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REVIEW

Controlling the Revolving and Rotating Motion Direction of Asymmetric Hexameric Nanomotor by Arginine Finger and Channel Chirality

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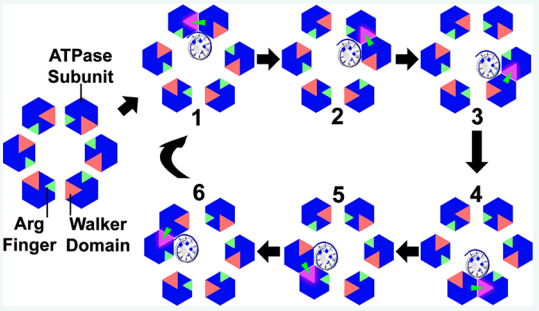
ABSTRACT: Nanomotors in nanotechnology are as important as engines in daily life. Many ATPases are nanoscale biomotors classified into three categories based on the motion mechanisms in transporting substrates: linear, rotating, and the recently discovered revolving motion. Most biomotors adopt a multisubunit ring-shaped structure that hydrolyzes ATP to generate force. How these biomotors control the motion direction and regulate the sequential action of their multiple subunits is intriguing. Many ATPases are hexameric with each monomer containing a conserved arginine finger. This review focuses on recent findings on how the arginine finger controls motion direction and coordinates adjacent subunit interactions in both revolving and rotating biomotors. Mechanisms of intersubunit interactions and sequential movements of individual subunits are evidenced by the asymmetrical appearance of one dimer and four monomers in high-resolution structural complexes. The arginine finger is situated at the interface of two subunits and extends into the ATP binding pocket of the downstream subunit. An arginine finger mutation results in deficiency in ATP binding/hydrolysis, substrate binding, and transport, highlighting the importance of the arginine finger in regulating energy transduction and motor function. Additionally, the roles of channel chirality and channel size are discussed as related to controlling one-way trafficking and differentiating the revolving and rotating mechanisms. Finally, the review concludes by discussing the conformational changes and entropy conversion triggered by ATP binding/hydrolysis, offering a view different from the traditional concept of ATP-mediated mechanochemical energy coupling. The elucidation of the motion mechanism and direction control in ATPases could facilitate nanomotor fabrication in nanotechnology.



KEYWORDS: *ATPase, biomotor mechanism, arginine finger, Walker A motif, Walker B motif, channel size, channel chirality, entropy driven*

Biomotors, also known as biological motors, are nanoscale machines ubiquitous in many biological processes,[1−3] such as cell mitosis, bacterial binary fission,[4,5] DNA replication,[6−8] DNA repair,[9−12] homologous recombination,[13−15] RNA transcription,[16] macromolecule trafficking,[17] and viral genome packaging.[18−41] Biomotors are essential protein devices that convert an energy source into different kinds of mechanical motions essential to cellular functions.[42] Many of them display a hexameric ring structure.[41,43−55] With the recent discovery of a revolving biomotor,[56,57] biological motors can be classified into three categories[1−3] based on the movement mode of transporting their substrates: linear, rotating, and revolving.[1,5,8,58,59] Specifically, in a rotating motor, the substrate rotates around its own axis, while in a revolving motor, the substrate revolves around the second object (Figure 1). The way that revolving motors work is distinct from rotating motors in that among the multiple parts, only the substrate is circumnavigating. Rotating refers to the action similar to the Earth turning around its own axis every 24 h, while revolving is akin to how the Earth circumnavigates around the Sun every 365 days but without self-rotation. Revolving rather than rotating avoids the coiling and tangling of long polymer chains, such as genomic dsDNAs during translocation. The well-studied rotating motors include F1/F0 ATPase,[43−47] DNA helicase,[48,49] Rho transcription

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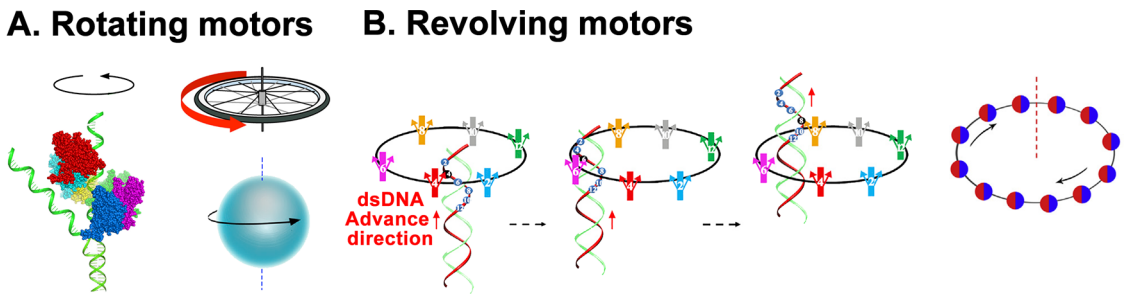

Figure 1. Illustration of two different types of motors. (A) Rotating motors are like a wheel and like the Earth rotating on its own axis round per day. Reprinted with permission from ref 3, Copyright 2016, American Society for Microbiology, and adapted with permission from ref 74. Copyright 2014 Springer Nature. (B) Revolving motors resemble the Earth revolving around the Sun one round per year without self-rotation. Reprinted with permission from ref 220. Copyright 2014 Elsevier.

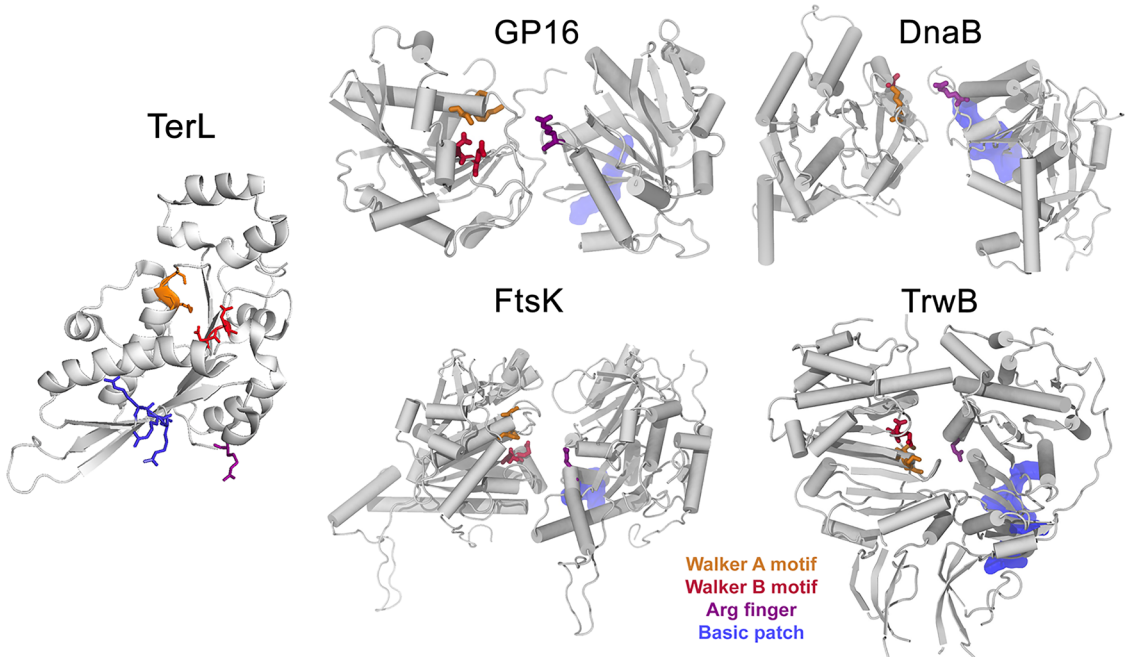

Figure 2. Structures of some ATPase domains in their dimer form. The Walker A and Walker B motifs, which form the active site, are colored in orange and red, respectively. The arginine finger is colored in purple. A solvent-exposed basic patch composed of positively charged residues is colored in blue. Adapted with permission from ref 29. Copyright 2015 National Academy of Sciences.

