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MuB gives a new twist to target DNA selection

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Transposition target immunity is a phenomenon observed in some DNA transposons that are able to distinguish the host chromosome from their own DNA sequence, thus avoiding self-destructive insertions. The first molecular insight into target selection and immunity mechanisms came from the study of phage Mu transposition, which uses the protein MuB as a barrier to self-insertion. MuB is an ATP-dependent non-specific DNA binding protein that regulates the activity of the MuA transposase and captures target DNA for transposition. However, a detailed mechanistic understanding of MuB functioning was hindered by the poor solubility of the MuB-ATP complexes. Here we comment on the recent discovery that MuB is an AAA+ ATPase that upon ATP binding assembles into helical filaments that coat the DNA. Remarkably, the helical parameters of the MuB filament do not match those of the bound DNA. This intriguing mismatch symmetry led us to propose a model on how MuB targets DNA for transposition, favoring DNA bending and recognition by the transposase at the filament edge. We also speculate on a different protective role of MuB during immunity, where filament stickiness could favor the condensation of the DNA into a compact state that occludes it from the transposase.

Keywords: phage Mu, AAA+ ATPase, nucleoprotein filament, symmetry mismatch, helical filament, DNA transposition

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Introduction

Behind the writing of a research paper there is a mess of cables feeding computers and other desktop-populating gadgets that keep us connected to the outer world. Not matter how tidy our draft looks, those cables entangle to the limit where an attempt to disconnect the coffee machine might end up ruining a few hours of inspired work. While restarting the mistakenly unplugged computer, I return to the train of thought of how some DNA transposons deal with a similar problem, while having to distinguish the host chromosome from their own DNA sequence in order to avoid inserting into themselves, causing self-destruction. This phenomenon observed in the transposition of phage Mu, Tn7 and in members of the Tn3-family, is called "transposition target immunity" because a copy of the transposon renders nearby sites "immune" to additional insertions by the same transposon (reviewed in ref. 1). The subject of this commentary is MuB, the protein that grants phage Mu with immunity against self-integration, and how the recent structural characterization of MuB unveils an unexpected and novel mechanism for selecting the target DNA.[2]

After initial integration into the bacterial chromosome, phage Mu uses a replicative-transposition mechanism to amplify its 37 kb genome up to 100-fold during a single growth cycle. By the end of this process, the Mu DNA content reaches nearly the size of the *E. coli* chromosome, and thus, the transposon must follow a precise mechanism of target DNA selection to prevent self-integration, a paramount task for the interplay of two phage proteins: MuA and MuB (reviewed in ref. 3). MuA is the transposase, which synapses the ends of Mu DNA and catalyzes the DNA strand cutting and joining into a target DNA. In turn, MuB is a small (35 kDa)

