Structure and mechanism of the ATPase that powers viral genome packaging

Brendan J. Hilbert, Janelle A. Hayes, Nicholas P. Stone, Caroline M. Duffy, Banumathi Sankaran, and Brian A. Kelch

Many viruses package their genomes into procapsids using an ATPase machine that is among the most powerful known biological motors. However, how this motor couples ATP hydrolysis to DNA translocation is still unknown. Here, we introduce a model system with unique properties for studying motor structure and mechanism. We describe crystal structures of the packaging motor ATPase domain that exhibit nucleotide-dependent conformational changes involving a large rotation of an entire subdomain. We also identify the arginine finger residue that catalyzes ATP hydrolysis in a neighboring motor subunit, illustrating that previous models for motor structure need revision. Our findings allow us to derive a structural model for the motor ring, which we validate using small-angle X-ray scattering and comparisons with previously published data. We illustrate the model’s predictive power by identifying the motor’s DNA-binding and assembly motifs. Finally, we integrate our results to propose a mechanistic model for DNA translocation by this molecular machine.

Significance

Many viruses use a molecular motor to pump DNA into a preformed protein shell called the capsid, a process that is essential for the formation of infectious virus particles. The ATPase machine powering this process is the strongest known biological motor. However, the structure and mechanism of this motor are unknown. Here, we derive a structural model of the ATPase assembly using a combination of X-ray crystallography, small-angle X-ray scattering, molecular modeling, and biochemical data. We identify residues critical for ATP hydrolysis and DNA translocation, and derive a mechanistic model for the translocation of DNA into the viral capsid. Our studies introduce a model for ATPase assembly and illustrate how DNA is pumped with high force.

Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 4ZNI, 4ZNJ, 4ZNK, and 4ZNL).

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isolation to enable mechanistic analyses in a simplified system. We anticipated that TerL from a thermophilic phage would have higher stability, solubility, and propensity to form a pentamer. Moreover, we expected that the features necessary for DNA translocation would be more exaggerated in a thermophilic motor because packaging is expected to be more difficult at high temperature due to the increased entropic penalty of ordering DNA within the capsid (3).

Here, we describe the TerL protein from the phage P74-26 (hereafter known as TerLP74-26), a thermophilic siphovirus that infects Thermus thermophilus (25). We show that the TerLP74-26 assembles into a pentamer that has ATPase and DNA-binding activity. We report the structure of the TerLP74-26 ATPase domain in both the apo and ADP•beryllium-bound states, and observe a large conformational change in a subdomain in response to ATP hydrolysis and release. We also show that ATP hydrolysis is catalyzed by a conserved arginine finger residue that is provided in trans by a neighboring TerL subunit. Finally, a combination of biochemical, biophysical, structural, and computational data is used to build a structural model for the TerL ring and a mechanistic model for how ATP hydrolysis results in translocation of DNA.

Results

Characterization of TerLP74-26. We recombinantly expressed and purified TerLP74-26 in Escherichia coli (Fig. S1A). TerLP74-26 predominantly elutes in size exclusion chromatography (SEC) at a volume consistent with a pentamer [molecular mass by SEC (MMSEC) of ~270 kD, MMpentamer of 285 kD] with a small monomer peak (MMSEC of ~57.3 kD, MMmonomer of 57.1 kD) (Fig. S1B). This observation is notable, because other TerL proteins are predominantly monomeric in isolation (16–19), only forming a pentameric ring when bound to the procapsid (8). TerLP74-26 is an active ATPase that exhibits apparent cooperativity in ATP hydrolysis, suggesting that the active sites are coupled (Fig. S1C–E). TerLP74-26 tightly binds DNA when locked into an ATP-bound state by the ATP mimic ADP•beryllium trifluoride (BeF₃) (26, 27) (Fig. S1F). Thus, because the isolated TerLP74-26 protein can assume a pentamer consistent with its functional form, we argue that TerLP74-26 is an excellent minimal model for understanding packaging motor structure.

Structure of the TerL ATPase Domain. We next sought to elucidate the structural mechanism of genome packaging by TerLP74-26. Because DNA translocation is ATP-dependent, considerable insight can be obtained from the structure of the isolated ATPase domain. Therefore, we expressed, purified, and crystallized a construct that corresponds to the N-terminal ATPase domain of TerLP74-26 (residues 1–256; Fig. 1B). The structure in the absence of nucleotide was determined by single-wavelength anomalous dispersion (SAD) (28) using selenomethionine (SeMet)-labeled protein, and phases were extended to 2.1 Å using diffraction data from native protein (Table S1). A second crystal form yielded another structure of the apo TerLP74-26 ATPase domain to 1.9 Å. The structures from each crystal form are very similar (C₅ rmsd of 0.8Å; Fig. S24 and Table S2). Unlike the full-length TerLP74-26, the isolated ATPase domain does not form a pentameric arrangement in either crystal form.

We note three prominent features of the TerLP74-26 ATPase domain (Fig. 2). First, TerLP74-26, as in other terminase ATPase domains (20, 21), contains a C-terminal subdomain (residues 221–251 in TerLP74-26) that sits above the ATPase active site. We call this region the “lid” subdomain with reference to the structurally unrelated but analogous lid subdomains found in other ASCE ATPases (29). Lid subdomains are often used in contacts between adjacent subunits in ASCE oligomers (11, 30), and their conformation can be modulated by ATP hydrolysis (11, 31). Second, we observe a large patch of six basic residues (R100, R101, R102, R104, R128, and K130) in the turns and strands of the antiparallel section of the beta-sheet, a region that is specific to the terminase ASCE subfamily (6). A basic residue is conserved at the Arg101 position in the other large terminase structures [Lys223 and Arg82 in T4 (8) and Sf6 (20), respectively] (Fig. S2C). We hypothesize that this region binds to the DNA backbone...
during packaging. In support of this hypothesis, we observe a sulfate ion bound to Arg101 in all of our crystal forms. The third region of note is a conserved arginine that is on the opposite face from the active site. We propose that this residue is used to activate ATP hydrolysis in a neighboring subunit in the assembly (discussed below).

