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Authors
Tsai, Chi-Lin
Williams, Gareth J
Perry, J Jefferson P
et al.

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An AAA+ ATPase Clamshell Targets Transposition

Chi-Lin Tsai[1], **Gareth J. Williams**[1], **J. Jefferson P. Perry**[2], and **John A. Tainer**[1,3,*]

[1]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

[2]Department of Biochemistry, University of California, Riverside, Riverside, CA 92521, USA

[3]Department of Molecular and Cellular Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA

Abstract

DNA transposition plays key roles in genome diversity, pathogenesis, and evolution. Yet, structural and mechanistic information on transposition targeting and regulation is limited. Arias-Palomo and Berger now define the decameric organization of the AAA+ ATPase IstB, unveiling key insights into its targeting and regulation of IstA transposase activity.

Transposition controls movements of discrete gene segments from a donor genome to a target location for RNA, ssDNA, or dsDNA. These transposable elements (TEs) are also called jumping genes or transposons. TE transposition can be triggered by environmental stress and can create new functions for adaptation and evolution. DNA arising from TEs account for ~45% of the human genome. Although few TEs are active, these contribute to genetic diversity and disease (Mills et al., 2007). Furthermore, TEs also generate genetic diversity in prokaryotes and contribute to pathogenesis mediated through the transfer of antibiotic resistance genes. Thus it is key to understand the mechanism of DNA transposition and integration. Genetic and biochemical results have identified different types of DNA transposition, including “cut-and-paste” or “copy-and-paste” (Curcio and Derbyshire, 2003). Yet, limited structural information available on transposase complexes restricts our current mechanistic understanding. In this issue of *Cell*, Arias-Palomo and Berger (2015) combine X-ray crystallography and cryo-electron microscopy (cryo-EM) to define how IstB, the helper protein for IstA transposase, functions (Figures 1A and 1B). These results extend our knowledge of macromolecular assembly states for both AAA+ (ATPase associated with various cellular activities) ATPase superfamily members and of transposition processes.

One of the best characterized transposition models comes from bacteriophage Mu, including a crystal structure of the MuA-DNA complex (Montaño et al., 2012) and EM reconstructions of the MuB helper (Mizuno et al., 2013). The MuB-ATP structure revealed the helical filament assembly on DNA requires ATP binding, but not hydrolysis (Mizuno et al., 2013). A MuA transposase tetrameric complex brings Mu transposon ends together with target DNA bent by 140°. The positively charged coiled-coil domains from adjacent MuA subunits

*Correspondence: jatainer@lbl.gov.

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wrap around target DNA. For transposition, MuA disassembles the MuB filament near the target DNA binding site by stimulating ATP hydrolysis and binds target DNA. Either MuA binding causes target DNA to bend or MuB bends DNA during ATP hydrolysis before disassembly. Transposition is completed when Mu DNA ends join target DNA, and gaps are filled by a non-replicative doublestrand break repair pathway (Jang et al., 2012) (Figure 1B, top).

Arias-Palomo and Berger have studied IstA and IstB that belong to the bacterial IS*21* Insertion sequence (IS) family, which encodes *istA* transposase and *istB* helper genes (Berger and Haas, 2001). IstA possesses an RNaseH-like catalytic domain likely important for endonucleolytic DNA cleavage during the transposase reaction that transfers the TE into the genome. IstB, an IstA transposase partner protein, is an AAA+ ATPase superfamily member, and has DNA binding activities key to the transposition reaction. IstB is important for IS strand transfer, for targeting the DNA accurately, and possibly for preventing repeated insertion of the IS into the same target DNA, a phenomenon known as target immunity. However, the mechanism by which IstB and related proteins such as MuB regulate transposase activity and the roles of its DNA binding and nucleotide state in these processes has been enigmatic.

Arias-Palomo and Berger have combined structural insights from crystallography and cryo-EM, along with biochemical analyses of the assembly state of IstB variants with nucleotide to unveil the structural basis for IstB functions. The IstB AAA+ ATPase crystal structure (Ist-B_{AAA+}, lacking the N-terminal domain) reveals that, in addition to a typical AAA+ ATPase topology consisting of a core αβα-fold, $IstB_{AAA+}$ forms a right-handed six-subunit repeat helical filament. In this respect, IstB resembles the replication initiator proteins including DnaA from bacteria and Cdc6/Orc1 from archaea and eukaryotes, as well as the bacterial helicase loader DnaC (Mott et al., 2008) and bacteriophage DNA binding protein MuB (Mizuno et al., 2013).

