Abstract

Tn7 and Tn7-like elements are mobile genetic elements notable for their specific target-site selection mechanisms and precise insertion. The prototypical Tn7 element is the best-studied element in the family. It employs two distinct transposition pathways using alternative target-site selection proteins, TnsD or TnsE, and a conserved core transposition machinery composed of the heteromeric transposase (TnsAB) and an ATPase adaptor (TnsC). TnsABC+TnsD directs Tn7 insertions into a specific chromosomal attachment site (*attTn7*), whereas TnsABC+TnsE preferentially facilitates transposition into conjugating plasmids. TnsD belongs to the TniQ family, which is commonly found in Tn7-like elements. TniQ is integrated with various target-site selection modules, allowing for the targeting of new genomic locations or the association with CRISPR-Cas for RNA-directed transpositions. Despite their differences, all target-site selection proteins recruit TnsC to assemble a targeting complex, which in turn activates the transposase.

Target-site selection proteins also distort the target DNA as part of the TnsC recruitment mechanism. To understand how TnsC preferentially recognizes DNA distortions, I determined the high-resolution cryo-electron microscopy (cryo-EM) structure of a gain-of-function variant of TnsC bound to DNA with a central mispaired region. TnsC forms asymmetric, open heptameric rings on DNA. The N-terminal face of the TnsC ring recognizes the DNA distortion and contains the region of the protein predicted to interact with TnsD, while the C-terminal face contains the regions that recruit the transposase. As a result, TnsC physically segregates interactions with the target-site selection proteins and the transposase, and imposes a strict spacing between the target and integration sites.

To determine how TnsC is recruited to a pre-selected genomic location, I then characterized the interaction between TnsC and the TniQ domain of TnsD ($TnsD^{NTD}$). High-resolution cryo-EM structures of $TnsD^{NTD}$ bound to TnsC and the ternary TnsC-$TnsD^{NTD}$-DNA complex demonstrate that $TnsD^{NTD}$ facilities the unidirectional polymerization of TnsC around the target DNA by interacting with the terminal TnsC protomer and enhancing nucleotide exchange within TnsC. These findings, together with previous studies, suggest that TnsD-dependent oligomerization of TnsC determines the downstream insertion of Tn7 at a fixed distance from the TnsD binding site.

Collectively, my thesis provides molecular insights into how TnsC regulates target-site selection, facilitates the crosstalk between different target-site selection proteins and the TnsAB transposase, and suggests a mechanism for transposition immunity.

McGill University

Graduate and Postdoctoral Studies

Final Oral Examination
for the Degree of
Doctor of Philosophy
of **YAO SHEN**

of the Department of Biochemistry**, on April 12, 2023 @ 2:00 PM** Hybrid. **In-person**: Room 501, Rosalind & Morris Goodman Cancer Institute & **via Zoom**.

COMMITTEE:

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Members of Faculty and Graduate Students are invited to be present

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PUBLICATIONS:

Shen Y, Petassi MT, Peters JE, Guarné A. Structural basis for the formation of the Tn7 targeting complex. *Manuscript in preparation*.

Shen Y, Gomez-Blanco J, Petassi MT, Peters JE, Ortega J, Guarné A. Structural basis for DNA targeting by the Tn7 transposon. *Nature Structural & Molecular Biology*, 29, 143-151 (2022). (Recommended in Faculty Opinions)

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Pilaev M, **Shen Y**, Carbonneau J, Venable MC, Rhéaume C, Lavigne S, Couture C, Guarné A, Hamelin MÈ, Boivin G. Evaluation of pre- and post-fusion Human metapneumovirus F proteins as subunit vaccine candidates in mice. *Vaccine*, 38, 2122-2127 (2020).

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SELECTED ABSTRACTS:

Structural basis of TnsC recruitment to TnsD binding sites in Tn7 transposition. CSHL transposable elements, Cold Spring Harbor Laboratory, NY, USA, 2022

Structural basis for DNA targeting by the Tn7 transposon. 3DEM Gordon Research Conference, Waterville Valley, NH, USA, 2021

TnsC determines the strict spacing between the target and insertion sites in Tn7 transposition. 2nd Annual CRBS Symposium virtual meeting, 2020

The heptameric assembly of TnsC bound to DNA reveals the activation mechanism of Tn7 transposition. 23rd Annual Buffalo DNA Replication & Repair Symposium, Buffalo, NY, USA, 2019