Investigating the specificity of *oriT* recognition by MOB_T relaxases

Hicham Sekkouri Alaoui[1, 2], **Zoé Bruchon[1]**, Haifa Laroussi[1], Louise Thiriet[1], Frédérique Favier[2], Claude Didierjean[2], Nathalie Leblond-Bourget[1], Nicolas Soler[1]

[1] Université de Lorraine, INRAE, DynAMic, Nancy, France
[2] Université de Lorraine, CNRS, CRM2, Nancy, France

hicham.sekkouri-alaoui@univ-lorraine.fr, nicolas.soler@univ-lorraine.fr

Abstract:

Integrative and Conjugative Elements (ICEs) are crucial drivers of bacterial evolution, promoting the dissemination of genetic traits such as antibiotic resistance, virulence factors, and novel metabolic capabilities. ICEs encode the machinery necessary for their own excision, horizontal transfer via conjugation, and integration into the host genomes. Relaxase proteins are central to the initiation of this DNA transfer, by recognition of the origin-of-transfer (*oriT*) sequence.

In Gram-positive bacteria, the ICE*St3*/Tn*916* superfamily encodes a unique class of relaxases, known as MOB_T, which are related to *Rep_trans* rolling-circle replication initiators. This study investigates the molecular mechanisms by which MOB_T relaxases recognize and interact with their DNA substrates. Using recent insights into the *oriT* binding site of the ICE*St3* relaxase (RelSt3), we examined the DNA-binding behavior of three related MOB_T relaxases: RelSt3 behavior was compared to the relaxases encoded by Tn*916* from *Enterococcus faecalis* (Orf20) and by ICE*_515_tRNA*^{Lys} from *Streptococcus agalactiae* (Rel515). Electrophoretic mobility shift assays and endonuclease activity assays were conducted using *oriT* sequences from these three ICEs.

We investigated the structural and genomic similarities among the relaxases RelSt3, Orf20, and Rel515, all of which share an N-terminal helix-turn-helix (HTH) domain. Despite this conserved architecture, they exhibited distinct patterns of DNA interaction, while all three retained single-stranded endonuclease activity on various DNA substrates. These findings highlight a potential functional divergence within the MOB_T relaxase family, suggesting the existence of distinct DNA recognition mechanisms.

Keywords: *Gram-positive, Integrative Conjugative Elements (ICE), relaxase, MOB_T, oriT*

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