagnod CD. 2000. Powering an inorganic nanodevice with a biomolecular motor. Science 290:1555–1558. http://dx.doi.org/10.1126/science.290.5496.1555.
9. Hu B, Margolin W, Molineux IJ, Liu J. 2013. The bacteriophage φ7 virion undergoes extensive structural remodeling during infection. Science 339:576–579. http://dx.doi.org/10.1126/science.1231887.
10. Crozat E, Graingle I. 2010. FtsK DNA translocase: the fast motor that knows where it’s going. Chembiochem 11:2232–2243. http://dx.doi.org/10.1002/cbic.201000547.
11. Guo P, Peterson C, Anderson D. 1987. Prohead and DNA-gp3-dependent ATPase activity of the DNA packaging protein gp16 of bacteriophage φ29. J Mol Biol 197:229–236. http://dx.doi.org/10.1016/0022-2836(87)90121-5.
12. Walker JE, Saraste M, Gay NJ. 1982. E. coli F1-ATPase interacts with a membrane protein component of a proton channel. Nature 298:867–869. http://dx.doi.org/10.1038/298867a0.
13. Walker JE, Saraste M, Runswick MJ, Gay NJ. 1982. Distantly related sequences in the α- and β-subunits of T4 synthesize, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J 1:945–951.
14. Jurado KA, Engelman A. 2013. Multimodal mechanism of action of allosteric HIV-1 integrase inhibitors. Expert Rev Mol Med 15:e14. http://dx.doi.org/10.1017/erm.2013.15.
15. Zhao Z, Khismatudinov E, Schwartz C, Guo P. 2013. Mechanism of one-way traffic of hexameric phi29 DNA packaging motor with four electropores relay layers facilitating anti-parallel revolution. ACS Nano 7:4082–4092. http://dx.doi.org/10.1021/nn400277s.
16. Schwartz C, De Donatis GM, Zhang H, Fang H, Guo P. 2013. Revolution rather than rotation of AAA+ hexameric phi29 nanomotor for viral dsDNA packaging without coiling. Virology 443:228–339. http://dx.doi.org/10.1016/j.virol.2013.04.019.
17. Schwartz C, De Donatis GM, Fang H, Guo P. 2013. The ATPase of the phi29 DNA-packaging motor is a member of the hexameric AAA+ superfamily. Virology 443:20–27. http://dx.doi.org/10.1016/j.virol.2013.04.004.
18. De-Donatis G, Zhao Z, Wang S, Huang PL, Schwartz C, Tinsdkov VO, Zhang H, Haque F, Guo P. 2014. Finding of widespread viral and bacterial revolution dsDNA translocation motors distinct from rotation motors by channel chirality and size. Cell Biosci 4:30. http://dx.doi.org/10.1186/2045-3701-4-30.
19. Guo P, Zhao Z, Haak J, Wang S, Wu D, Meng B, Weitao T. 2014. Common mechanisms of DNA translocation motors in bacteria and viruses using one-way revolution mechanism without rotation. Biotechnol Adv 32:853–872. http://dx.doi.org/10.1016/j.biotechadv.2014.01.006.
20. Neuwald AF, Aravind L, Spouge JL, Koonin EV. 1999. AAA+ : a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. Genome Res 9:27–43. http://dx.doi.org/10.1101/gr.9.1.27.
21. Karata K, Inagawa T, Wilkinson AJ, Tatsuta T, Ogura T. 1999. Dissecting the role of a conserved motif (the second region of homology) in the AAA family of ATPases. Site-directed mutagenesis of the ATP-dependent protease FtsH. J Biol Chem 274:26225–26232.
22. Hilbert BJ, Hayes JA, Stone NP, Duffy CM, Sankaran B, Kelch BA. 2013. Structure and mechanism of the ATPase that powers viral genome packaging. Proc Natl Acad Sci U S A 110:E3792–E3799. http://dx.doi.org/10.1073/pnas.1306951112.
23. Enemark EJ, Joshua-Tor L. 2008. On helicas and other motor proteins. Curr Opin Struct Biol 18:243–257. http://dx.doi.org/10.1016/j.sbi.2008.01.007.
24. Iyer LM, Makarova KS, Kooinv EV, Aravind L. 2004. Comparative genomics of the FtsK-HerA superfamily of pumping ATPases: implications for the origins of chromosome segregation, cell division and viral capsid packaging. Nucleic Acids Res 32:5260–5279. http://dx.doi.org/10.1093/nar/gkh828.
25. Joly N, Zhang N, Buck M. 2012. ATPase site architecture is required for self-assembly and remodeling activity of a hexameric AAA+ transcrip- tional activator. Mol Cell 47:484–490. http://dx.doi.org/10.1016/j.molcel.2012.06.012.
26. Wendler P, Ciniawsky S, Kock M, Kube S. 2012. Structure and function
Vale RD, Funatsu T, Pierce DW, Romberg L, Harada Y, Yanagida T. 1996. Direct observation of single kinesin molecules moving along microtubules. Nature 380:451–453. http://dx.doi.org/10.1038/380451a0.