termination factor,[50−53] TrwB,[60−65] MCM,[66,67] and RepA or RuvB,[68−73] all of which have a channel diameter of 1−2 nm.[74] Revolving motors include the DNA translocases Ftsk in Gram-negative bacteria,[54] SpoIIIE or SftA (YtpS) in Gram-positive bacteria,[75] A32 ATPase of poxvirus,[76−80] DNA packaging enzyme of adenovirus,[81−83] the genome segregation enzymes of mimivirus,[2,84−87] as well as the DNA packaging motors of herpesvirus,[88−103] SPP1,[27] T7,[104] HK97,[105] P22,[106] and Phi29.[107] The three classes of biomotors differ in structure and function, but utilize similar mechanisms for force generation to perform mechanical work. More information about the linear, rotating, and revolving motors can be found in recent reviews.[1−3]

The common feature of a multisubunit ring-shaped structure of ATPase motors[108,109] has raised an intriguing question on how these biomotors control the direction of their motion and how the sequential action of their individual subunits is regulated. The key driving force in a viral DNA packaging motor is a DNA-dependent ATPase. Although this was first reported more than 30 years ago,[18] literature on mechanisms of directional control of ATPase motors has just begun to emerge.[31,110−115] The common ATP binding domain[116,117] contains highly conserved motifs that form an ATPase activity pocket.[117] Previous modeling work on the phi29 gp16 ATPase[118] suggested that a conserved arginine residue plays a critical allosteric role in coordinating the sequential hydrolysis on the multisubunit ring, as found in both RNA and DNA packaging motors.[110,119−124] This arginine residue was defined as the arginine finger. In this review, we summarize the most recent discoveries on the arginine finger, focusing on its role in motion direction control, sequential intersubunit coordination, and asymmetrical multimer assembly. We also discuss the chirality and size of the DNA transport channel, conformational changes, and entropy conversion of the motors involved in the revolving mechanism. Finally, we present a different perception on ATP chemical energy conversion into physical motion in the hexameric biomotors. The understanding of motor structure, motion mechanism, and direction control of oligomeric ATPases will provide a prototype model for future manufacturing of nanomotors in nanotechnology.[110,125,126]

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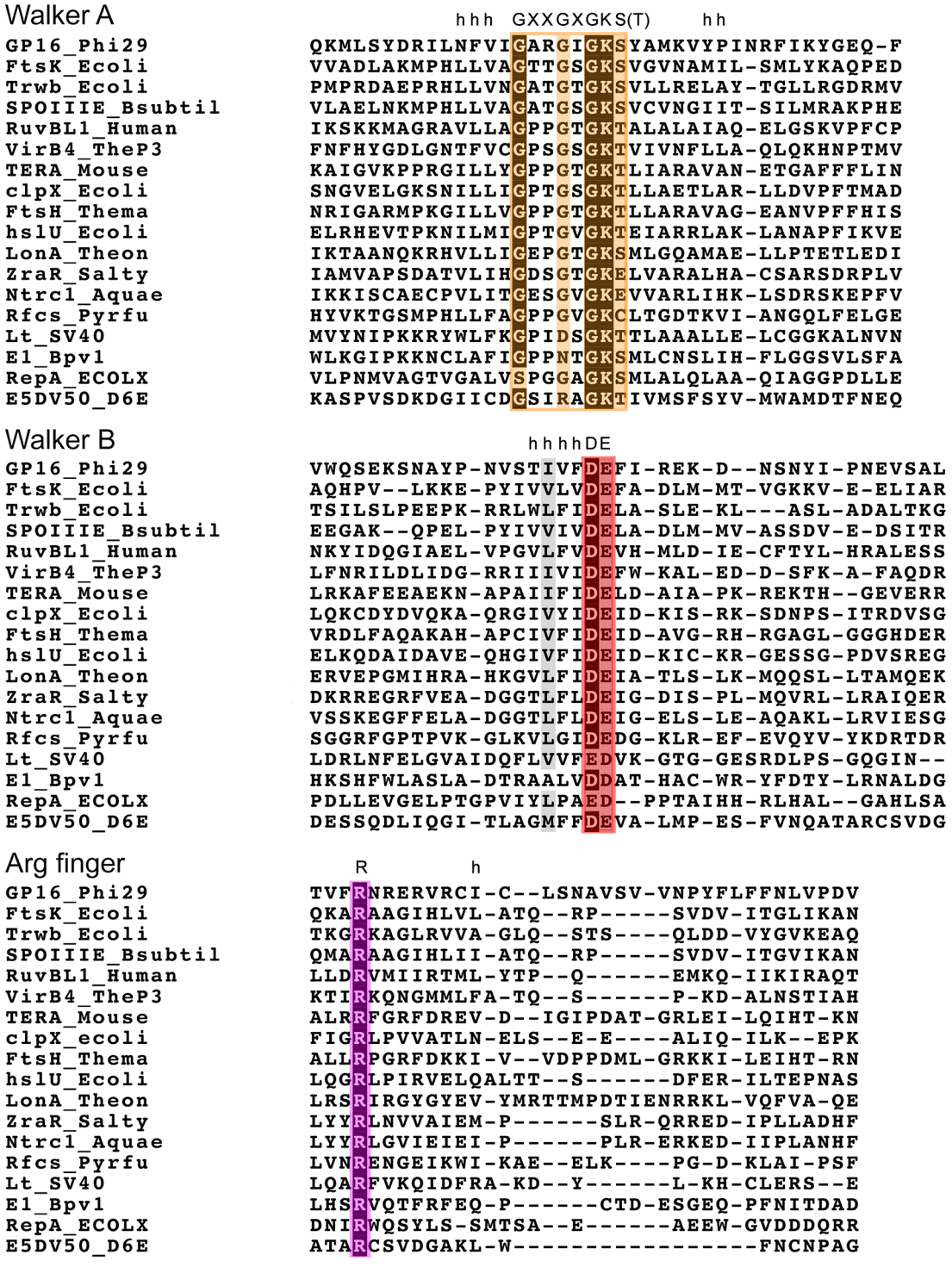

Figure 3. Sequence alignment reveals conserved motifs[18,110] (Walker A motif, Walker B motif,[116,117] and Arg finger[110,125]) across different types of ATPases. Highly conserved residues are highlighted as follows: Orange for Walker A with darkness representing the rate of homology; red for Walker B; and purple for Arg finger. The letter h above the column denotes conserved hydrophobic residues.

