An ATPase filament bridge How a transposon and CRISPR stick together

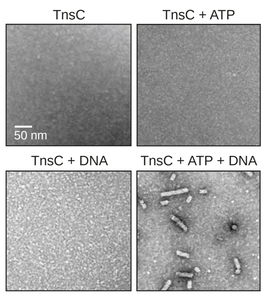
Irma Querques, Michael Schmitz, Seraina Oberli, Christelle Chanez and Martin Jinek

Department of Biochemistry, University of Zurich, 8057 Zurich, Switzerland

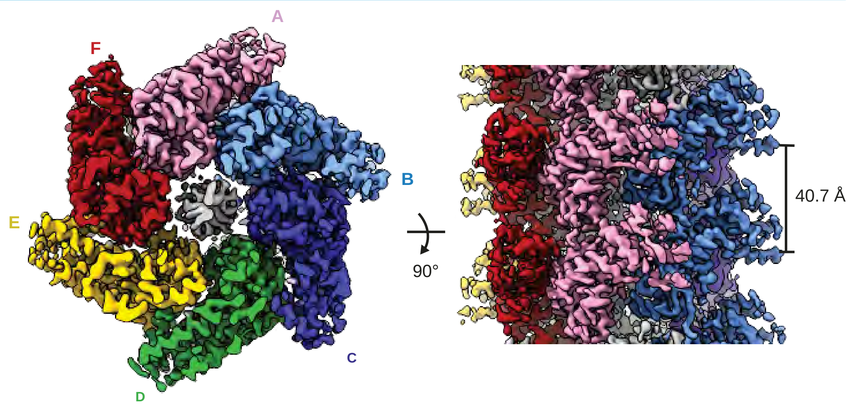
BACKGROUND

Since the discovery of bacterial adaptive immunity, CRISPR-Cas systems have been mainly regarded as a mechanism to counteract horizontal transfer of mobile genetic elements including transposons in prokaryotic genomes. Conversely, a distinct family of Tn7-like elements co-opted CRISPR-Cas RNA-guided machineries to direct transposon insertion into specific target sites[1-6].
In type V CRISPR-associated transposons, RNA-directed transposition relies on the cross-talk between the pseudonuclease Cas12k, the transposase TnsB, the zinc-finger protein TniQ and the ATPase TnsC[3]. Yet, the molecular mechanisms underpinning this interplay have remained unknown. Here we present biochemical and structural studies of a *Scytonema hofmannii* type V CRISPR-associated transposon system.

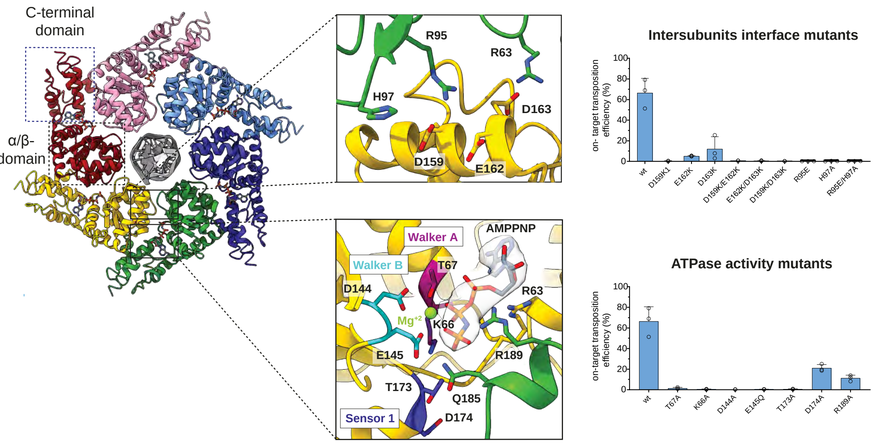
The AAA+ ATPase TnsC forms helical filaments



Negative stain electron micrographs showing ATP- and DNA-dependent polymerization of TnsC.