To determine the conformational changes induced by ATP binding, we solved a 2.0-Å crystal structure of the TerLP74-26 ATPase domain cocrystallized with the ATP mimic, ADP-BeF₃. The packing of the ADP-BeF₃ cocystal is unrelated to the apo crystals, with three ADP-BeF₃-bound TerLP74-26 ATPase domains in the asymmetric unit (Fig. S2B). The residues of the Walker A and B motifs are in the active conformation (Fig. 3A). The largest conformational change between the apo and ADP-BeF₃ states occurs in the lid subdomain. Upon binding the ATP analog, the lid rotates ∼13° in a rigid body motion (Fig. 3B and Movie S1). Upon binding the ATP mimic, the P-loop residues Arg39 and Gln40 rotate to contact the gamma-phosphate mimic directly. The P-loop’s interactions with several lid residues (Ser221, Thr225, Arg228, and Tyr232) result in a commensurate rotation of the lid toward the ATP adenine ring (Fig. 3C). Thus, the motion appears to be induced by the P-loop, which bridges the gamma-phosphate mimic and residues at the base of the lid. The rotation of the lid upon ATP binding results in a reorientation of the lid’s hydrophobic residues such that they are now less solvent-exposed, potentially altering TerL’s interactions within the motor (Fig. 3D).

**Identification of the Trans-acting Arginine Finger.** The structures of TerL ATPases reported here and elsewhere (20, 21) display incomplete active sites, presumably because all were crystallized without formation of an active ring. These monomeric TerL structures have only two positively charged residues within the P-loop that contact the gamma-phosphate of ATP to stabilize the transition state for hydrolysis. However, other ASCE family members have at least three positive residues that contact the gamma-phosphate (11–15, 31, 32). Indeed, our ATPase domain construct lacks ATPase activity (Fig. S3A), confirming that the active site in our structure is incomplete. In ASCE-type ATPases, the third positive charge is provided in trans by a residue known as the “arginine finger,” which is most often arginine, although it can also be lysine (29). The location of the arginine finger within the Rossman fold varies widely across the ASCE family, which means that ASCE subfamilies often have different relative orientations of their ATPase domains within the oligomer (33). Thus, the identification of the trans-acting arginine finger in TerL is necessary both for elucidation of the ATPase mechanism and for determining the architecture of the TerL ring assembly.

We used a two-step strategy to identify the arginine finger of TerLP74-26. First, we screened mutants of surface-exposed arginines in full-length TerLP74-26 for ATPase activity. For mutants that showed no ATPase activity in this initial scan, we tested whether an inactive TerLP74-26 protein (mutated Walker B motif) could restore activity by donating its intact arginine finger.

We measured steady-state ATPase activity for 11 different arginine mutants in full-length TerLP74-26 (Fig. 4A). The R39A, R139A, and R235A variants completely abrogate ATPase activity, making them candidates for the arginine finger. Of these candidate residues, Arg39 and Arg235 reside within or near the active site, suggesting that they may affect catalysis in cis. Conversely, Arg139 is located ∼25 Å away from the gamma-phosphate of ATP (Fig. 2).

To assess which of our candidates is the arginine finger, we developed a biochemical complementation assay. We mixed each of our three candidate arginine finger mutants with the E150A variant whose active site is disabled in cis (Fig. S1C) but can still donate an intact arginine finger to an adjacent subunit in the assembly. Thus, E150A should restore activity to an arginine finger mutant, but not to a mutant whose ATPase activity is disabled in cis. Mixing E150A with R139A results in restoration of ATPase activity, whereas mixtures of E150A with R39A or R235A exhibit no significant ATP hydrolysis (Fig. 4B). ATPase activity is maximal at a 1:1 ratio of E150A to R139A (Fig. S3B), as expected for complementation of an arginine finger mutant. In contrast, a double mutant combining both the R139A and E150A mutations in the same protein has no activity, and cannot be complemented by either the E150A or R139A single mutant. Our complementation results mirror the results of similar experiments from Cox et al. (34) definitively establishing that the ASCE family member RecA uses trans-interactions to catalyze ATP hydrolysis.
We verified that the R139A mutation does not perturb ATPase domain structure by solving the 2.5-Å resolution structure of the isolated ATPase domain with the R139A mutation. This structure is essentially identical to the WT structure (Cα rmsd = 0.2 Å; Fig. 3C), suggesting the observed deficit is not due to structural changes in cis. In support of this finding, we observe that arginine or lysine is conserved at this position in the large terminase family (Fig. 4C and Fig. S3D). Hence, we expect that the arginine finger is playing a similar role throughout the family of large terminases. Taken together, our results indicate that Arg139 is the arginine finger, which mediates ATP hydrolysis in TerL in trans. Our identification of the arginine finger illustrates that (i) ATP hydrolysis is catalyzed in trans and (ii) the previously proposed structural model (8) must be substantially refined to account for these trans-interactions within the TerL ring.

Modeling of the TerL Ring. Our two key results, the TerLP74-26 ATPase domain structure and identification of the arginine finger residue, allow us to create a model for the TerL ring assembly. The structure provides a basis for molecular docking, and the arginine finger provides a spatial restraint that must be satisfied for the TerL assembly. We used the program M-ZDOCK (35, 36) to model the TerL ATPase ring. Fivefold symmetry was the only constraint applied. We used the trans-arginine finger interaction to validate the docking results independently. Although many ATPase rings are asymmetrical during function (12, 37), we assume fivefold symmetry for simplicity. Regardless, most ringed ATPase structures were first modeled as symmetrical assemblies (32, 38), and these models were refined later to show asymmetry during function (9, 10, 12, 37). After docking of the ATPase ring, we used the full-length crystal structure of T4-TerL (8) to orient a homology model of the P74-26 nuclease domain, which results in minimal steric clashes between the two domains. The soft energetic potential used in M-ZDOCK results in slightly shorter interatomic distances (35), and therefore a constricted ring; thus, we treat the molecular docking results in a qualitative fashion.

Molecular docking produced a TerL assembly that is consistent with our identification of the arginine finger. Arg139 contacts the ATP gamma-phosphate in a neighboring subunit, despite no restraint for this interaction imposed during the calculation (Fig. 5 A and B). Thus, these models satisfy a critical spatial interaction identified by our biochemical results. The lid subdomains interact with an adjacent subunit, with the nuclease domains arranged radially (Fig. 5C).