DEFINITION AND LOCATION OF THE ARGININE FINGER IN ATP REGULATING COMPLEXES

Characteristics of ATP-Activity Pocket. The ATP activity pocket in an ATPase complex typically comprises the following components: arginine finger, Walker A motif, Walker B motif, P-loop, and lid subdomain (Figure 2).[127] Hexameric ATPases each contain a conserved core domain, which consists of two conserved sequence motifs termed Walker A and Walker B[45,128,129] with a sequence of GXXGXGKS/T and hhhhDE (Figure 3),[18] respectively. The Walker A and Walker B motifs have been identified to be responsible for the ATP binding and ATP hydrolysis.[18,109,116] Given the conservation of the Walker motifs, it is not surprising that most residues interacting with ATP are intolerant of amino acid alterations. The invariant lysines in the Walker A motif have been intensively studied, revealing their roles in coordinating the ATP β and γ phosphates and structuring the P-loop in related

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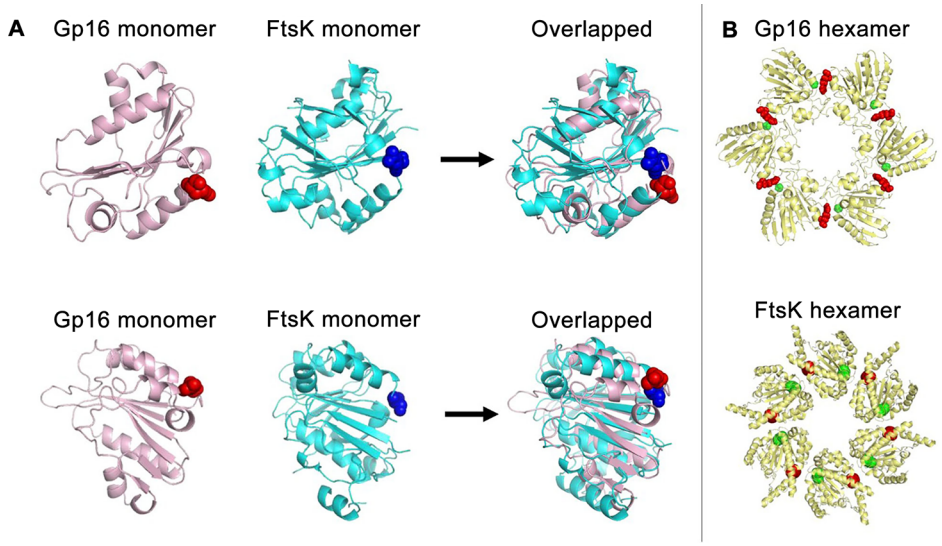

Figure 4. Adjacent location of arginine fingers and Walker A motifs within gp16 and FtsK ATPases. (A) Comparison of the crystal structure of FtsK and the computed gp16 monomers, which represent a single subunit of a hexameric ATPase, revealing the alignment of the two ATPase subunits even though they are from different species. The arginine finger (red sphere) and the Walker A (blue sphere) overlap when the gp16 and FtsK ATPases are superimposed. (B) Comparison of gp16 and FtsK hexamer models. The green sphere represents Walker domains. The red sphere represents the arginine finger. Based on the proximity of the green and red spheres, the figure reveals that the arginine finger interacts with the adjacent ATPase subunit. Reprinted with permission from ref 110. Copyright 2016 American Society of Microbiology.

NTPases.[43,130] Mutation of lysine to a polar amino acid generally will eliminate the wild-type ATPase function.[96] As for the Walker B motif, the conserved negatively charged residues such as glutamate and aspartate act to polarize a water molecule to nucleophilically attack the γ phosphate group of ATP. Most commonly studied mutations are substitutions of glutamate and aspartate by glutamine or alanine.[56,110,125,131,132] Upon these mutations, ATP hydrolysis is prevented, but ATP binding is retained.

For some ATPases, the sensor 1 and 2 motifs have also been reported to play important roles in the ATPase function; however, sensor 1 is not strictly conserved in ATPase proteins, thus whether these two sensors are common features of all ATPases or just an alternate description of the arginine finger requires future verification.[29,133−138] Sensor 1 motif is located in the loop connecting the β4 strand to α4 helix. It is often a polar residue thought to interact with the γ-phosphate of ATP. Due to this interaction, it is believed that the sensor 1 motif senses the binding of ATP and orients a water molecule for a nucleophilic attack on the γ-phosphate of the bound ATP molecule. It has been shown in p97 D2 that the shift of the sensor 1 residues, upon nucleotide binding, induces displacements at the distal end of the ATP binding domain.[3] Sensor 2, located near the beginning of α7, is conserved in many ATPase proteins. It contains a conserved arginine residue, which, together with the Walker B motif, engages the bound ATP and mediates conformational changes that sequester the catalytic site from water.[33,133] Mutations of the sensor 2 residues led to a loss or decrease of ATP binding and/or ATP hydrolysis.[110,125,135−138]

Besides the Walker A, Walker B, and sensors 1 and 2, another common component in the ATP-regulating complexes is the lid domain (Figure 2). The lid subdomain (residues 221−251 in the TerL ATPase) is a short peptide with negatively charged amino acids that might interact with the positively charged arginine residues. Crystal structure revealed that the lid is displayed adjacent to the ATP binding site on the surface of the ATPase protein.[29] Despite its relevance to ATPase activity, the study on the lid domain has been limited, probably due to its low degree of conservation among ATPases.

ATP binding and hydrolysis in ATPases are attributed to residues occupying two or more of the four key sites near the ATP molecule. These residues are located in the ATP-binding pocket or at the adjacent intersubunit interface. Among them, arginine possesses an extended and flexible side chain with a planar and positively charged guanidine group at its extremity. The positive charge is distributed over the three side-chain nitrogens, which is advantageous for hydrogen-bond and electrostatic interactions with groups of opposite charge and polarity, for example, ATP phosphate groups. The multidentate character of arginine allows for strong inter- and intraprotein interactions, as seen in phosphorylation-driven signal transduction pathways.[139]

Definition and Location of the Arginine Finger. "Arginine finger" means a particular arginine residue coordinated to the β- or γ-phosphate of ATP in the ATPase catalytic reaction center.[140] The location of representative arginine fingers in a certain monomer (Figure 2 left), dimer (Figure 2 right), and hexamer (Figure 4) is illustrated. Although there are multiple arginine residues present throughout the ATPase protein, the arginine finger can be identified using knockout experiments.[141] For example, an arginine finger knockout study was used to determine the role and necessity of the arginine finger in F1-ATPase.[43] It was found that the substitution of the arginine residue in the arginine finger motif by a lysine analogue called Lyk resulted in reduced catalytic function. In another case, the identification of the arginine finger was achieved *via* the mutation of the basic arginine residue to a neutral residue, alanine, in phi29 motor ATPase gp16. Mutated gp16 was found to lose the capability

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to incorporate into the hexameric ring, to bind dsDNA, or to package DNA.[110,125]

The arginine residue is a recurrent characteristic of the active sites and subunit interfaces of many ATPase proteins.[139] Sequence alignment of different ATPases shows that the arginine finger motif is conserved across the ATPase families, but its location varies. Depending on the family it belongs to, the ATPase protomers can orient differently in the oligomeric assembly, leading to a different location of the arginine finger. In most ATPase proteins, the arginine finger contains one or more arginine residues and is often found at the end of the α4 helix.[135] In order to identify the location of the arginine finger in TerL ATPase, investigators screened mutants of surface-exposed arginine residues for ATPase activity.[29] This experiment identified residue Arg139 as the arginine finger that interacts with the γ-phosphate of the ATP molecule binding in the adjacent subunit and helps catalyze ATP hydrolysis. The arginine finger in the gp16 ATPase was identified to be Arg146, located after α4, as is the case in other ATPases in the same family with consensus sequence and confirmed structural information (Figures 2 and 3).[110,142] This motif interacts with ATP in a highly specific fashion, binding to the γ-phosphate of ATP that is also coordinated by the neighboring subunit. The arginine finger is essential for ATP hydrolysis, as even conservative mutations led to the abolition of ATPase activity.[143,144]

GENERAL FUNCTION OF THE ARGININE FINGER

The conserved arginine finger plays essential functional roles in many ATPases.[145−156] Positive residues in the active site are necessary for ATP hydrolysis, which are involved in stabilizing the transition state during the reaction.[157] The arginine finger provides some of this necessary charge. Substitution mutations that replace the arginine residue with neutral residues result in the loss of ATPase function.[29,110,125]

