Assembly dynamics of the replicative transposition complex of Tn4430 in vitro and in vivo

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Abstract

Tn4430 is the paradigm of a widespread family of bacterial transposons, the Tn3 family, that are notorious for their contribution of the dissemination of antibiotic resistances. The effectiveness of these elements largely relies on their replicative mode of transposition which allows them to duplicate each time they move within the host genome. Recent genetic, biochemical, and structural studies from our laboratory have led to substantial progress into the understanding of this mechanism. The data support a new model for replicative transposition termed "replication hijacking" according to which transposons integrate into replication intermediates to recruit the host machinery that is necessary for their duplication.
The goal of ma thesis project is to deepen the characterization of this mechanism along two complementary axes. The structural role of the donor and target DNA and the contribution of DNA replication will be studied by exploiting the biochemical assays that I have developed during my Master thesis. The prospect of this part of the project is to reconstruct the full replicative transposition reaction *in vitro*. In parallel, interaction between the transposition partners will be examined in life cells using real-time fluorescence microscopy. Together, the two approaches will provide an integrated view of the "replication hijacking" mechanism *in vitro* and *in vivo*.
Previously, the *in vitro* transposition reaction was performed with simple oligonucleotides that were not really the natural substrates of the transposase. These reactions were carried out with oligonucleotides forming the ends of pre-cleaved transposons and with oligonucleotides forming a replication fork (the best substrate for integration reactions). In this poster, I will present my results showing that transposase can integrate a transposon end found in a non-pre-cleaved plasmid into a replication fork. In addition, experiments showing that transposon flanking sequences in the plasmid can influence integration will be presented.

Keywords: Tn3, Tn4430, Replicative transposition, Electrophoresis, In vitro, Replication hijacking, In vivo

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