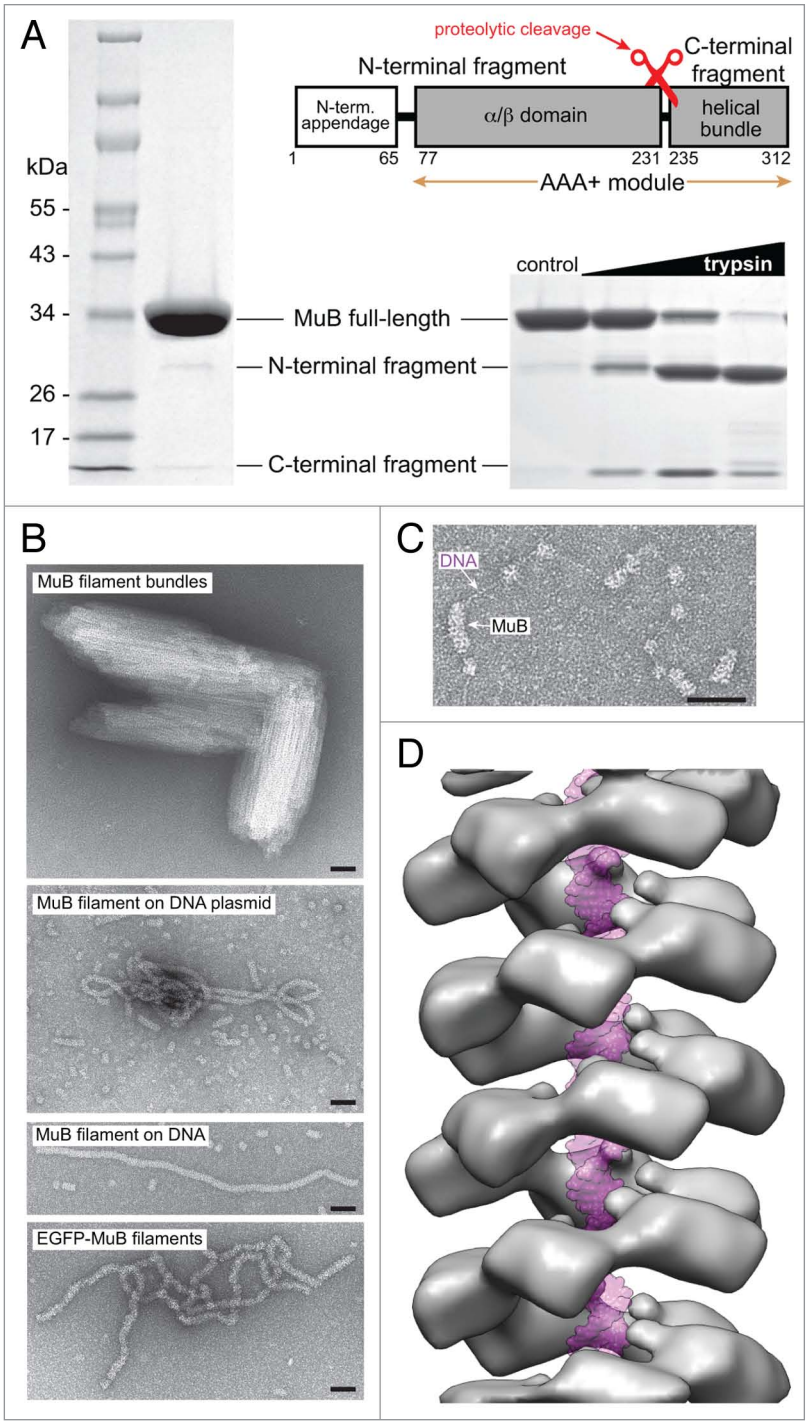

Figure 1. MuB is an AAA+ ATPase that forms helices on the DNA. (**A**) Scheme of MuB protein architecture. MuB is composed of an AAA+ module preceded by an N-terminal appendage. The AAA+ module has a central α/β-domain and a C-terminal helical domain connected by a linker that is prone to cleavage, as shown in the SDS-PAGE. (**B**) Negative staining EM images of MuB filaments formed under different conditions. (**C**) Small patches of MuB filaments partially covering a double stranded DNA molecule. Scale bars: 500 Å. (**D**) 3D reconstruction of the MuB filament with a simulated DNA molecule fitted in the axial channel.

ATP-dependent DNA binding protein that plays multiple functions during transposition, activating the catalytic activity of MuA, enhancing the efficiency of MuA-mediated Mu end synapsis, and selecting the target DNA for transposition. In the absence of MuB, MuA is not only much less efficient, but it selects the transposable element or the nearby sequences as the preferred target for transposition, often leading to self-destruction.[4,5] Although the details are elusive, the current mechanistic explanation of Mu transposition immunity is based on a biased distribution of MuB on the DNA caused by the interaction with MuA.[6,7] Upon ATP binding, MuB forms a broad mixture of oligomers[5] that bind to the DNA and somehow present it as a better target for MuA strand transfer reaction.[6] At the same time, MuA interacts with MuB stimulating ATP hydrolysis and the release of MuB from the DNA. Thus, by the time MuA assembles an active complex and prepares the Mu ends for insertion into a new target DNA, MuB has been cleared out from the nearby sequences and accumulates at DNA regions 5–25 kb away from the original insertion site that become preferred targets for transposition.[8]