We tested our model’s plausibility by comparing our TerL assembly with other TerL crystal structures and a cryo-EM reconstruction. First, orthologous TerL ATPase crystal structures (8, 20) can be identically positioned without significant steric clashes of the ATPase domains (Fig. S4 A and C). In fact, the N-terminal extension that is unique to T4 is placed on the outside of the ring rather than lining the pore of the ring as in the previous proposal (8). Moreover, the pores of our Sf6-TerL and T4-TerL ATPase models are positively charged (Fig. S4 E and F). This fits the ATPase domain ring distortion observed in our recent reconstruction (41). Third, our T4-TerL ring model is a bowl-like as-
ab initio calculation of the TerLP74-26 molecular envelope using our SAXS data and imposing fivefold symmetry. The calculated molecular envelope fits the scattering data well ($\chi^2 = 0.79$) and is roughly shaped like a bowl, with a pore in the center and a V-shaped cross-section. The overall bowl-like shape of the TerLP74-26 ring model fits the SAXS reconstruction well; the ATP ligand is shown as colored spheres. (B) Side view of the TerLP74-26 ATPase ring derived from symmetry-constrained docking. Colors and the image are produced as in A. (C) Schematic depiction of the TerL ring. The TerL ring is shown from the perspective of the pore, with the ring artificially “broken” and flattened on the page so that all subunits can be viewed simultaneously. Each subunit is colored as shown in other panels in this figure. The nucleosome domains are depicted as translucent squares to represent the ambiguity of the placement of the nucleosome domain within the TerL ring. The loops protruding from the sides of each subunit represent the conserved basic patch (Arg101 in TerLP74-26). The gamma-phosphate of ATP is contacted by the arginine finger from a neighboring subunit.

We tested various aspects of our structural model to determine whether it has predictive power. Our model predicts that the lid’s hydrophobic patch (Trp231 and Tyr238) contacts an adjacent subunit for arginine finger positioning (Fig. 6B). To test the hypothesis that these residues are important for ATPase activity, we individually mutated Trp231 and Tyr238 to alanine. Mutation at each of these residues results in severe loss of ATPase activity (Fig. 6C). Thus, the lid’s hydrophobic patch is critical for TerL activity.

As an additional test of our model’s predictive power, we used our model to identify the DNA-binding motif. Basic residues line the pore of the TerL ring, with a central pore residue (Arg101) that is conserved (Fig. 6D and Fig. S2C). A positive electrostatic environment in the pore is similar to other ASCE nucleic acid translocases (12, 13, 15). We hypothesize that Arg101 binds DNA during packaging. To test this hypothesis, we mutated several surface arginines in full-length TerLP74-26 and measured the ability of these mutants to interact with DNA in the presence of ADP•BeF₃ (Fig. 6E). Mutation of Arg101 causes loss of DNA binding, whereas mutation of residues distal to the pore (Arg39 and Arg58) has no measurable effect (Fig. 6F). Although a defect in binding ATP could cause loss of DNA binding, the R101E mutant retains WT levels of ATPase activity (Fig. S5D), indicating that the DNA-binding defect is not due to loss of ATP.

Fig. 5. Derivation of the TerLP74-26 ring assembly structure. (A) Model of the TerLP74-26 ATPase ring derived from symmetry-constrained docking. A bottom-up view shows each of the five subunits in different colors, with the ATP lid in green. The arginine finger and the conserved basic site (Arg101) are shown as colored spheres. (B) Side view of the TerLP74-26 ATPase ring derived from symmetry-constrained docking. Colors and the image are produced as in A. (C) Schematic depiction of the TerL ring. The TerL ring is shown from the perspective of the pore, with the ring artificially “broken” and flattened on the page so that all subunits can be viewed simultaneously. Each subunit is colored as shown in other panels in this figure. The nucleosome domains are depicted as translucent squares to represent the ambiguity of the placement of the nucleosome domain within the TerL ring. The loops protruding from the sides of each subunit represent the conserved basic patch (Arg101 in TerLP74-26). The gamma-phosphate of ATP is contacted by the arginine finger from a neighboring subunit.

Fig. 6. Validation of the TerL ring assembly model. (A) Model of TerLP74-26 fits the ab initio SAXS envelope. The modeled TerLP74-26 ring structure (colored cartoon) is superposed onto the SAXS envelope calculated using GASBOR (47) (imposing fivefold symmetry). Dummy atoms for the SAXS envelope are shown as gray spheres. (B) Hydrophobic patch on the lid subdomain mediates critical intersubunit interactions. The structural model suggests that Trp231 and Tyr238 of the lid hydrophobic patch (ball-and-stick representation) mediate critical interactions with a neighboring subunit for positioning the arginine finger. (C) TerLP74-26 lid hydrophobic patch is critical for TerL function. Steady-state ATPase activity is shown for 0.5 μM TerL. Mutation of either W231 or Y238 results in a large decrease in ATPase activity. Error bars are the SD from at least three replicates. (D) Electrostatic map of the docked TerLP74-26 ATPase ring, with positive and negative surface potentials shown in blue and red, respectively. Note the positive charge lining the pore of the TerLP74-26 ATPase ring. The electrostatic surface was calculated using the APBS plug-in for PyMOL (DeLano Scientific) (48). Figure is colored by electrostatic potential (kBT/ec) as indicated. (E) DNA binding for multiple arginine mutants. EMSA was carried out as in Fig. S1F, including several arginine mutants. Arg101 is required for DNA binding, whereas Arg39 and Arg58 are dispensable. (F) Mutation of the conserved pore arginine abrogates DNA binding. The ability of three different arginine mutants to bind DNA tightly in the EMSA assay was mapped onto the model of the TerLP74-26 ring. As predicted by the structural model, Arg101 is necessary for DNA binding and mutations distal to the pore (R39A and R58A) have no measurable effect on DNA binding. A 100-bp DNA ladder (New England Biolabs) is used as a standard.
binding. Thus, we have identified the critical DNA-binding motif in TerL, supporting our structural model for the TerL ring.

Discussion

Our results suggest a substantially different organization of the TerL ring than the initial model that was proposed previously in a ground-breaking study of the T4 terminase. The previous model, which is based on fitting the T4-TerL crystal structure to a cryo-EM reconstruction with a resolution of 34 Å, proposed that the TerL ATPase domains form a ring that contacts portal through the lid subdomains (8). The pore of the ATPase ring has a net negative charge and is lined by a portion of TerL that is unique to T4. The nuclease domain was proposed to form a ring distal to portal that grips and translocates DNA through the pore. Because the ATPase active sites do not contact a neighboring subunit, Sun et al. (8) proposed that an invariant arginine in the P-loop (Arg162 in T4 TerL, Arg39 in TerLP74-26) acts as the arginine finger to catalyze ATP hydrolysis.