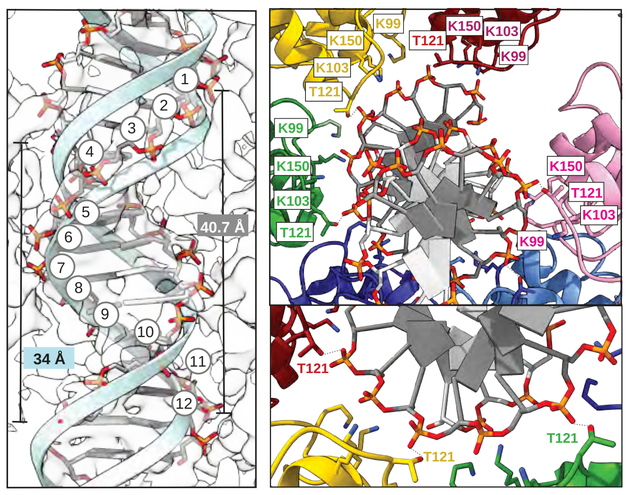


Cryo-EM density map of TnsC•AMPPNP•dsDNA filaments at 3.6 Å resolution.

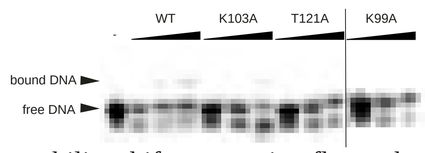


Structural model of the TnsC filament (left). Zoomed-in views of the inter-protomer interface and the ATPase catalytic site (middle). Functional analysis of TnsC mutants in transposition assays[3] as measured by digital droplet PCR (right).