Although more detailed structural, biophysical, and biochemical characterization of the arginine finger in motor ATPases is needed, significant evidence has led to the speculation that the arginine residue is part of the Walker A and Walker B domains. The characteristic Walker A and B motifs in ATPases are involved in coordinating the β and γ phosphates of ATP and a water-activating magnesium ion during ATP hydrolysis. The ATP hydrolysis is also aided by sensor 1 and 2 motifs. Crystal structures of the biomotor ATPase domains reveal a highly conserved arginine residue in the proximity of the sensor 2 motif (Figure 2). In these structures, an ATP molecule comes into contact with the Walker A and B motifs of one subunit, while the arginine finger coordinates the ATP from the adjacent subunit (Figure 4).[158]

PERCEPTION ON *TRANS* ACTION BUT NOT *CIS* ACTION OF THE ARGININE FINGERS IN ATPASES OR MOTOR COMPLEXES

It is believed that the arginine finger facilitates the ATP hydrolysis in a *trans* manner.[159] The term *trans* originates from the Latin root "*trans*" meaning "across from", which is relative to "*cis*", meaning "the same side as". Specifically, a *trans*-acting arginine finger refers to an arginine residue from one ATPase subunit that regulates the ATP hydrolysis in the adjacent subunit. A *cis*-acting arginine finger, on the other hand, refers to an arginine residue that regulates the ATP hydrolysis in the same subunit. The classification of the *trans*-acting arginine finger is important for both understanding the ATPase mechanism and defining the structure of the active ring assembly.[29] To investigate this, the crystal structures of the ATPase activity domains of biomotors have been employed for comparison and analysis. The overall structural features of the core domains are conserved in all ATPases of the superfamily with a conserved arginine residue near the sensor 2 motif; however, the helicase superfamily III proteins lack the sensor 2 arginine due to an atypically formed α-helical domain. A majority of ATPases are arranged in such a way that the nucleotide binding pocket is positioned at the interface between two protomers.[110,160] This structural arrangement supports the notion that, in an active ATPase complex, the arginine finger of one subunit should be positioned near the nucleotide bound in the neighboring subunit. A structure of the hexameric ring of phi29 gp16 ATPase was modeled by aligning with the hexameric FtsK DNA translocase of *Escherichia coli*.[110] The arginine finger of one subunit was shown to outstretch to the active site of the adjacent subunit, in agreement with other ATPases, such as TerL and ClpX, in which the arginine finger is positioned in the ATP binding pocket for cooperative behavior among subunits.[29,161] This structural feature is evident in various ATPase hexamers (Figure 2). Mutants that showed no ATPase activity were tested to determine if proper function could be restored by adding ATPase monomers with an intact arginine finger. Biochemical complementation assays thus revealed that the mutant whose arginine is disabled in *cis* (within the same subunit) does not restore activity, but that disabled in *trans* (not in the same subunit) does restore activity.

Nonetheless, the literature on the arginine finger is still inconsistent. Some reports suggest that the arginine finger is a *cis*-acting component that functions within a single subunit of the ATPase ring,[43] while others report that the arginine finger is a *trans*-acting factor that bridges two adjacent subunits.[29,110,125,159,162−164] Some studies even suggest that there are two arginine fingers in each ATPase subunit.[109,139] It has also been reported that the reduction in ATPase activity upon arginine finger mutation is due to an effect on catalysis but not ATP binding.[143] The complexity and the controversy may be due to the fact that some ATPases are a circular-shaped, multiple component ring,[47,165−169] but some ATPases are present as a single subunit.[170,171]

COMPARISON OF THE ARGININE FINGERS ACROSS VARIOUS ATPASE TYPES

Arginine fingers are mostly conserved in ATPase proteins (Figure 3). Oligomeric ATPases contain one arginine finger per monomer subunit. It has been shown that ATPases from SF1 and SF2 contain a tandem fold and bind the nucleotide at the interface between two domains. Similar to many ATPases,[172,173] the N-terminal provides the Walker A and Walker B motifs, and the C-terminal provides other elements, some of which are for binding of the substrate, such as dsDNA. Mutations of these arginine residues are lethal and lead to loss of *in vivo* and/or *in vitro* activity, suggesting that these residues are imperative in ATP metabolism.[110,139,159,162,174]

Although the arginine finger is, in general, involved in the proper functioning of ATPases, its function may vary slightly across different ATPase types. The arginine residue is able to interact with the γ-phosphate of ATP and is required for ATP binding, hydrolysis, and intersubunit communication. It is positioned near the γ-phosphate of ATP and plays a catalytic

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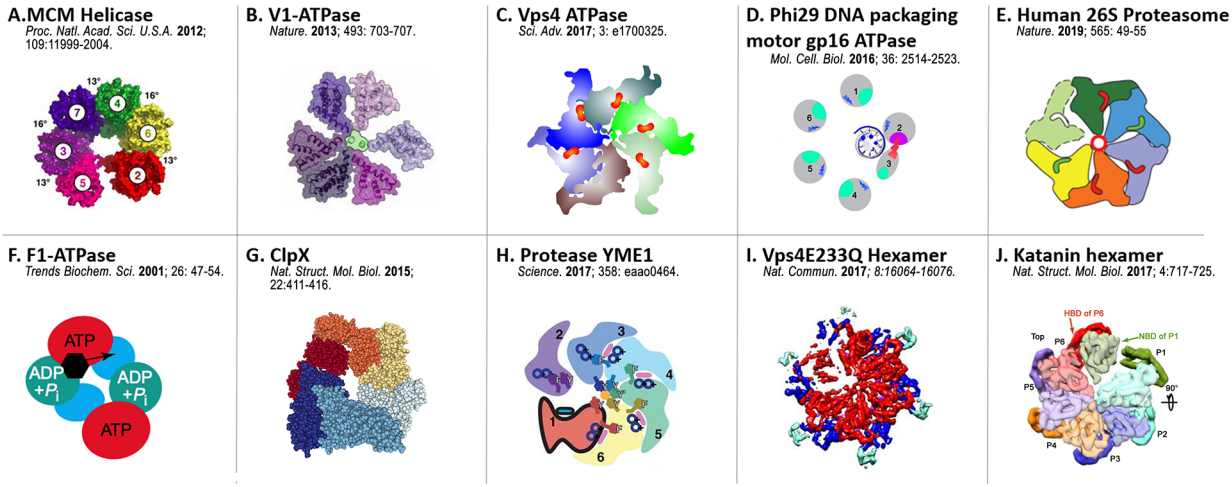

Figure 5. Asymmetrical crystal or Cryo-EM structures of various ATPase hexamers: (A) MCM helicase. Reprinted in part with permission from ref 179. Copyright 2012 National Academy of Science. (B) V1-ATPase. Reprinted by permission from ref 180. Copyright 2013 Springer Nature. (C) Vps4 ATPase. Illustration adapted from ref 181. (D) Phi29 DNA packaging motor gp16 ATPase. Reprinted in part with permission from ref 110. Copyright 2016 American Society for Microbiology. (E) Human 26S Proteasome ATPase. Reprinted with permission from ref 182. Copyright 2019 Springer Nature. (F) F1-ATPase. Reprinted with permission from ref 183. Copyright 2001 Elsevier. (G) ClpX. Illustration adapted from ref 110. (H) Protease YME1. Reprinted with permission from ref 185. Copyright 2017 American Association for the Advancement of Science. (I) Vps4E233Q Hexamer. Reprinted in part with permission from ref 186. Copyright 2017 Springer Nature. (J) Katanin hexamer. Reprinted with permission from ref 187. Copyright 2017 Springer Nature.

role in properly positioning the ATP molecule within the ATP binding site.[175] It is believed that the arginine finger may also play a role in stabilizing the transition state during hydrolysis.[176] Mutational studies concluded that the main role of the arginine finger in the F1-ATPase is to catalyze ATP hydrolysis and mediate efficient energy conversion.[43,44] Mutations of Walker A and arginine finger yield a similar phenotype, indicating that the arginine finger is also involved in nucleotide binding.[110,159]

Additionally, the arginine finger may also aid in stabilizing the ATPase hexamers[135] due to its role in dimer formation and intersubunit interaction.[110,125,177] Arginine finger mutations[178] in HslU, p97 VCP, ClpB D1, ClpC D1, and Hsp104 D1 prevent oligomer formation even in the presence of ATP, supporting the proposal that the arginine finger is involved in formation of a dimer in the hexameric ring.[110] Nevertheless, arginine finger mutations have led to different results from those in Ras/RasGAP proteins, where complex formation was not affected upon arginine subsititution. This mystery suggests that the identification of the arginine finger in Ras/RasGAP requires rescrutiny.