Our identification of the conserved trans-acting arginine finger indicates that the previous model requires significant modification. Although the P-loop arginine previously proposed as the cis-acting arginine finger is necessary for packaging (42) and ATPase activity (8) (Fig. 4-I), our data do not support the hypothesis that it is the arginine finger. Instead, we propose that this residue, Arg39 in TerLP74-26, is conceptually analogous to the sensor II arginine found in the family of ATPases known as the ATPases associated with diverse cellular activities (AAA+), which aids ATP hydrolysis in cis and confers movement of the AAA+ lid subdomain upon ATP hydrolysis (11, 31). In order for the previous model to accommodate the trans-acting arginine finger, a rotation of the ATPase domain of ∼110° is necessary. Although formally possible, this rotation of the ATPase domain does not fit the cryo-EM density for the actively translocating motor (8) and would disrupt portal interactions. Therefore, we disfavor this model.

Our docked model for the TerL ring satisfies the distance constraint imposed by the arginine finger and revises the orientation of TerL relative to portal. We propose that the nuclease domains form a radially arranged ring that is proximal to portal, whereas the ATPase ring is distal. Our model positions the lid subdomains at the interface between adjacent ATPase subunits, where they assist in positioning the arginine finger, as supported by our mutagenesis data (Fig. 6C). In the previously proposed model, the lid residues interact with portal (Fig. S5 E and F). However, because our sample lacks portal, the observed ATPase defects in lid mutants are not due to a disruption of portal interactions, but are consistent with our proposed role in TerL assembly.

Our updated model accounts for functional conservation across the TerL family. First, our model contains a largely basic patch lining the pore. Within this patch, we identify Arg101 as a critical component of the DNA-binding motif. With conservation of a basic residue at this position, our model is congruent with the terminase family. Notably, in the previously proposed structural model, the equivalent residue in T4-TerL is positioned distal to the pore and the nuclease domain was hypothesized to bind DNA (8) (Fig. S5 E and F), both of which are inconsistent with our observations. Second, our identification of the arginine finger in TerL brings the mechanism of ATP hydrolysis into accord not only with the terminase family but also with the rest of the ASCE family. Conservation of the DNA-binding and catalytic mechanism therefore supports our updated structural model.

Although our model allows accurate predictions for regions of function, the overall model is qualitative in nature. As previously mentioned, the ring dimensions are slightly constricted due to the docking algorithm, with the pore’s smallest inner diameter measuring ∼16 Å, as calculated from the Cβ positions of surface-exposed arginines lining the pore (we use the Cβ position because it is rigidly fixed). Thus, the modeled pore is too small to accommodate dsDNA. We propose that the TerL ring is expanded relative to the docked model because the docked model exhibits contacts that are clearly too close with several overlapping atoms, which would artificially constrict the ring. Furthermore, our model positions the nuclease domain based on the full-length T4-TerL structure, which crystallized as a monomer (8). The nuclease domain position may be ortholog-dependent or may be altered upon ring formation and/or upon portal binding. Nuclease position is important because the nuclease domain may be playing a key role in pentamer assembly, considering that the isolated TerL(P74-26) ATPase domain crystallizes as a monomer. Although ongoing and future investigations will refine the details of our structural model, we have established that it captures essential aspects of the TerL ring through its consistency with prior data, as well as its predictive power. Our structural model, combined with our nucleotide-dependent structural changes, allows us to derive a preliminary mechanistic model for DNA translocation in this family.

Based on our TerL ring model and the observed conformational changes upon ATP hydrolysis and release, we propose a mechanistic model for DNA translocation. During genome packaging, DNA is gripped in the center of the TerL ring. In this model, it is assumed that the TerL ring hydrolyzes ATP one subunit at a time, and not in a concerted all-or-none mechanism.

![Fig. 7. Proposed model for the mechanism of DNA translocation by TerL.](image-url)
This assumption is consistent with the mechanism of ATP hydrolysis in other ringed ASCE ATPases (12, 13, 15, 43). When one subunit hydrolyzes ATP, it undergoes a conformational rearrangement such that the lid pivots 13° around the Rossmann fold (Fig. 3B). If we further assume that the lid remains bound to the neighboring subunit throughout packaging, as seen in homologs (44), the result would be the Rossmann fold pivoting outward and upward toward the capsid (Fig. 7D, Movie S2).

We propose that the conformational change from one ATP hydrolysis event propagates to an adjacent subunit, sterically exerting force on the adjacent subunit such that the two subunits move in concert. Because TerL only binds DNA tightly in the ATP-bound form (Fig. S1F), ATP hydrolysis at one subunit will lead to that individual subunit releasing DNA. During a hydrolysis event, the ATP-hydrolyzing subunit loses its grip on DNA but initiates motion that is propagated to the adjacent ATP-bound subunit, which is still gripping DNA through interaction with Arg101. The conformational change of the ATP-hydrolyzing subunit results in an upward translocation motion of DNA at the adjacent subunit and resets the motor for unidirectional translocation. We estimate that this motion would translocate DNA perpendicularly to the plane of the ring by ~8 Å per hydrolysis event, or about 2.4 bp. In addition, we predict that DNA would rotate in the plane of the ring by ~2.3° for each step. Because our model is qualitative, our estimated step rotation and size should be viewed with reservation. However, these values compare favorably with the 2.5-bp translocation (43) and ~3.5° rotation per step (45) measured for the phi29 motor at low packaging force. Our proposed “lever-like” mechanism for force generation is in contrast to the previous model, wherein a “spring-like” motion of DNA-bound nuclease domains translocates DNA through the pore (8). Because several other studies have evaluated DNA packaging in the context of the previous TerL structural and mechanistic models, our work illustrates that these studies should be reinterpreted within the context of the updated TerL model.