TnsC filaments assemble on structurally remodeled DNA

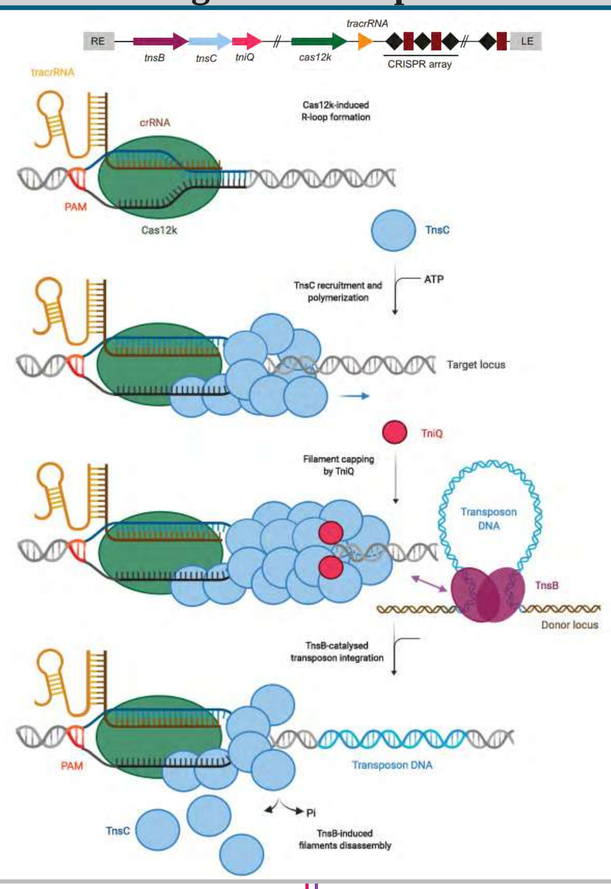


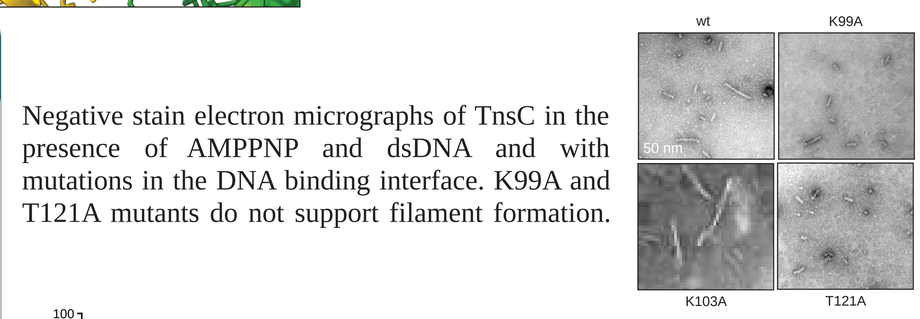
Overview of the bound DNA duplex (backbone in stick representation) and comparison with an ideal B form dsDNA (light blue cartoon backbone). One strand only of the duplex is tracked by consecutive TnsC protomers with a two-nucleotide periodicity, resulting in a DNA helix with 12 base pairs per turn.



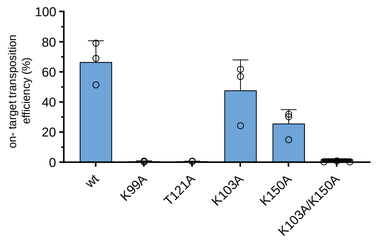
Electromobility shift assay using fluorophore-labeled dsDNA in the presence of AMPPNP, showing that TnsC mutants bind DNA with lower affinity than the wild-type protein.

Mechanistic model of RNA-guided transposition



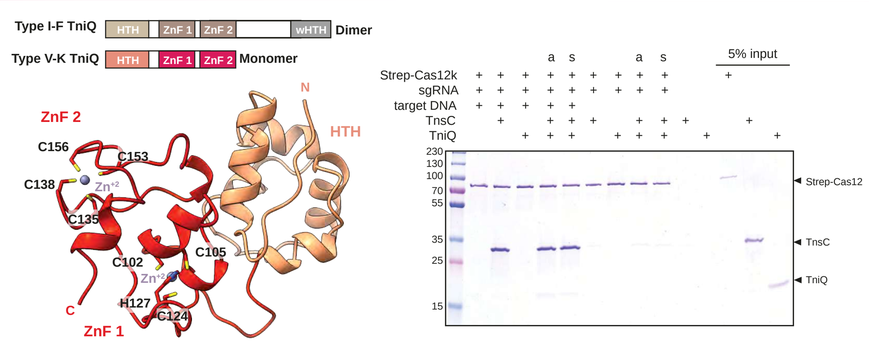


Negative stain electron micrographs of TnsC in the presence of AMPPNP and dsDNA and with mutations in the DNA binding interface. K99A and T121A mutants do not support filament formation.

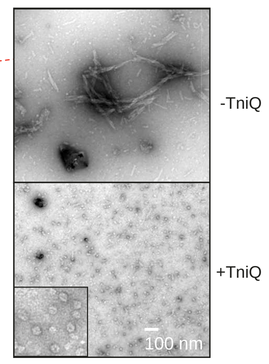


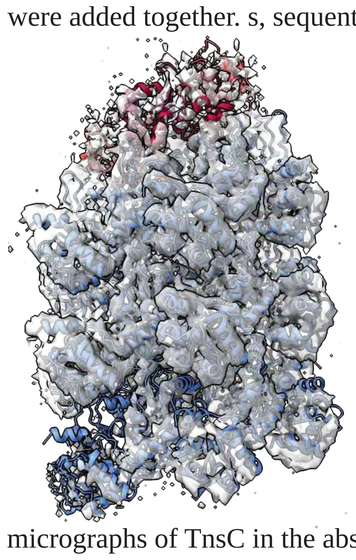
Functional analysis of TnsC DNA binding mutants in transposition assays[3] as measured by digital droplet PCR.