Another ATPase, which differs in its role in the cell but contains similar structural motifs and ATP hydrolysis mechanisms, is RuvB ATPase.[137] RuvB and motor ATPases are both hexameric proteins. In *E. coli*, the cross-shaped Ruv family proteins function in genetic recombination through processing Holliday junctions. RuvB contains an arginine residue (Arg174) that is located between sensor 1 and 2 motifs. Mutagenesis experiments reveal that this arginine residue is essential for ATP hydrolysis and proper ATPase function.[70−73] The arginine finger in RuvB is also responsible for intersubunit interaction during the ATP hydrolytic cycle, similar to that of the arginine finger in the phi29 biomotor.

AN ASYMMETRIC ATPASE HEXAMER MADE UP OF ONE "DIMER" AND FOUR MONOMERS

In many hexameric ring-shaped ATPases, the arginine finger serves as the bridge between two of the ATPase subunits; the two adjacent subunits thus form a more compact dimer configuration that may appear as a monomer in low-resolution cryo-EM images. This caused the hexameric ring to appear asymmetrical, as shown for the phi29 motor ATPase by the Guo group.[56,57] This asymmetric hexameric structure has been observed in X-ray diffraction and cryo-EM imaging of many ATPases in addition to the phi29 motor ATPase gp16 (Figure 5).[110,179−187] This hypothesis of one interchanging dimer and four monomers is supported by the profile of gp16 in ultracentrifugation, showing the presence of both monomers and dimers in the mixture. However, ATPase motors have for a long time been reported as a pentameric configuration by cryo-EM, probably due to the interchanging dimers that display close contact between two adjacent ATPase subunits. Traditional cryo-EM is an ensemble measurement by averaging over many configurations, thus the dimer with close contact might show as one instead of two subunits. Moreover, the low and featureless EM density maps of gp16 in recent cryo-EM imaging of the entire motor complex[115] have precluded the possibility of obtaining an unambiguous fit for five or six copies of gp16, adding another layer of ambiguity to the ongoing debate.

Each subunit of the ATPase hexamer has the capability of binding an ATP molecule; however, saturation of the ATPase with ATP reveals that at least two of the subunits are not bound with ATP. Even when not all subunits are able to bind ATP, the ATPase function is retained.[188] These observations suggest that the functional ATPase hexamer is asymmetrical and the subunits in the ATPase vary in conformation during ATP hydrolysis. To investigate the role of the arginine finger in the dimer formation within the hexameric ATPases, arginine knockout experiments were performed. It was found that mutation of the arginine finger in phi29 gp16 resulted in loss of

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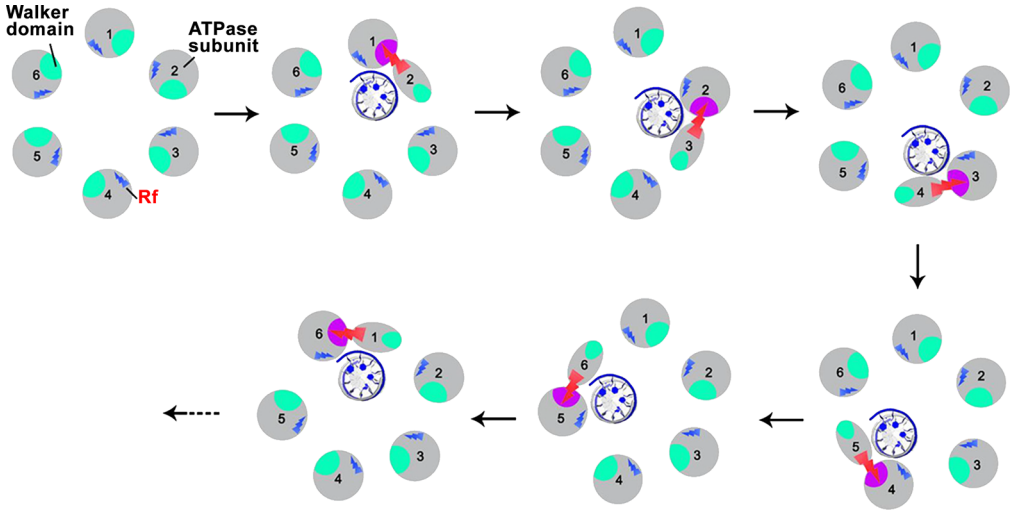

Figure 6. Arginine finger (red arrow) regulates dimer formation and sequential action during ATP hydrolysis. Green: The five inactive Walker domains for ATP-binding (the P-loop, Walker A and B motifs). Pink: The one active ATP-binding center after activation by arginine finger. The *trans*-acting arginine finger acts as a bridge between two ATPase subunits when ATP is bound. As hydrolysis continues, ATP binds to the subsequent subunit and dimer formation occurs in a sequential manner. Reprinted with permission from ref 110. Copyright 2016 American Society for Microbiology.

dimer assembly and DNA packaging ability; however, dimer formation was rescued with the addition of either a wild-type gp16, a Walker A mutant, or a Walker B mutant, which all contain a functional arginine finger. An inhibition assay in which the arginine finger function is knocked out revealed that a single arginine mutant subunit led to inactivation of the entire ATPase ring. These results suggest that the arginine finger is a necessary component for coordinating the formation of the ATP binding pocket and intersubunit communication in the revolving motor ATPases.[110,139]

Further evidence that the arginine finger motif drives the formation of dimers is provided by glycerol gradient centrifugation and electromobility shift assays (EMSA) experiments, where both monomeric and dimeric subunits are present in the ATPase population.[110,125] In order to determine the ratio of monomer to dimer during DNA packaging, investigators tested the packaging activity of the different fractions recovered from the sucrose gradient. It was observed that the fraction containing the dimer alone did not have DNA packaging activity, while DNA packaging activity was retained in the fractions that also contained monomers. This finding is also supported by a previous report that the addition of fresh gp16 monomer and ATP is necessary for re-initiating the activity of DNA packaging intermediates, which contained gp16 dimers, into an infection virus.[189]

OUTSTRETCHING TO ADJACENT ATP POCKETS AND FORMATION OF DIMERIC SUBCOMPLEXES TO REGULATE SEQUENTIAL ACTION OF ATPASES

Recently, the way the arginine finger regulates the motion direction of the ATPase within the phi29 DNA packaging motor has been proposed.[110] The model assumes that ATP/DNA binding and ATP hydrolysis are coupled with conformational changes of the gp16 ATPase. These changes occur in a sequential manner and are coordinated by the arginine finger. The arginine finger acts as a bridge between two adjacent subunits, leading to the formation of a transient dimer. The conformational changes of the ATPase subunit will in turn lead to the displacement of the dsDNA to the adjacent monomer. During this process, the formation of the dimer results in an asymmetric hexamer, which explains why many previous studies showed asymmetric structures of various ATPase hexamer models (Figure 5).[110,179−187] The arginine finger functions in intersubunit interaction by extending from one subunit to the adjacent one, which facilitates the formation of a dimer.