It is unclear how this conformational change will affect subunits further downstream than the two moving subunits. The breakage of symmetry at the ATP hydrolysis site necessitates at least one other site of symmetry breaking to maintain a closed ring structure. Alternatively, the TerL assembly may form an open lock washer shape that dynamically alternates which subunits cap the ends of the lock washer, as has been proposed for the ASCE helicase DnaB (12). Although our current model is fivefold symmetrical, recent studies of ASCE family members illustrate that significant asymmetry exists during motor function (12, 13, 37). Future refinement of our structural model will identify how TerL symmetry drives DNA translocation.

Materials and Methods

Cloning, Expression, and Purification of TerLP74-26. The TerLP74-26 gene was synthesized by Genscript Corporation and subcloned into a modified pET24a vector (Novagen). Standard procedures. TerLP74-26 was recombinantly expressed in BLR(DE3) E. coli cells and purified by nickel affinity, ion exchange, and SEC (details are provided in SI Materials and Methods).

ATPase and DNA-Binding Assays. ATPase activity was measured using a standard coupled enzyme assay. DNA binding was measured by EMSA (details are provided in SI Materials and Methods).

Cristallization, Diffraction Data Collection, SAXS, and Docking. Semet-labeled, apo, and ADP•BeF₃-bound TerLP74-26 ATPase domain were crystallized with the hanging drop vapor diffusion method in solutions containing buffered sodium sulfate. Crystals were frozen in a cryogenic buffer containing elevated respective precipitant concentrations supplemented with 30% (v/vol) ethylene glycol. Diffraction data were collected at Advanced Light Source (ALS) beamline 5.0.1, at Advanced Photon Source beamline 23-ID-B, at Brookhaven National Laboratory beamline X25, or using a home source. SAXS data were obtained on filtered R104E-TerLP74-26 at the Structurally Integrated Biology for Life Sciences (SiBYS) beamline at the ALS. The TerLP74-26 ATPase ring was modeled using M-ZDOCK (35) (details are provided in SI Materials and Methods).

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Supporting Information

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SI Materials and Methods

Cloning. The gene for TerLP74-26 was synthesized with codon optimization for expression in *E. coli* by Genscript Corporation. This gene was cloned into the BamHI and XhoI sites of a modified pET24a vector with an N-terminal T7-gp10 expression tag and prescision protease cut site, and a C-terminal non-cleavable His10 tag (31). Site-directed mutagenesis was performed using a mutagenesis protocol similar to QuikChange (49). Enzymes were purchased from New England BioLabs. Oligonucleotides were obtained from IDT. The ATPase domain was constructed by deleting the codons encoding the C-terminal 229 residues using the aforementioned mutagenesis protocol (49). This deletion site was chosen based on limited proteolysis and bioinformatic analysis (data not shown).

Expression and Purification of TerLP74-26. All proteins were expressed in *E. coli* BLR-DE3 cells containing the pET24a-TerLP74-26 plasmid. Bacterial cultures were grown in Terrific Broth (Research Products International) supplemented with 30 μg/mL kanamycin at 37 °C until an OD600 of 0.7 was reached. Cells were then placed at 4 °C for 20 min, after which overnight expression at 18 °C was induced by the addition of isopropyl-β-D-thiogalactopyranoside to 1 mM. Cells were pelleted by centrifugation and resuspended in buffer A [500 mM NaCl, 20 mM imidazole, 50 mM Tris (pH 8.5), 5 mM β-mercaptoethanol (βME), 10% (vol/vol) glycerol] before being flash-frozen in liquid nitrogen for storage. BLR-DE3 cells with SeMet-labeled ATPase domain (residues 1–256) were grown in minimal media. When cultures reached an OD600 of 0.5, they were supplemented with amino acids, except for Met, and grown to an OD600 of 0.8. Cultures were then supplemented with SeMet. Induction and harvesting were performed as above.

For full-length constructs, thawed cells were lysed in a cell disruptor and pelleted to clear the lysate. Lysate was loaded onto a 10-mL centrifuge spin-column (Thermo Scientific) containing Ni-affinity beads (Thermo Scientific) preequilibrated with buffer A. Protein was eluted with buffer B [500 mM NaCl, 500 mM imidazole, 50 mM Tris (pH 8.5), 5 mM βME, 10% (vol/vol) glycerol] and dialyzed overnight at room temperature into buffer QA [25 mM Tris (pH 8.5), 125 mM NaCl, 2 mM DTT, 10% (vol/vol) glycerol]. Before dialysis, prescision protease was mixed with eluate to cleave the T7 expression tag. The sample was then loaded onto spin-columns containing Q resin (GE Healthcare) preequilibrated with buffer QA. After protein loading, the Q resin was washed with buffer QA and contaminants were eluted with buffer QA1 [25 mM Tris (pH 8.5), 200 mM NaCl, 2 mM DTT, 10% (vol/vol) glycerol]. Protein was eluted with buffer QB [25 mM Tris (pH 8.5), 450 mM NaCl, 2 mM DTT, 10% (vol/vol) glycerol] and dialyzed overnight at room temperature into buffer QA before concentrating.

For purification of the TerL-ATPase domain, cells were lysed and pelleted as above. After pelleting, all purification steps proceeded at room temperature. Lysate was loaded onto a preequilibrated 5-mL His-Trap column (GE Healthcare). The column was washed with buffer A and eluted with buffer B. Eluate was diluted fourfold with buffer A, and prescision protease was added. The protein was dialyzed overnight in buffer QA. The protein was then filtered and flowed over a 5-mL HiTrap Q-HP column (GE Healthcare) preequilibrated in buffer QA. The flow-through was collected, concentrated, and flash-frozen.

Coupled Enzyme ATPase Assays. Coupled enzyme assays were performed at room temperature (50 °C) using the following concentrations (unless otherwise noted): 6 U/mL pyruvate kinase, 6 U/mL lactate dehydrogenase, 1 mM phosphoenolpyruvate, 340 μM NADH, 50 mM Tris (pH 7.5), 500 μM Tris(2-carboxethyl)phosphine, 100 mM potassium chloride, 5 mM magnesium chloride, 1 mM ATP, and 0.5 μM TerL. Absorbance was measured in a 96-well format with a Perkin–Elmer Victor 1420 multichannel counter using an excitation filter centered at 355 nm, with a bandpass of 40 nm. In this assay, every NADH oxidized to NAD⁺ corresponds to one ATP hydrolyzed. To convert the measured absorbance directly to ATP concentration, we performed a standard curve so as to measure the NADH extinction coefficient directly under our experimental conditions.