A more detailed view into the DNA target selection mechanism has been hampered by the difficulties in characterizing MuB, since the abundantly produced recombinant protein readily undergoes cleavage into two proteolytic fragments[9] (**Fig. 1A**), and the addition of ATP triggers the formation of oligomers with great tendency to precipitate.[10] Furthermore, MuB has defied all crystallization attempts by many groups in the past 15 years, and it lacks significant sequence similarity with other proteins that could shed light on its structure. On the other hand, the characterization of the proteolytic fragments, including the NMR model of the re-folded C-terminal proteolytic fragment,[11] provided limited insight into protein functioning. The usage of fluorescent microscopy, however, meant a breakthrough in understanding MuB behavior.[12-14] By using very diluted fluorescently labeled MuB on single surface-immobilized linear DNA, it was possible to observe ATP-induced MuB oligomers binding non-specifically to DNA. MuB

formed many short segments along the DNA, which elongated to form an apparently continuous polymer fully coating the DNA molecule. These experiments revealed that the functions of MuB are closely related to its polymeric state, and while monomeric MuB suffices to stimulate the MuA activity and assembly at the Mu ends, the selection of the target DNA requires the ATP-dependent formation of MuB polymers. Then, the questions were: How do the MuB oligomers look like? How does MuB bind to the DNA? Why is an ATP/ADP switch needed for DNA selection? How does MuB transform the DNA into a preferred substrate for transposition?

Revealing the True Nature of MuB

Intrigued by the low solubility of MuB-ATP complexes, we looked at the protein precipitates by negative staining electron microscopy (EM). Strikingly, EM images revealed that upon adding ATP, MuB assembles into helical filaments (**Fig. 1B**). The filaments are quite "sticky" and show a great tendency to aggregate into large bundles, which illustrate well the poor solubility of the sample (**Fig. 1B**, top). In the presence of DNA, MuB wraps around the DNA forming filaments that matched the expected length of the DNA molecules, but without causing an appreciable distortion of the DNA, as we corroborated by topology analysis of MuB-bound DNA samples. Filament nucleation starts at different points along the DNA with the formation of short MuB segments (**Fig. 1C**), which extend by the incorporation of additional subunits to completely cover the DNA molecule (**Fig. 1B**, middle panels).

Higher detail on the filament architecture was obtained by cryo-EM of vitrified samples and 3D image reconstructions, revealing a right-handed solenoid 150 Å wide with a pitch of 48 Å and ~5.4 subunits per helical turn, with a hollow axial channel of a diameter that easily accommodates a B-form DNA molecule (**Fig. 1D**). The reconstructions of the MuB filament free or bound to DNA have similar parameters. However, the reconstructions did not show a clear density for the DNA. This is due to the important fact that the symmetry of the MuB helix differs from the helical parameters of the B-form DNA within, and thus, the density of the DNA is averaged out during the reconstruction procedure.

Further insight into the filament assembly came from the search for MuB distant homologs using protein fold recognition tools, which revealed significant similarity of MuB residues 72–312 with members of the AAA+ (ATPases Associated with diverse cellular Activities) ATPase superfamily. This family groups proteins with diverse functions that present a characteristic AAA+ module composed by an N-terminal α/β-domain and a C-terminal helical bundle.[15] Typically, AAA+ proteins bind ATP in a cleft between both domains, each providing characteristic elements for ATP binding and hydrolysis (Walker A and Walker B motifs and ATP sensors I in the N-domain and sensor II in the C-domain). In addition, the AAA+ active site is often completed by at least one residue (arginine finger) from an adjacent subunit that interacts with the ATP. Hence, AAA+ proteins are typically ring-like or helical oligomers with a central hole to accommodate the protein or DNA substrate. According to our predictions MuB folds into a characteristic AAA+ module, with an N-terminal α/β-domain (77–231) and a C-terminal helical domain (236–312) connected by a linker that matches the sequence prone to proteolytic cleavage (**Fig. 1A**). Indeed, removal of the first 65 residues, which are not part of the predicted AAA+ module, only had a modest effect on ATPase activity, did not impede filament formation and conferred target immunity. In turn, truncation of the C-terminal domain renders an inactive protein that does not form filaments, nor stimulates MuA activity, thus proving that the complete AAA+ module is needed and sufficient for these functions. To further confirm that MuB is an AAA+ ATPase we identified and mutated the characteristic AAA+ elements, demonstrating that the mutant proteins lost the ATPase activity. Furthermore, those mutants that cannot bind ATP also loose the capacity to form filaments, proving that ATP glues the subunits together and triggers filament formation. These findings explain previous kinetic studies showing that the ATPase activity of MuB is stimulated in the oligomeric forms, and that the protein as a monomer might not be able to bind and/or hydrolyze ATP.[10] It also explains why studying the proteolytic fragments of MuB provided little understanding of the protein function,[9,11] since the two AAA+ domains act as a single unit.