The demonstration of a sequential mechanism raises the question of how the different subunits of the ATPase can sense the ATP/DNA binding state of the adjacent subunits. Investigators addressed this question by studying the behavior of gp16 mutants in which the arginine fingers were mutated. Mutated gp16 eliminated its capability to assemble into dimers, and the mutant was unable to hydrolyze ATP, bind DNA, or package DNA in an ATP-dependent manner.[110,125] Thus, the arginine finger was implicated to regulate conformational changes, dimer formation, DNA binding, and ATP hydrolysis and thus eventually to orchestrate force generation for DNA translocation in the phi29 motor.[110,125,190,191] This finding was further confirmed recently, reported as a switch-like regulator.[192]

In the sequential mechanism of gp16 action (Figure 6), it was proposed that the hydrolysis of ATP causes a conformational change to the ATPase subunit, which destabilizes the "active" ATPase dimer and may simultaneously trigger a conformational change (strike) of the arginine finger. This outstretch of the arginine finger to the adjacent ATP binding pocket facilitates the formation of the next in-line "active" dimer. Mutagenesis studies were conducted with phi29 ATPase, HslU, p97 VCP, and others in which both ATP hydrolysis and oligomer formation were impaired due to mutation of the arginine finger. This leads to the conclusion that these arginine fingers function in motor subunit communication as well as ATP hydrolysis.[109,110,125,138] The mutation of the arginine finger in gp16 led to impaired function in DNA binding in the presence of γ-S-ATP. Hence, the arginine finger appears to regulate the sequential action of the gp16 ATPases by carrying the ATP/DNA binding/hydrolysis information from one subunit to another, adding

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an extra level of cooperativity in gp16 as seen in other mutants, such as in Walker B mutations.[56,57,110]

Interestingly, it was reported that hydrophobic residues in the catalytic site of an ATPase may play a role in controlling the motor speed.[193] These hydrophobic residues are thought to be responsible for controlling the number of water molecules within the catalytic space and altering the network of water interactions. Natural evolution has selected the optimal speed variants that ultimately improve the fitness of organisms or phages, which may be the reason why these hydrophobic residues are considered nonconserved motifs.

ENTROPY-DRIVEN PROHEAD- AND DNA-DEPENDENT CONFORMATIONAL CHANGES OF ATPASES TO TRIGGER ATP HYDROLYSIS AND MOTION IN RELEVANCE TO ALLOSTERIC EFFECTS OF THE ARGININE FINGER

Besides providing necessary positive charges for ATP binding and hydrolysis, the arginine finger plays an indispensable role in regulating the conformational changes and coordinating the sequential motions in the ATPase complexes.[194,195] In 1986, Guo *et al.* reported[18] that viral DNA packaging enzymes, including gp16 of phi29, gp19 of T7, gp17 of T4 and gpA of λ, all contain a conserved A-type sequence of "basic-hydrophobic region-G-X2-G-X-G-K-S-X7-hydrophobic" (X represents any amino acid) for ATP binding. After the construction of the first defined *in vitro* DNA packaging system with all purified components[40] and the discovery of the pRNA as the motor-gearing component,[38] they were able to elucidate that the two enzymes involved in DNA packaging have distinct functions; the enzyme with larger molecular weight is a prohead and DNA-dependent ATPase, while the other with smaller molecular weight is responsible for DNA binding. In the same paper, it was reported that the gp16 of bacteriophage phi29 DNA packaging motor is a prohead and DNA-dependent ATPase.[18] The mechanism of "prohead and DNA-dependent ATPase" has been scrutinized for 30 years and is now clear. It suggests that the interaction of the gp16 ATPase with other motor components leads to a change in conformation (entropy) of the ATPase subunit, resulting in a higher affinity for dsDNA. The subsequent DNA binding leads to a second conformational change of the ATPase subunit that is activated to hydrolyze the bound ATP. Hydrolysis of ATP leads to another conformational change of the ATPase (higher entropy) that resumes a low affinity for dsDNA, thus pushing the DNA to the adjacent ATP-bound subunit of a high affinity for dsDNA. Such alternative high and low affinities for DNA are the forces that drive the motion of the dsDNA substrate in the DNA packaging motor.

In the ATPase catalytic cycle, ATP binding causes the first round of conformational (entropy) changes of the ATPase subunits, a positive allosteric effect that results in a higher affinity for dsDNA. The interaction of ATP and ATPase has been studied using a variety of assays. EMSA demonstrated that nonhydrolyzable γ-S-ATP qualitatively stalls and fastens the formation of ATPase/dsDNA complex, indicating that ATPase undergoes conformational (entropy) changes upon ATP binding and leads to a higher affinity for dsDNA.[56,57,110] Similar results were observed from Förster resonance energy transfer (FRET) analysis, showing increased energy transfer from eGFP-ATPase to Cy3-dsDNA upon addition of γ-S-ATP.[56] Sedimentation studies also revealed a high prevalence of the gp16-dsDNA complex with γ-S-ATP. As expected, such conformational changes are abolished by the site-directed mutation of the Walker A motif,[165] which has been identified[18] and confirmed[18,56,172] to be responsible for ATP binding.[133]

ATP is hydrolyzed only after dsDNA binding, which then causes a conformational (entropy) setback of the ATPase subunit, a negative allosteric effect on the ATPase subunit that leads to a lower affinity toward dsDNA, pushing the dsDNA toward the next adjacent ATPase subunit that has already bound with an ATP. The dsDNA advances by dsDNA by 0.54 nm or 0.27 nm for each of the 12 steps in the connector channel. That is, each ATP molecule packages 1.75 bp of dsDNA. The process repeats six times as the DNA moves by a helical pitch, that is six ATP molecules are consumed for one DNA revolving cycle, corresponding to the packaging of 10.6 bp.[56] The translocation from one subunit to another subunit is regulated by the action of the arginine finger. The hydrolysis of ATP was confirmed by adding ATP to the purified ATPase/dsDNA/γ-S-ATP complex. ATP replaced the γ-S-ATP, leading to the release of dsDNA from the complex. ADP had a lesser effect on dsDNA release, whereas AMP was incapable of releasing dsDNA from ATPase.[56] The release of inorganic phosphate from the P-loop stimulates an entropy gain in ATPase, which is accompanied by a conformational shift that forces the substrate DNA away from the interior pocket of the ATPase, resulting in the movement of the genomic DNA toward the next ATPase subunit. Given that Walker B mutants bind ATP but do not hydrolyze ATP,[165] introduction of a mutation to the Walker B motif eliminates the catalytic step and thus halts DNA translocation.

THE LEFT- AND RIGHT-HANDED CHIRALITY BETWEEN THE REVOLVING AND ROTATING MOTORS OFFERS ADDITIONAL DIRECTION CONTROL COUPLED WITH THE ARGININE FINGER

Sequential action of the arginine finger is critical for controlling the motion direction in the phi29 DNA package motor; however, an additional component, the 12-subunit connector, also plays a significant role in controlling the motion direction. For example, the chirality is the way to ensure "the push through a one-way valve".[193,196] Revolving motors show left-handed chirality, which is distinct from the right-handed chirality of rotating motors. Genomic dsDNA generally is B-type right-handed. The revolving of the dsDNA along the channel wall without self-rotating requires the surrounding track to have an opposite chirality to match contours of the DNA and the channel (Figure 8).[1,74] Hence, the left-handed channel wall is a necessary factor for a revolving motor, as it facilitates the threading motion of one strand of the dsDNA. This antichiral arrangement between the DNA helix and the channel is also seen in SPP1, T7, HK97, and P22 motors.[27,104−107] The connector channels in these motors are made up of 12 subunits that are oriented in a 30° tilt, leading to the opposite chiral arrangement to reach a configuration match during DNA translocation. Since the phage genome moves along the channel wall *via* only one strand in the 5′-3′ direction, as seen in Phi29,[2,37] the 30° tilt to the left ensures the continuous engagement and contact of this strand when the DNA shifts to the next subunit of the dodecamer. Three hundred sixty degrees in one turn during the 12-step motion results in 30° per step (360°/12 = 30°) (Figure 7).[1,74] This configuration avoids coiling and torsional