To understand how TerL concentration affects ATPase activity, we measured ATPase activity over a wide concentration range of TerL. The rise in ATPase rate is slow and nonlinear at low TerL concentrations, but it increases linearly at higher concentrations (Fig. SI D). The nonlinear activity at low TerL concentrations is not due to the limits of our assay, because we are able to measure ATPase rates reliably below the rate observed here using a different ATPase. The slow increase in ATPase rate at low concentration is most likely due to assembly of a TerL pentamer. It is noteworthy that the gp16 ATPase from the packaging motor of the phi29 phage shows a similar nonlinear increase in ATPase rate (51), suggesting that an assembly of the ATPase subunits may be a necessary step in full ATPase activity for other viral motors. Regardless, the experiments that follow were performed at a concentration of TerL (0.5–1 μM) that is well within the linear range. Data comparing the rate of ATP hydrolysis to [ATP•MgCl₂] were fit using the Hill equation: V = \( V_{\text{max}} \cdot [\text{ATP}\cdot\text{MgCl}_2]^n/(K_{\text{half-maximal}}^n + [\text{ATP}\cdot\text{MgCl}_2]^n) \), where \( V_{\text{max}} \) is the maximum rate, n is the Hill coefficient, and \( K_{\text{half-maximal}} \) is the substrate concentration that yields half-maximal activity.

DNA-Binding Assays. Full-length WT (0.13 nmol) or mutant TerLP74-26 (0.13 nmol) was incubated with 125 ng of 100-bp ladder (New England BioLabs) for 30 min at room temperature in apo, ADP, ADP•BeF₃, or ATP buffer as indicated. Apo buffer was composed of 50 mM Tris (pH 7.5), 150 mM KCl, and 1 mM DTT. ADP buffer was composed of 50 mM Tris (pH 7.5), 150 mM KCl, 1 mM DTT, 1 mM ADP, and 10 mM MgCl₂. ADP•BeF₃ buffer was composed of 50 mM Tris (pH 7.5), 150 mM KCl, 1 mM DTT, 1 mM ADP, 10 mM NaF, 4 mM BeCl₂, and 10 mM MgCl₂. ATP buffer was composed of 50 mM Tris (pH 7.5), 150 mM KCl, 1 mM DTT, 1 mM ATP, and 10 mM MgCl₂. Samples were then loaded onto a 1.5% (wt/vol) agarose gel with a 1:14,000 dilution of GelRed dye (Phenix Research) and run at 80 V for 2–2 h.

Crystallization. SeMet-labeled, apo, and ADP•BeF₃-bound TerLP74-26 ATPase domain were crystallized with the hanging drop vapor diffusion method. Drops were set with final protein concentrations of 3–8 mg/mL apo, SeMet-labeled, and ADP•BeF₃-bound samples were preincubated with 10 mM magnesium chloride and 10 mM DTT before crystallization. The R139A variant was preincubated only with DTT. ADP•BeF₃ crystals were preincubated with 2 mM ADP, 10 mM sodium fluoride, and 4 mM beryllium fluoride. ADP (2 mM) was preincubed with SeMet-labeled protein. SeMet-labeled crystals formed in 0.9 M ammonium sulfate and 0.1 M trisodium citrate (pH 5.0). Cubic apo crystals formed in 0.7 M ammonium sulfate and 0.1 M
trisodium citrate (pH 5.0). Apo R139A cubic crystals formed in 0.5 M ammonium sulfate and 0.1 M trisodium citrate (pH 4.5). Hexagonal apo crystals formed in 11% (wt/vol) PEG 2000, 0.15 M ammonium sulfate, and 0.1 M trisodium citrate (pH 5.0). ADP•BeF₃-bound crystals formed in 19% (wt/vol) PEG 3350, 0.05 M ammonium sulfate, and 0.1 M trisodium citrate (pH 5.0). Single crystals formed only after microseeding. Cryogenic buffer conditions for each crystal type consisted of elevated respective precipitant concentrations supplemented with 30% (vol/vol) ethylene glycol. Cryogenic conditions also contained all reagents that were premixed with each respective protein sample before crystallization.

**Data Collection and Structure Solution.** SeMet and apo ATPase domain datasets were collected at ALS beamline 5.0.1 at a wavelength of 0.9774 Å. SeMet structure experimental phases were calculated by SAD (28) using the PHENIX Autosolver pipeline (52). The SeMet model was used for molecular replacement for the apo structure. The second apo ATPase crystal data were collected at Advanced Photon Source beamline 23-ID-B at a wavelength of 1.033 Å. ADP•BeF₃ crystal data were collected at Brookhaven National Laboratory beamline X25 at a wavelength of 1.100 Å. ADP•BeF₃ data were truncated due to anisotropy in the diffraction. After scaling, data were processed using the University of California, Los Angeles Molecular Biology Institute Diffraction Anisotropy Server (53). The apo R139A mutant crystal data were collected on a home source MicroMax007-HF/ Saturn 944 CCD detector X-ray diffraction system at a wavelength of 1.54 Å. A C-terminal–truncated apo structure was used for molecular replacement. All datasets were processed with HKL3000 (54). Model building and refinement were performed with Coot (55) and PHENIX (56).

**SAXS Data Collection and Analysis.** Samples were dialyzed into buffer [25 mM Tris (pH 8.5), 125 mM NaCl, 4 mM DTT, 2% (vol/vol) glycerol] using dialysis buttons (Hampton Research) and filtered through a 0.45-μm membrane (Millipore). WT TerL₉₄₋₂₆ exhibited low levels of aggregation, which hampered interpretation of data. We hypothesized that the basic patch (Fig. 2) contributes to the aggregation of TerL₉₄₋₂₆. To avoid aggregation, we made the R104E mutant. Indeed, this mutant shows no signs of aggregation (Fig. S5 A–C). Samples were shipped at 4 °C to the ALS, Lawrence Berkeley National Laboratory, for data collection at the SIBYLS beamline (57). Data were collected at 10 °C with exposures of 0.5 s, 1 s, and 6 s and at several concentrations of TerL, and buffer blanks were subtracted to give protein scattering. Due to radiation sensitivity at longer exposures, only the 1-s exposure was used for analysis. Data were processed using the ATSAS software package (58). The SAXS reconstruction was calculated using GASBOR (47) in real-space mode using fivefold symmetry. The docked model was optimally fit to the SAXS envelope by converting the GASBOR dummy atoms to a map using the “molmap” and “fit to map” commands in UCSF Chimera (59).