One might therefore expect that IstA/IstB may share its transposition mechanism with the Mu system. Fascinatingly, the full-length IstB ($IstB_{FL}$) cryo-EM structure bound to DNA reveals that with its N-terminal domain present IstB behaves very differently. Full-length IstB forms a clam-shaped assembly that sandwiches the DNA between a pentamer of IstB dimers (Figure 1A). This architecture is a unique assembly state for an AAA+ ATPase, as these proteins are generally observed as spiral assemblies, including open locked-washer or helical filaments (Erzberger and Berger, 2006), or closed rings, as seen in the exemplary hexameric RuvB Holliday junction branch migration motor where intersubunit activation occurs by an arginine finger motif that participates in both ATP hydrolysis and inter-subunit communication (Putnam et al., 2001). The distinct IstB assembly places conserved arginine 221 to interact with the active site of a neighboring AAA+ domain for this function. Within the clamshell, IstB contorts the DNA into a U-shape, bending it nearly 180° as revealed by difference analysis of cryo-EM structures with and without DNA. The beautiful structural work defining distinct IstB assembly states is nicely complemented by biochemical analyses with various nucleotides and IstB active site mutations to define the molecular basis for IstB assembly. Importantly, this large IstB assembly state is essential for its interactions with IstA, and IstA stimulates IstB ATPase activity. In essence, the data support a model in which

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ATP-induced IstB oligomerization and the subsequent DNA bending promote IstA association and completion of transposition.

Details about how IstB recruits IstA remain to be elucidated. However, as the presented results place IstB into the initiator clade of AAA+ ATPases, which includes the DnaC helicase loader and DnaA replication initiator proteins, insights may be gained by considering how these proteins work. In DnaC, a helical filament is formed in the presence of ATP and is stabilized by an initiator/loader specific protein motif (ISM). Mutation of hydrophobic residues in the ISM impair DnaC assembly and compromise cell growth (Mott et al., 2008). DnaC helical filament assembly is important for interactions with the replication initiator DnaA helical oligomer and for recruiting/loading DnaB helicase onto DNA. The IstB AAA+ ATPase domain also has an ISM, which may play a similar role for recruiting/loading IstA onto target DNA. Indeed, the IstB ISM has several positively charged residues that are juxtaposed to DNA in the cryo-EM structure, and future mutational studies of these residues may determine if they affect IstA/IstB assembly, DNA binding, and transposition activities.

Collectively, the results presented here allow a new mechanism for transposition to be proposed (Figure 1). Previous results showed that transposases generally oligomerize to bring transposon ends together. These new findings show that IstA specifically interacts with IstB in its ATP-bound large oligomeric form, not the ADP-bound dimer, to stimulate its ATPase activity. ATPase stimulation accelerates ATP-turnover by IstB, triggering the disassembly of IstB decamers to dimers. The IstB dimers likely dissociate from DNA following ADP release, although this needs to be tested. Following IstB dimer dissociation from target DNA, IstA-DNA can facilitate the strand transfer process with target DNA and complete the transposition reaction (Figure 1B, bottom).

That IstB forms a decameric clamshell architecture extends the known assembly states for both proteins involved in transposition and the AAA+ ATPase superfamily members. The unique and stable complex of IstB with target DNA, which is recognized by IstA, generates an enabling system to capture the IstA transposase and IstB-DNA complexes together. Thus, this system promises new structures of IstA/IstB macromolecular assemblies to reveal how the transposition process is completed.