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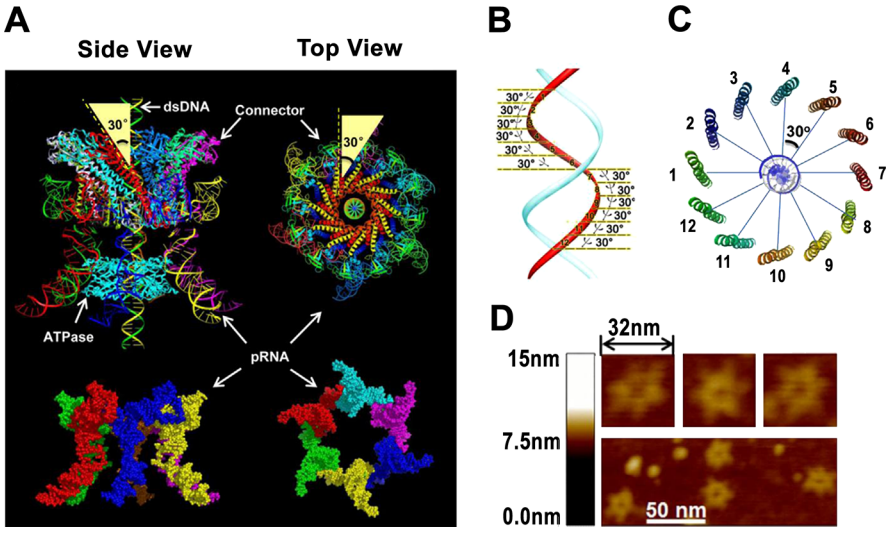

Figure 7. Structure of phi29 DNA packaging motor. (A) Structure of hexameric pRNA and the connector showing a 30° tilt. (B, C) dsDNA showing the shift of 30° angle between two adjacent connector subunits. Reprinted with permission from ref 56. Copyright 2013 Elsevier. (D) AFM images of hexameric pRNA with 7-nucleotide loops. Adapted with permission from ref 219. Copyright 2013 RNA Society.

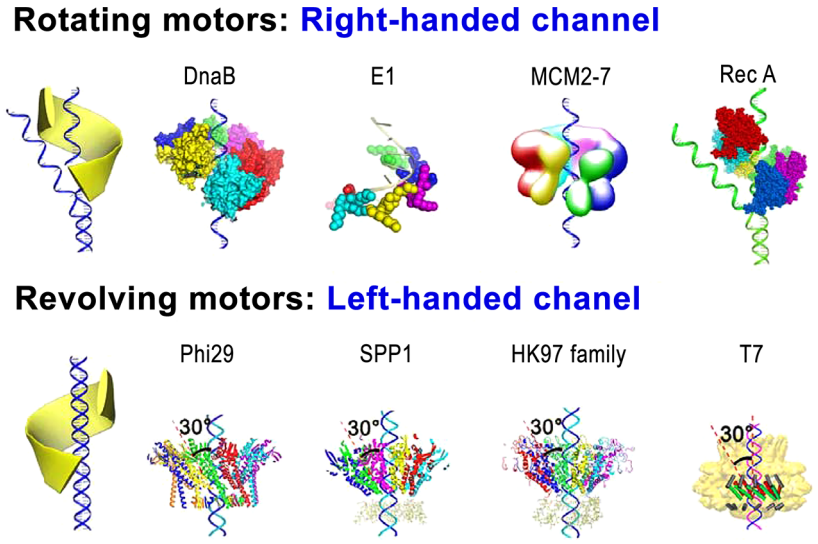

Figure 8. Different chiralities of rotating and revolving motors. Rotating biomotors exhibit right-handed chirality to drive the right-handed dsDNA similar to the nut driving the bolt or the screw driver turning the screw, whereas revolving biomotors exhibit left-handed chirality within the channel. Crystal structure analysis of viral DNA packaging motors reveals that this class of biomotors package DNA using the revolving mechanism. Reprinted with permission from ref 5. Copyright 2014 Springer Nature. Reprinted in part with permission from ref 74. Copyright 2014 Springer Nature.

forces as seen in rotating motors. Taken together, the left-handed antichiral arrangement of the motor channels of the revolving motor leads to a controlled threading motion of the substrate, supporting a revolving motor model (Figure 8 bottom).[74]

Although bacteriophage portal proteins from various families do not show significant sequence alignment nor similar size, they assemble to a similar overall structure. For example, bacteriophage Phi29, SPP1, and T7[104] have protein sizes of 36 kDa (Phi29 gp10),[107] 57 kDa (SPP1 gp6),[27] and 59 kDa (T7 gp8),[104] respectively. Bacteriophage P22 has a protein component, gp1, which is 94 kDa.[106] These portal proteins are all arranged in a propeller-like, 12-subunit structure with a central channel acting as a valve for DNA translocation. In addition to sharing similar three-dimensional structures, these bacteriophage motor proteins have analogous conserved regions that function in viral genome packaging. In nearly all portal proteins, the sequence stretch of α-β-α-β-β-α exists with a similar pattern of strands and helices and with similar spacing and length.

Analysis of the quaternary structures of various bacteriophages has revealed that the 30° tilted helix exists in all portal proteins. Evidence that the antichiral arrangement is integral in dsDNA packaging is seen in mapping studies, revealing that the 30° tilt occurs in the same conserved sequences in the last α helix of the α-β-α-β-β-α stretch. This highlights the importance of this 30° antichiral arrangement, as it has been conserved by evolution.[1] According to a parallel threading mechanism of bolt and nut,[1,74] rotating motors need to have right-handed channels in order to accommodate right-handed dsDNAs (Figure 8, top). Verification of the right-handed rotating motor is provided by crystal structures of helicase-

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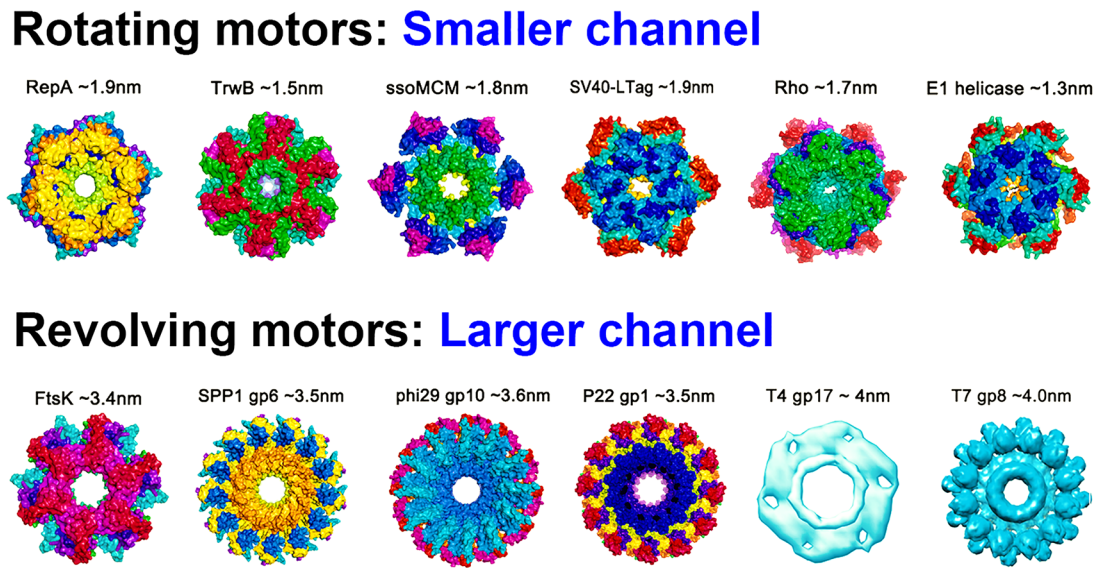

Figure 9. Channel size to differentiate rotating and revolving mechanism. Rotating motors have channel sizes all ≤2.0 nm in diameter to ensure full contact between DNA and channel wall similar to the nut driving the bolt, while revolving motors have channel sizes ≥3 nm to have room to accommodate the revolving motion. Reprinted in part with permission from ref 5. Copyright 2014 Springer Nature.