**Molecular Docking and Modeling of the TerL₉₄₋₂₆ Ring.** The model of the TerL₉₄₋₂₆ ATPase ring was calculated with the ADP•BeF₃-bound structure of the TerL₉₄₋₂₆ ATPase domain using M-ZDOCK (35). Fivefold symmetry was applied, but no other restraints were imposed during the docking. To model the TerL₉₄₋₂₆ nuclease domain, we used I-TASSER (60) to generate a homology model with the nuclease domains of Sf6-TerL and T4-TerL as structural templates. To model the full-length ring, we aligned the full-length T4 structure (8) [Protein Data Bank (PDB) ID code 3CPE] onto the ATPase domains of our model and then superposed the TerL₉₄₋₂₆ nuclease homology model onto the T4 nuclease domains.

To model the potential conformational changes that drive DNA translocation within the ring upon ATP hydrolysis, we first positioned the apo ATPase structure onto a subunit within the modeled ring, using only the lid subdomain for alignment. To model the effect of this change on downstream subunits in the ring assembly, a second ATPase domain (bound to ADP•BeF₃) was placed adjacent to the apo subunit in an orientation identical to the orientation in the ring model. To calculate the approximate movement of individual subunits that may be associated with ATP hydrolysis, we took advantage of the convenient output format of M-ZDOCK, in which all symmetry-related atoms are in the same x–y plane. Therefore, overall translation of a DNA-binding residue upon ATP hydrolysis and release (in this case, Cα of Arg101) can be simply calculated from the change in the z coordinate. To calculate the in-plane rotation angle per step, we translated the Cα of Arg101 of the “translocating” subunit back into the original plane. We then calculated the in-plane angle swept out by the motion of this subunit by measuring the angle between these three points: the original position of Arg101 Cα within the ring, the center of mass of all Arg101 Cα atoms, and the new (back-projected along the z axis) Arg101 Cα position.

**Modeling of the T4 TerL Ring and Fitting to Cryo-EM Reconstruction.** To make the model of the T4 TerL ring, we aligned the full-length T4-TerL structure (PDB ID code 3CPE) (8) onto the ATPase domains of our docked pentameric model using Pymol (DeLano Scientific). The resulting model was then fit into the cryo-EM reconstruction of the T4 capsid with the TerL ring bound (EMDB-1572) (8) in UCSF Chimera using the fit to map command (59).
Fig. S1. Characterization of TerL_{P74-26}. (A) SDS/PAGE analysis of TerL_{P74-26} purity. Diluted TerL_{P74-26} shows that the protein runs as a single band and that the undiluted protein shows no significant protein contaminants. Precision Plus Protein Unstained Standards (BioRad) are used as molecular mass markers. (B) TerL_{P74-26} elutes in SEC (S200) at a volume consistent with a pentamer. (C) TerL_{P74-26} shows significant ATPase activity as measured by a coupled-enzyme assay (50), whereas a variant that removes the catalytic glutamate (E150A-TerL_{P74-26}) is completely inactive, suggesting that the observed ATPase activity in WT TerL is not due to contaminants. The E150A variant’s active site is disabled in cis but can still donate an intact arginine finger to an adjacent subunit in the assembly. (D) Steady-state ATPase activity is shown for different concentrations of TerL. Error bars are the SD from at least three replicates. (E) Michaelis–Menten kinetic analysis of TerL_{P74-26}. Titration of ATP indicates that TerL_{P74-26} has high affinity for ATP\*MgCl\_2 (concentration for half-maximal activity of \(\sim 1.5 \pm 0.2 \, \mu M\)) and robust turnover (apparent catalytic rate constant \(k_{cat, app} \approx 0.091 \pm 0.003 \, s^{-1}\)). ATP hydrolysis is sigmoidal with respect to the concentration of ATP\*MgCl\_2, with an observed Hill coefficient of \(1.7 \pm 0.3\). This apparent cooperativity suggests that there is coupling between ATPase active sites within the TerL assembly. (F) TerL_{P74-26} binds DNA when incubated with an ATP mimic. One hundred twenty-five nanograms of 100-bp ladder (New England BioLabs) was incubated with either TerL_{P74-26} or a buffer control for 30 min at room temperature and run on a 1.5% (wt/vol) agarose gel at 80 V. Tight binding to DNA is only observed for TerL_{P74-26} that is preincubated with ADP\*BeF\_3. Slight shifts in the DNA migration are observed for TerL_{P74-26} in the apo state or incubated with ADP or ATP, which may indicate weak binding.
Fig. S2. Comparison of TerL<sub>P74-26</sub> structures. (A) Similarity between the two apo structures of the TerL<sub>P74-26</sub> ATPase domain (C<sub>α</sub> rmsd of 0.8 Å). The structures from both crystal forms are superposed with small deviations at the top of the lid and beta-hairpin loop. These regions make different lattice contacts in the two crystal forms. (B, Left) Superposition of the three individual ADP•BeF<sub>3</sub>-bound ATPase domains shows that there are only minor differences between each monomer. The loop linking beta-strands 4 and 5 displays significant (>1 Å) differences between all three conformers. This loop is longer than seen in the structures of the Sf6-TerL and T4-TerL proteins (20, 21), and it is in different packing environments in each monomer in the asymmetric unit. Thus, we conclude that this loop is relatively flexible in TerL<sub>P74-26</sub>. (B, Right) Omit map density for the ADP•BeF<sub>3</sub> ligands, magnesium ions, and waters in each active site. Omit maps contoured at 3σ (0.34 e/Å<sup>2</sup>) are shown for the active sites of all three ATPase domains in the asymmetric unit. Although molecule A has a slightly different electron density in the active site, its overall conformation is essentially the same as in molecules B and C, with a C<sub>α</sub> rmsd within active site residues of 0.17 Å and 0.16 Å, respectively. (C) Comparison of the ATPase domains of TerL orthologs. TerL ATPase domains from Sf6 (tan) and T4 (orange) are superposed onto P74-26 (purple), with C<sub>α</sub> rmsd values of ∼2.1 Å and 1.7 Å, respectively. Note that the gray region in T4-TerL is unique to T4 and closely related phages, and was not used for superposition. (Inset) Conservation of the TerL<sub>P74-26</sub> basic patch. Lys223 in T4-TerL is at the same position as DNA-binding residue Arg101 of TerL<sub>P74-26</sub>. Arg82 in Sf6-TerL is at the same position as Arg101 of TerL<sub>P74-26</sub>.
Fig. S3. Identification of the trans-acting arginine finger. (A) TerL<sub>P74-26</sub> ATPase domain is not catalytically active. Steady-state ATPase activity is shown for 0.5 μM TerL. Error bars are the SD from at least three replicates. (B) Mixture of the R139A and E150A single mutants has optimal ATP hydrolysis at a 1:1 ratio. TerL-R139A and TerL-E150A are mixed at the indicated ratios, with a final concentration of 1 μM total TerL, and steady-state ATPase activity is measured. (C) R139A mutation does not cause conformational changes in the ATPase domain. The 2.5-Å crystal structure of the R139A-TerL<sub>P74-26</sub> ATPase domain (yellow) is superposed onto the 2.0-Å structure of the WT structure (dark purple). Position 139 is shown in a ball-and-stick representation for both variants. (D) Arginine finger residue is conserved in other TerL ortholog structures. T4-TerL and Sf6-TerL (PDB ID codes 3CPE and 4IEE, respectively) were superposed onto TerL<sub>P74-26</sub> with the arginine finger residue shown in stick representation.
Fig. S4. Superposition of TerL orthologs onto the TerL\textsubscript{P74-26} ATPase ring model. (A) Model of the Sf6-TerL ATPase ring. The TerL ATPase domain from Sf6 was superposed onto the docked model of the TerL\textsubscript{P74-26} ATPase ring. (Left) Each subunit is shown in a different shade, and the ring is viewed from the side. There are no significant clashes in this model. (B) View of the electrostatic surface potential of the Sf6-TerL ring from the bottom shows a net positively charged pore as would be expected from the DNA-binding region. (C) Model of the T4-TerL ATPase ring. The TerL ATPase domain from T4 was superposed onto the docked model of the TerL\textsubscript{P74-26} ATPase ring. The region specific to the T4 phages is shown in gray, the main ATPase domain is shown in various colors, and the ring is viewed from the side proximal to the nuclease domains. There are no significant clashes in this model, and the T4-specific region is present on the outside of the ring, away from the core machinery. (D) Electrostatic surface potential of the T4-TerL ring pore, as viewed from the bottom of the ring. Again, the residues within the pore have a net positive charge, as would be expected for a region that binds DNA. (E and F) Model of the T4-TerL ring fits a cryo-EM structure of the intact motor (E is viewed from the side and F from the bottom). The revised model of the T4-TerL ring is shown as a colored cartoon, with the cryo-EM envelope of the T4 capsid and packaging motor, which was calculated without fivefold averaging (gray surface).
Fig. S5. Functional and biophysical analysis of the TerL<sub>P74-26</sub> pentameric assembly. (A) SAXS profile of a highly soluble variant of TerL<sub>P74-26</sub> (R104E). Scattering intensity is shown as a semilog plot vs. the scattering angle (q). (B) Guinier plot of low scattering angles shows no aggregation, and a radius of gyration of 48.0 ± 2.0 Å. (C) TerL<sub>P74-26</sub> construct is well-folded. A Kratky plot of the SAXS data is shown. The peak at low scattering angles and the relatively flat and low profile at high scattering angles are hallmarks of well-folded protein. (D) TerL<sub>P74-26</sub> R101E variant is catalytically active. Steady-state ATPase activity is shown for 0.5 μM TerL. E150A and WT are shown as points of comparison. Error bars are the SD from at least three replicates. (E) Key functional residues mapped onto the previous model for the TerL assembly. The view is as seen from the perspective of the capsid/portal. The TerL<sub>P74-26</sub> ATPase domain crystal structure and nuclease domain homology model were superposed onto the previous model for the T4 TerL ring (PDB ID code 3EZK). Key residues for testing each model are shown as colored spheres: The arginine finger (Arg139) is orange, the DNA-binding residue (Arg101) is blue, and the lid hydrophobic patch residues (W231 and Y238) are shown in rusty red. The nuclease domain homology model is semitransparent. (F) Same as in E, but viewed from the side. The procapsid and portal would be above the TerL ring.
Table S1. Crystallographic statistics