The similarity of MuB with other AAA+ members, also allowed us to identify the loop (loop-1) responsible for DNA binding. Interestingly, mutations in this loop did not only hamper interaction with DNA, but also conferred a higher ATPase rate, suggesting a coupling mechanism between DNA binding and ATP hydrolysis. It was already established that DNA binding had an inhibitory effect on the ATPase activity of MuB,[5] but the opposite is also true, the impediment to interact with the DNA releases the inhibitory effect over the active site, thus confirming the existence of an allosteric regulatory mechanism.

MuB Filaments: Some (dis)assembly Required

The accumulated knowledge on AAA+ ATPases and the biochemical data on MuB help to put the pieces together on how MuB filaments assemble on the DNA (**Fig. 2A**). MuB is a monomeric protein with a dynamic N-terminal appendage and an AAA+ module with relatively high flexibility between the N- and C-domains, which would explain the resistance of the protein to be crystallized and the high susceptibility of the linker to proteolytic cleavage. MuB can probably bind to the DNA through loop-1, but likely with low affinity. The binding of ATP between the N- and C-domains fixes the AAA+ module in a conformation that favors MuB oligomerization. Perhaps the formation of small MuB oligomers with an increased affinity for the DNA—provided by the sum of the subunits—precedes binding to the DNA, in agreement with previous fluorescent microscopy observations.[12] We hypothesize that the size of the immediate precursor for DNA binding must be ≤ 5 MuB subunits, otherwise the filament would form a complete helical turn and the DNA could not enter easily. Upon DNA binding, loop-1 might adopt a