DNA complexes that have a right-handed spiral configuration.[48] This is seen in RecA filament and DnaB, which function in a nonplanar hexameric conformation.[48] In this rotating-like mechanism, for example, RecA monomers assemble into an open washer shape in a concatemeric arrangement.[197] ATPases, however, remain as a symmetrical closed ring in the absence of dsDNA.[110] E1 helicase also adopts a right-handed staircase conformation when bound with dsDNA.[198] Crystallographic studies provide evidence that right-handed motor complexes use the rotating mechanism.[197]

The mechanism for the packaging of viral double-stranded genome into the protein shell with the aid of an elegant motor is an intriguing subject.[1−3,20,199−204] Significant progress on the study of the mechanisms of viral DNA packaging motors has been achieved in the poxvirus,[76−80] adenovirus,[81−83] herpesvirus,[88−103] and minivirus.[2,84−87] Studies have revealed that the revolving mechanism is a common feature shared by all the dsDNA packaging motors, including SPP1, P22, T7, the HK97 family phage, and poxvirus evidenced by the results from both structural and biochemical studies. Analysis of crystal structures of the motor channels (the connectors) of SPP1,[27] T7,[104] HK97,[105] P22,[106] and Phi29[107] revealed that all of the motor channels displayed an antichiral arrangement between the channel and the DNA helices. The primary amino acid sequences are not conserved; however, the 3D structures of the swivels are both conserved and aligned.[1,74] Structural analysis of the SPP1 and Phi29 channels reveals unidirectional flow loops that function in the one-way trafficking of dsDNA. Layers of positively charged lysine residues,[193] representative of all phage channels, interact with the electronegative phosphate backbone of a single DNA strand. The effectiveness of the viral DNA packaging motor is due in part to the coordination of these complementary forces.

Revolving ATPase motors move along one strand of the dsDNA in the 5′ → 3′ direction.[3,37,74,110,205] RecA ATPases also move along in the 5′ → 3′ direction. Unlike the revolving motors and RecA ATPases, some rotating ATPases move in the 3′ → 5′ direction.[206] Whether the DNA strand polarity is relevant to the revolving or rotating mechanism remains to be elucidated.

REVOLVING AND ROTATING MOTORS CAN BE DISTINGUISHED BY THEIR CHANNEL SIZE

The arginine finger is critical for controlling the motion direction; however, how could the similar arginine finger control the two kinds of motors (rotating and revolving) that are very different in motion mechanism? The two differential motion mechanisms are also dictated by an additional motor structure factor: the channel size, which can be used to distinguish revolving motors from rotating ones. Channel size also plays an important role in controlling the one way motion and the motion direction. For rotating motors, their channel diameter should be no larger than 2 nm (the diameter of a dsDNA) to allow for close contact between a DNA and the channel wall for threading, since a ssDNA within the channel displays an A form helical structure and is smaller than 2 nm in diameter.[48] Examples include rotating motors of DnaB,[48] Rho factor,[50−52] TrwB,[60−65] MCM,[66] and RepA or RuvB,[68−73] all of which have a channel diameter of 1−2 nm.[74]

For revolving motors, such as a Phi29 DNA packaging motor, their channel diameter is generally larger than 3 nm. The larger channel size of the revolving motors allows a dsDNA to revolve around the channel wall, while precluding the possibility of a bolt and nut tracing mechanism, characteristic of rotating motors. Cryo-EM images of a tilted T7 dsDNA core relative to its axis reveal that in revolving motors, dsDNA advances by touching the channel wall rather than passing through the center of the channel.[207,208]

The difference in channel size has been confirmed by crystal structure analysis, cryo-EM measurement, and single-channel conductance assays. The diameter of dsDNA is 2 nm, while the diameters of the narrowest region of the connector channels of Phi29,[107] SPP1,[27] HK97,[105] the ATPase ring of T4,[33,205] as well as the dsDNA translocase FtsK[54] of bacteria, are all larger than 3 nm (Figure 9). To prove the revolving mechanism, the connector of bacteriophage Phi29 DNA packaging motor was used as the channel for the single pore translocation of folded,

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double-stranded, or tetra-stranded DNA. A current blockage of 32% was observed for translocation of dsDNA through the connector channel,[209] consistent with the ratio of the cross-sectional areas of dsDNA; $A = \pi r^2$, dsDNA ($(2/2)^2 \times 3.14 = 3.14$ nm^2), and channel ($(3.6/2)^2 \times 3.14 = 10.2$ nm^2, 10.2 nm^2/3.14 nm^2 = 32%). While for tetra-stranded DNA, passage through the connector channel of Phi29 yields a blockage of ~64%. This blockage data show that the cross-sectional area at the narrowest region of the Phi29 funnel is 3-fold the area of the dsDNA. The much larger width of the nut, in comparison to the bolt, precludes the possibility of a bolt and nut threading mechanism, but rather suggests that, at any translocation step, the dsDNA can be in contact with only one (or two) ATPase subunit.[74]

In contrast, the channels of rotating motors, such as replicative DNA helicases TrwB, E1, and DnaB,[48,64,167,210−212] are smaller than 2 nm in diameter (Figure 9). For these motors, the channel is expected to have a width similar to that of ssDNA. Nonetheless, for certain rotating motors, local unwinding fluctuations of the dsDNA lead to separation of the double helix, and thus only one strand enters the channel, while the other remains outside.[168,213−218] Given that the ssDNA within the channel displays an A form helical structure,[48] the channel diameter should be no larger than 2 nm so that the ssDNA can make full contact with the channel. Overall, the above data indicate that the revolving motor can be distinguished from the rotating motor by the size of their motor channels.

CONCLUSION

The arginine finger is an indispensable part of the ATP-activity pocket of the ring-shaped ATPase motors with revolving or rotating mechanisms. It is believed to be involved in initiating and coordinating the sequential action within the motor, which eventually leads to the pulling and pushing motions of the substrate during translocation. The arginine finger is also implicated to play a role in controlling the motion direction of the motor. All of these are achieved through a *trans*-action mechanism in promoting dimer formation, direct involvement in regulating ATP binding and hydrolysis, and allosteric effects associated with protein conformational changes.

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Notes

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ABBREVIATIONS

ATPase, a class of enzymes that catalyze the hydrolysis of ATP to provide the driving force for different kinds of mechanical motions essential to cellular functions; **biomotor mechanism**, concerns how biomotor proteins harness energy to drive the mechanical motions of their substrates; **arginine finger**, a particular arginine residue coordinated to the β- or γ-phosphate of ATP or interacting with some components/motifs in the ATPase catalytic reaction center; **Walker A motif and Walker B motif**, two conserved sequence motifs in ATPases responsible for ATP binding and ATP hydrolysis; **channel size**, refers to the diameter of the substrate translocation pore in biomotors, which can be used to distinguish revolving motors from rotating ones; **channel chirality**, the orientation of the pore-lining secondary structures tilted to either the left or the right, which is an attribute of asymmetry in many channel structures; **entropy driven**, the biological processes are driven by the increase or the decrease of entropy. High entropy refers to the product state that is conformationally more dynamic or disordered.

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