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<td>100.00 (100.00)</td>
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**CC**, correlation coefficient; I/σ(I), intensity divided by error of intensity; R, residual factor; R-meas, redundancy independent merging R factor; R-pim, precision indicating merging R factor.

Table S2. Cα rmsd

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<td>1.127</td>
<td>0.897</td>
<td>0.784</td>
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</tr>
<tr>
<td>BeF3-A</td>
<td>0.526</td>
<td>0.461</td>
<td>1.231</td>
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<tr>
<td>BeF3-B</td>
<td>0.641</td>
<td>1.119</td>
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<tr>
<td>BeF3-C</td>
<td>1.053</td>
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*Values are measured in angstroms.*
**Movie S1.** Conformational rearrangements upon binding ADP•BeF$_3$ in the TerL$_{P74,26}$ ATPase. An interpolation between TerL apo and ADP•BeF$_3$-bound structures using the UCSF Chimera "Morph Conformations" tool (default parameters) (59) was performed. The movie demonstrates the effects of substrate binding and release on the lid subdomain (pink). A closer view reveals how residues in the P-loop move to engage ADP•BeF$_3$ (Fig. 3C). Finally, a wide view reveals the large-scale movement of the Rossmann fold upon ADP•BeF$_3$ binding and release if the lid is held stationary (Fig. 3D).

**Movie S2.** Animation describing the proposed mechanism for coupling ATP hydrolysis to DNA translocation. Initially, a single ATPase domain of the TerL ring is shown. In the context of the ATPase ring, all subunits are initially bound to ATP. We focus on three of the five subunits (blue, magenta, and red) and show the DNA-binding residue (Arg101) as spheres. The magenta subunit then hydrolyzes and releases ATP, triggering the conformational change. The magenta lid subdomain remains bound to an adjacent subunit (blue). The conformational change shifts the Rossmann fold of the magenta subunit, as well as the adjacent red subunit, resulting in translocation of the red subunit's DNA-binding motif by $\sim$8 Å perpendicular to the plane of the ring (Fig. 7).