

**Stony Brook University  
The Graduate School**

Doctoral Defense Announcement

**Abstract**

RNA interference by 3'-tRFs: small RNA tailing, trimming, and 2'-O methylation determine transposon silencing

By

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While RNA interference (RNAi) is an essential pathway well known for gene silencing and transposon control in eukaryotes, emerging roles in chromosome and genome stability suggest a far broader function. Growing evidence supports the existence of highly conserved, Dicer-independent pathways. One such pathway involves 18 and 22 nucleotide long fragments derived from the 3' end of mature tRNAs (3'-tRFs). These small RNAs (sRNAs), which are ubiquitously expressed across all organisms, tissues and cell-types, help maintain genome stability by inhibiting endogenous retroviruses (ERVs) that use 3' ends of full-length tRNAs to prime reverse transcription at the 5' UTR primer binding site (PBS). While a perfectly complementary PBS may aid reverse transcription, such an element also becomes susceptible to silencing by tRFs. Considering that extensive complementarity to mRNA targets can induce small RNA tailing and decay, this study sought to determine whether 3'-tRFs are 2'-O methylated, a 3' terminal modification known to prevent sRNA degradation. Indeed, HENMT1 mediates 2'-O methylation of 3'-tRFs and in absence of HENMT1, non-templated tailing of 3'-tRFs occurs by terminal nucleotidyl transferases known to modify miRNAs. This study also examined how 3'-tRFs target ERVs, prompting the design of a massively parallel reporter assay (MPRA) to gain a deeper understanding of tRF silencing at the PBS in MusD6, a highly active ERV in mice. Analysis of 2000 unique PBS variants revealed region specific effects with mismatches in the first four positions of the PBS seed yielding greater repression of MusD6. This finding suggests that 3'-tRFs may silence their targets in a manner like PIWI-interacting RNAs (piRNAs), which target transposons in the germline with relaxed seed complementarity. Consequently, 3'-tRFs may occupy a unique niche at the crossroads of miRNA and piRNA biology, exhibiting characteristics akin to both classes of sRNAs.

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**Dissertation Advisor:** Rob Martienssen

**Place:** Cold Spring Harbor Laboratory, Wendt Building, Hawkins Conference Room

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