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AFM-based force spectroscopy unravels stepwise formation of the DNA transposition complex in the widespread Tn3 family mobile genetic elements

by Maricruz Fernandez et al.

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Hello researcher,

Greetings from the BioAFM Journal Club. Today I'd like to share a recent article with you from the journal *Nucleic Acids Research*.

In this article, single-molecule force spectroscopy AFM (SMFS-AFM) was performed on model surfaces to probe the binding of TnpA transposase to DNA molecules and extract the thermodynamic and kinetic parameters of a DNA transposition complex assembly. DNA transposable elements (transposons) are specific DNA sequences that can move from one location of the genome to another. The process is facilitated by recombination enzymes (transposases) that can cut and join DNA sequences via different mechanisms. As transposons, in particular, the widespread Tn3 family, have been associated with the spread of antibiotic resistance among pathogens, they pose a serious health risk. Nevertheless, the specific molecular mechanisms remain poorly understood, i.e., the interaction of TnpA with DNA partners to assemble an active transposition complex and catalyze the reaction.

The authors used Bruker's ForceRobot 300 AFM to study the binding of TnpA to DNA molecules with either one or two transposon ends to form either a single-end complex (SEC) or a pair-end complex (PEC). Initially, small-angle x-ray scattering and cryo-electron microscopy studies showed that both free wild-type (WT) and mutant TnpA molecules exist as closed dimers in solution. It also showed that PEC formation involves relocation of the dimer and tight bonding of the two transposon ends into the active form. SMFS measurements showed that both WT and mutant TnpA bind to a spatially constrained single transposon end and that the thermodynamic and kinetic parameters are very similar, indicating that the mechanisms for SEC formation within both groups of molecules are very alike. Kinetic analysis with two-end substrates showed that the expected rate-limiting step of PEC formation occurred at a much faster rate for mutants than for WT TnpA. This is attributed to specific structural differences in mutants that lead to enhanced TnpA flexibility and potentially to more stable activated PEC configurations. The authors suggest a synapsis-by-naked-end-capture (S-NEC) model involving a mechanism by which an inactive TnpA dimer first binds to a single

transposon end to form an inactive SEC then undergoes a conformational change to capture the other end and adopt the active PEC configuration.

I hope you find this article interesting and useful for your work!

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