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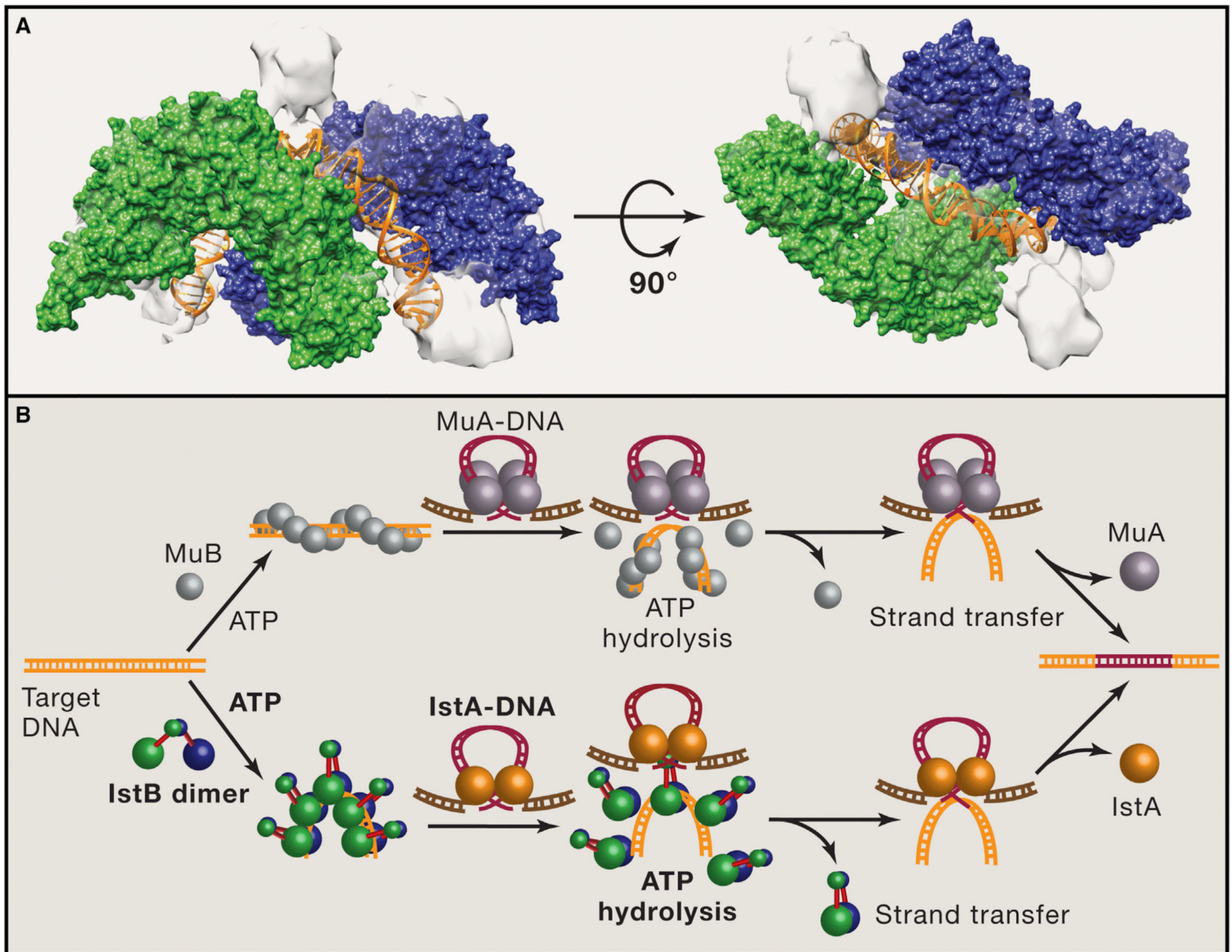

Figure 1. Structure-based Transposition Models

(A) IstB-DNA oligomeric structure (Arias-Palomo and Berger, 2015). Full-length IstB forms a pentamer of dimers (shown in green and blue) and sandwiches a target DNA duplex (orange). The oligomeric structure was reconstructed by docking the ATPase domain structure of IstB onto the cryo-EM density (gray). (B) Structure-based schematic of DNA transposition models. (Top) ATP binding induces MuB helical filament formation on the target DNA while the MuA transposase tetramer brings the Mu-DNA ends to the MuB-DNA complex. MuA stimulates ATP hydrolysis by MuB, and MuB dissociates from target DNA, allowing MuA to complete the strand transfer reaction to the target site. (Bottom) ATP binding induces IstB oligomerization to form a pentamer of dimers that sculpts the target DNA into a U-shape. The IstA transposase dimer, carrying the insertion sequence, selectively recognizes the IstB-DNA complex. IstA then stimulates ATP hydrolysis by IstB, IstB dissembles from target DNA and IstA completes the strand transfer.