Structure and mechanism of the ATPase that powers viral genome packaging

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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.


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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•BeF3-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. S1 C–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 (Cₚ rmsd of 0.8 Å; 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 crystal 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, Trp225, 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 TerLP74-26 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 Rossmann 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.
different TerL variants at 0.5 μM each are mixed either with an alternate TerL mutant in a 1:1 ratio (as indicated by the “+” sign) or with buffer, and their steady-state ATPase activity is measured. Note that the R139A/E150A double mutant is a single variant and not a mixture. Only a mixture of the R139A and E150A mutants significantly restores activity. Schematic illustrations illustrate that the Walker B mutant (E150A) has a debilitated active site but lacks ATPase activity due to loss of the trans-acting residue. (C) Arginine finger residue is conserved in other TerL ortholog sequences. A logo diagram (46) was made from a sequence alignment of 70 TerL proteins. The residue numbering is shown for TerLP74-26.

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. S3C), 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 ATPase domain 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.

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 B and D). Second, our structural model predicts that for other TerL proteins that are monomeric in isolation, ring assembly would be enhanced by ATP mediating cross-subunit interactions. Indeed, this dependency has been observed for T4 (39), T3 (40), and lambda (41). Third, our T4-TerL ring model is a bowl-like assembly that reasonably fits the cryo-EM map (8) for an actively packaging T4 phage (Fig. S4 E and F). However, this fitting positions the ATPase domain ring distal to the capsid, whereas the nuclease domains interact with portal (Fig. S4E), an interaction that has been observed recently (10). Thus, our revised TerL ring model is a different arrangement than that previously proposed.

We examined whether our structural model is globally consistent with a molecular envelope, as calculated from small-angle X-ray scattering (SAXS) data. Whereas WT TerLP74-26 exhibits slight aggregation, we obtained high-quality SAXS data by exploiting the R104E-TerLP74-26 mutant, a more soluble variant than the WT protein (Fig. S5 A and B). R104E-TerLP74-26 is well folded and has a radius of gyration of 48.0 ± 2.0 Å (Fig. S5 B and C), consistent with a pentameric assembly. We performed an
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 ligand 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 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 binding and mutations distal to the pore (R39A and R58A) have no measurable effect 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. 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 sub-domain 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 plugin in PyMOL (DeLano Scientific) (48). Figure is colored by electrostatic potential (k_BT/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 nuclelease 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. 4I), 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 nuclelease 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, each lid residue interacts 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 Cα positions of surface-exposed arginines lining the pore (we use the Cα 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 nuclelease domain based on the full-length T4-TerL structure, which crystallized as a monomer (8). The nuclelease domain position may be ortholog-dependent or may be altered upon ring formation and/or upon portal binding. Nuclelease position is important because the nuclelease domain may play a key role in pentamer assembly, considering that the isolated TerLP74-26 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 Rossman 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 Rossman fold pivoting outward and upward toward the capsid (Fig. 7 and 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 perpendicular 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 TerL24-26. The TerL24-26 gene was synthesized by Genscript Corporation and subcloned into a modified pET24a vector to generate standard expression constructs. TerL24-26 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).

Cryostallization, Diffraction Data Collection, SAXS, and Docking. Semelt-labeled, apo, and ADP·BeF3-bound TerL24-26 ATPase domain were cryocrystallized with the hanging drop vapor diffusion method in solutions containing buffered aqueous solutions of 10% PEG 4000 and 0.1 M sodium sulfate. Crystals were frozen in a cryogenic buffer containing elevated respective precipitant concentrations supplemented with 30% (volvoll) 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-TerL24-26 at the Structurally Integrated Biology for Life Sciences (SIBYLS) beamline at the ALS. The TerL24-26 ATPase ring was modeled using M-ZDOCK (35) (details are provided in SI Materials and Methods).

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