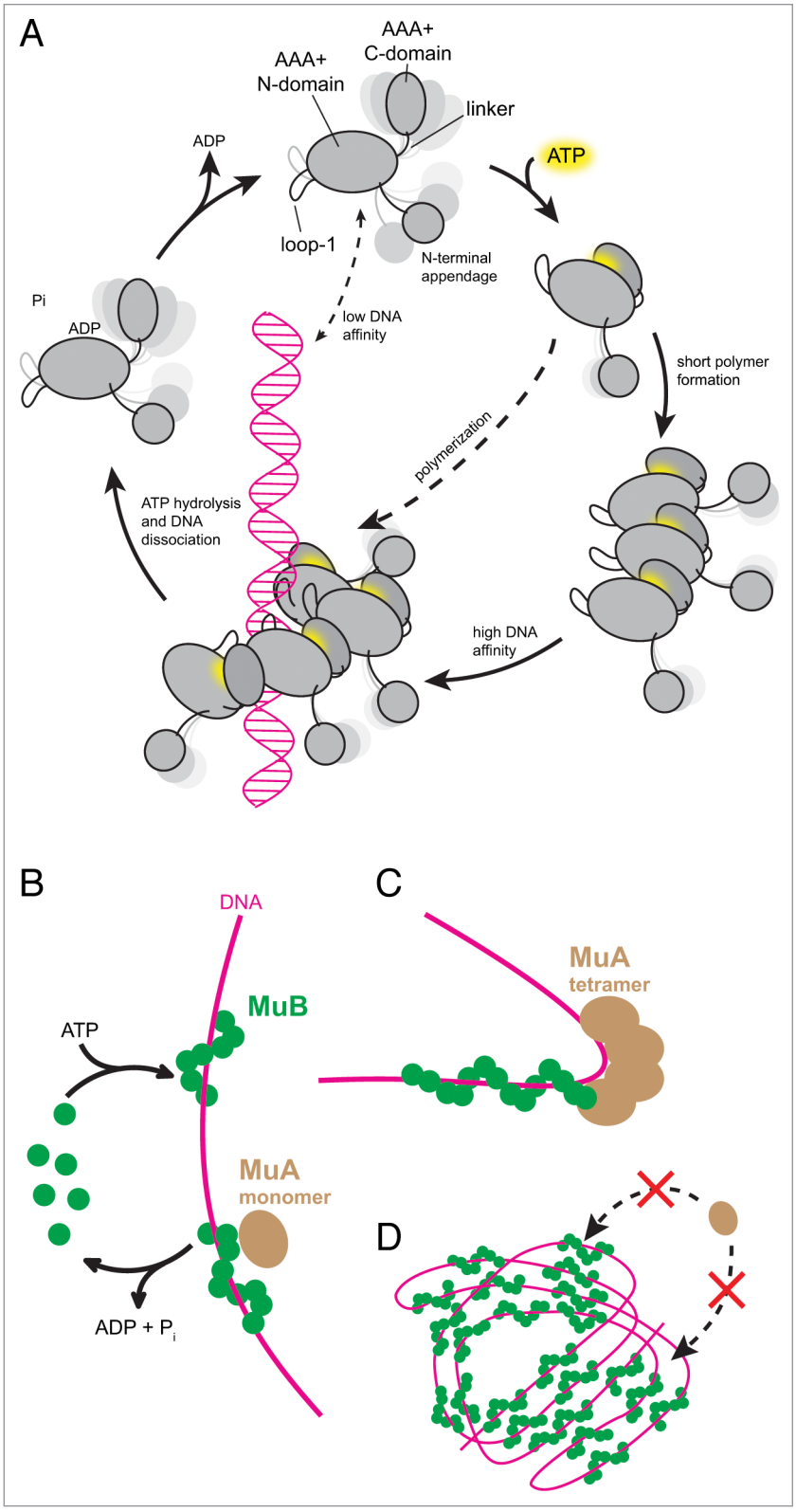

Figure 2. MuB filaments target DNA for transposition. (**A**) ATP-dependent formation of MuB filaments on the DNA. (**B–D**) Different models for the interaction of MuA and MuB. (**B**) MuA as a monomer triggers ATP hydrolysis and MuB dissociation from the DNA. (**C**) A MuA tetramer bound to the Mu ends stimulates multiple MuB subunits at the filament edge, favoring DNA distortion and bending. (**D**) MuB filament-filament interactions promoting DNA condensation and protection against the action of MuA.

conformation that decreases the ATPase rate, and thus, favors the stability of the filament. Then, the filament extends by the coupling of additional MuB-ATP subunits. However, due to the different symmetries between the protein and DNA helices, the interactions of MuB subunits with the DNA change along the filament axis with the consequent variations on the ATPase activity. This is perhaps an important contributing factor for the formation of short rather than long MuB segments. Fluorescent experiments indicated that the size of the polymers varies between 10–60 MuB subunits,[13] which would correspond to ~2–11 helical turns. The relatively weak cooperativity in MuB-DNA polymerization together with the weak DNA binding might be advantageous so that MuB can be easily disperse by MuA coupled ATP hydrolysis during transposition immunity, and perhaps it also favors the displacement of MuB filaments by other DNA interacting proteins, so that MuB does not interfere too much with normal cellular processes.

The dissociation of MuB filaments from the DNA is prompted by the interaction with MuA and the stimulation of ATP hydrolysis (**Fig. 2A and B**). The group of G. Chaconas identified a patch of three positively charged lysines as the interacting surface with MuA.[11,16] We observed similar results and mapped these residues in the linker region between the N- and C-domains, which in our model occupies an exposed position on the filament surface. In other AAA+ members, the conformational changes in this linker correlate with the ATP hydrolysis cycle,[17] suggesting that the interaction of MuA could alter the conformation of the linker and activate the ATPase activity of MuB. ATP hydrolysis would trigger the rapid dissociation of MuB from the DNA due to the change of the DNA binding loop to a low affinity conformation, the increased flexibility between the N-and C-domains and the loss of interactions with the adjacent subunits.