Harada Y, Ohara O, Takatsuki A, Itoh H, Shimamoto N, Kinosita K, Jr. 2001. Direct observation of DNA rotation during transcription by Escherichia coli RNA polymerase. Nature 409:113–115. http://dx.doi.org/10.1038/35051126.

Doering C, Ermentrout B, Oster G. 1995. Rotary DNA motors. Biophys J 69:2256–2267. http://dx.doi.org/10.1016/S0006-3495(95)80096-2.

Arita T, Masaike T, Noji H, Yoshida M. 2002. Stepping rotation of F1-ATPase with one, two, or three altered catalytic sites that bind ATP only slowly. J Biol Chem 277:24870–24874. http://dx.doi.org/10.1074/jbc.M202582200.

Guo P, Schwartz C, Haak J, Zhao Z. 2013. Discovery of a new motion mechanism of biomotors similar to the earth revolving around the sun without rotation. Virology 446:133–143. http://dx.doi.org/10.1016/j.viro.2013.07.025.

Pi F, Zhao Z, Chelikani V, Yoder K, Kvaratskhelia M, Guo P. 29 June 2016. Development of potent antiviral drugs inspired by viral hexameric DNA packaging motors with revolving mechanism. J Virol http://dx.doi.org/10.1128/JVI.00508-16.

Wendler P, Shorter J, Sneed D, Plisson C, Clare DK, Lindquist S, Saibil HR. 2009. Motor mechanism for protein threading through Hsp104. Mol Cell 34:81–92. http://dx.doi.org/10.1016/j.molcel.2009.02.026.

Kravats AN, Tondstad-Navaei S, Bucher RJ, Stan G. 2013. Asymmetric processing of a substrate protein in sequential allosteric cycles of AAA+ nanomachines. J Chem Phys 139:121921. http://dx.doi.org/10.1063/1.4817410.

Morita M, Tasaka M, Fujisawa H. 1993. DNA packaging ATPase of bacteriophage T3. Virology 193:748–752. http://dx.doi.org/10.1006/viro.1993.1183.

Arai S, Saito S, Suzuki K, Mizutani K, Kakinuma Y, Ishizuka-Katsura Y, Ohsawa N, Terada T, Shirouzu M, Yokoyama S, Iwata S, Yamato I, Murata T. 2013. Rotation mechanism of Enterococcus hirae V1-ATPase based on asymmetric crystal structures. Nature 495:703–707. http://dx.doi.org/10.1038/nature11778.

Ye Q, Rosenberg SC, Moeller A, Speir JA, Su TY, Corbett KD. 2015. TRIP13 is a protein-remodeling AAA+ ATPase that catalyzes MAD2 conformation switching. eLife 4:e07367. http://dx.doi.org/10.7554/eLife.07367.

Stinson BM, Baytshtok V, Schmitz KR, Baker TA, Sauer RT. 2015. Subunit asymmetry and roles of conformational switching in the hexameric AAA+ ring of ClpX. Nat Struct Mol Biol 22:411–416. http://dx.doi.org/10.1038/nsmb.3012.

Sun SX, Wang H, Oster G. 2004. Asymmetry in the F1-ATPase and its implications for the rotational cycle. Biophys J 86:1373–1384. http://dx.doi.org/10.1016/S0006-3495(04)74208-3.

Lyubimov AY, Costa A, Bleichert F, Botchan MR, Berger JM. 2012. ATP-dependent conformational dynamics underlie the functional asymmetry of the replicative helicase from a minimalist eukaryote. Proc Natl Acad Sci USA 109:11999–12004. http://dx.doi.org/10.1073/pnas.1209406109.

Souttas P, Wigley DB. 2001. Unwinding the “Gordian knot” of helicase action. Trends Biochem Sci 26:57–54. http://dx.doi.org/10.1016/S0968-0004(00)01734-5.

Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. 2015. The Phyre2 web portal for protein modeling, prediction and analysis. 2015. Nat Protoc 10:845–858. http://dx.doi.org/10.1038/nprot.2015.053.