



GRADUATE SCHOOL OF BIOMEDICAL SCIENCES
BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY/RTI

Ph.D. THESIS DEFENSE

BO HAN

Mentor: Phillip Zamore, PhD

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"Using Experimental and Computational Strategies to Understand the Biogenesis of microRNAs and piRNAs"

miRNA are expressed ubiquitously in tissues and fine-tune gene expression by regulating the stability and translation of mRNAs. In contrast, piRNAs are mainly expressed in animal gonads and their major function is repressing transposable elements to ensure the faithful transfer of genetic information from generation to generation. My thesis research focused on the biogenesis of miRNAs and piRNAs using experimental and computational strategies.

The biogenesis of miRNAs involves sequential processing of their precursors by the RNase III enzymes, Drosha and Dicer, to generate miRNA/miRNA* duplexes, which are subsequently loaded into Argonaute proteins. We discovered that, after assembly into Ago1, more than a quarter of *Drosophila* miRNAs undergo 3' end trimming by the 3'-to-5' exoribonuclease Nibbler. Such trimming protects miRNAs from non-templated nucleotide addition to their 3' ends, and ultimately enhances target messenger RNA repression.

In *Drosophila* germ line, piRNAs associate with three PIWI proteins, Piwi, Aub, and Ago3. piRNAs bound by Aub and Ago3 are generated by reciprocal cleavages of sense and antisense transposon transcripts, which amplifies piRNA abundance and degrades transposon transcripts in the cytoplasm. On the other hand, Piwi-piRNA represses the transcription of transposons in the nucleus. We discovered that Aub- and Ago3-mediated transposon RNA cleavage not only generate piRNAs bound to each other, but also produces substrates for the endonuclease Zucchini, which processively cleaves those substrates in a periodicity of ~26 nt and generates piRNAs that predominantly load into Piwi. Without Aub or Ago3, the abundance of Piwi-bound piRNAs drops and transcriptional silencing is compromised.

Mentor(s)

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