To further understand how MuB filaments control DNA target selection, we investigated the size of MuB-DNA filament needed to stimulate MuA strand transfer reaction. We demonstrated that although the efficiency of the transposition

increases with the concentration of MuB, when the DNA is fully covered by MuB, it becomes a poor substrate for transposition. Hence, strand-transfer reaction is favored at DNA sites adjacent to the MuB filament ends, whereas the DNA within the filament is not accessible to the transposase.

MuB Filaments Play Offense and Defense

Altogether, these results support that MuB plays a double immunity role, first turning the DNA at the edge of the filament into a better substrate for transposition, and second, protecting the DNA within the filament from the transposase.

How can MuB alter the structure of the DNA? The fact that MuB helix wraps the DNA without seemingly altering its structure, and that the helical parameters of the MuB polymer do not match those of the B-form DNA is a property not observed in other nucleoprotein filaments. Other protein filaments either follow the parameters of the DNA helix, or impose their different symmetry deforming the DNA. The unique symmetry mismatch between MuB and DNA implies that individual MuB monomers must face DNA in different ways. This intriguing observation led us to propose a model where MuA tetramer bound to the Mu ends stimulates simultaneously a patch of MuB subunits at the filament end (**Fig. 2C**). We propose that the coordinated ATP hydrolysis and concomitant movement of the DNA binding loops could impose a symmetry match on the DNA, inducing a local deformation that favors DNA bending and capturing by the transposase, as recently observed in the crystal structure of MuA transposase with a highly bent target DNA bound in the active site.[18]

On the other hand, MuB has been proposed to play a prominent role in binding and protecting the Mu DNA from self-insertion.[19] However, the protection of the DNA by the formation of a linear MuB filament, as observed by EM, would be quite ineffective, since the number of MuB proteins required to coat the DNA in the distances observed in the immunity mechanism would clearly exceed those available during viral infection. Therefore, the images of DNA saturated with MuB (**Fig. 1B**) are not likely to happen in vivo, and a more realistic scenario would be that shown in **Figure 1C** with patches of filaments distributed at different positions along the DNA. Thus, is there a mechanism by which a limited number of MuB molecules could occlude lengthy DNA sequences from the action of the transposase? One possibility is that the observed tendency of the filaments to stick to each other and form bundles is a physiologically relevant characteristic of MuB. In this way, a limited number of more or less short filaments distributed along the DNA could interact with each other, compacting the DNA into a condensed state that might turn into a poor substrate for transposition (**Fig. 2D**). A similar strategy is used during retroviral infection, where the cellular protein BAF (Barrier to autointegration factor) binds and occludes the viral DNA protecting it from self-integration.[20-23] Thus the poor solubility of the MuB-ATP assemblies, which gave so many troubles for the characterization of MuB function, could indeed hide an important cross-bridging capability that might protect the Mu genome from the transposase. This filament stickiness was not observed by fluorescent microscopy, but we could argue that the EGFP attached to the N-terminus of MuB must provide the filament with an outer crust that would prevent the interaction with other filaments. Indeed, EGFP-MuB forms filaments in the presence of ATP (**Fig. 1B**, bottom), but these do not tend to aggregate into bundles. We did not observe this filament stickiness neither with the N-terminal truncated form of MuB, suggesting that perhaps the N-terminal 70 residues appended to the AAA+ module could be involved in filament-filament interactions. If and how the N-terminal appendage promotes filament aggregation and what would be the importance of the filament stickiness for occluding the DNA from the action of the transposase are important questions that will have to be tested.

Final Remark

At the time of concluding this commentary, I bought a little plastic spiral to wrap around the cables of my computer. It looks like a good system to keep them organized and away from the coffee machine cord, but it is still far from the beauty of Nature's solutions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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