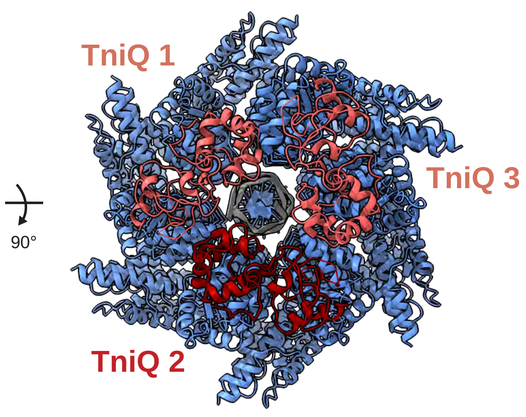
The Zn finger protein TniQ restricts TnsC polymerization



Left. Crystal structure of TniQ at 1.3 Å resolution. HTH, helix turn motif. wHTH, winged HTH motif. ZnF, zinc-finger motif. Right. Co-precipitation of TnsC and TniQ by immobilized StrepII-fused Cas12k-sgRNA complex. a, TnsC and TniQ were added together. s, sequential addition.

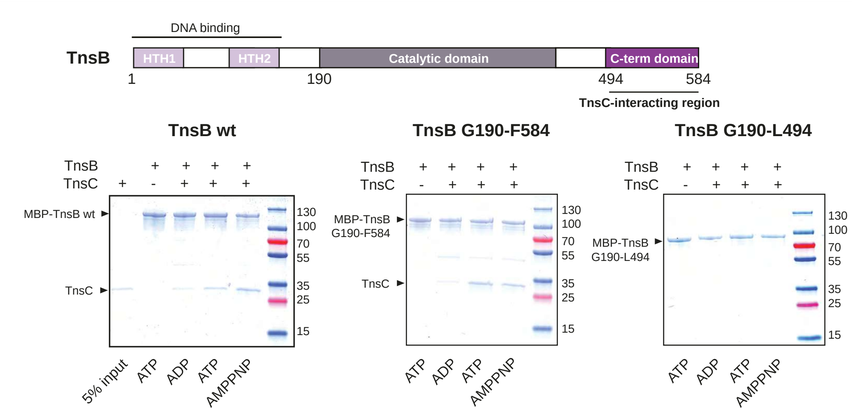


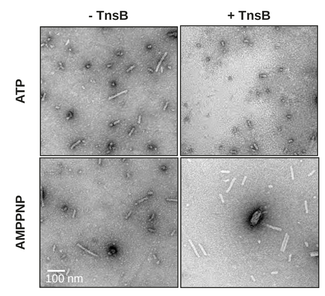




Left. Negative stain electron micrographs of TnsC in the absence or presence of TniQ. Right. Cryo-EM density map and structural model of TniQ-TnsC•ATP•dsDNA filaments at overall 3.4 Å resolution. TniQ caps the TnsC filament.

TnsB recruitment triggers filament disassembly





Top. Co-precipitation of TnsC by TnsB constructs fused to maltose binding protein (MBP) in the presence of different nucleotides. TnsB interacts with TnsC filaments via its C-terminal domain. Left. Negative stain electron micrographs showing ATP hydrolisis-dependent disassembly of TnsC filaments triggered by TnsB.

CONCLUSIONS

- TnsC oligomers bridge between the RNA-guided target selector Cas12k and the TnsB transposase, promoting target DNA remodeling and ultimately transposon integration.

- TnsB and TniQ directly interact with the TnsC filaments, regulating their assembly via two distinct mechanisms. While TniQ restrict filament growth by capping the Cas12k-distal end, TnsB induces ATP hydrolysis and filament disassembly.

- This work discloses first mechanistic insights into regulation of type V CRISPR-associated elements and will guide the rational design of these systems as programmable, site-specific gene insertion tools.

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Complementary work in: Park, J.-U. et al. Science 373, 768-774, doi:10.1126/science.abi8976 (2021).

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