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Ph.D. THESIS DEFENSE

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Understanding the Production and Stability of Mouse PIWI-Interacting RNAs

PIWI-interacting RNAs (piRNAs) are small non-coding RNAs unique to animals that guard the germline genome integrity by regulating transposons, viruses, and genes. In mice, piRNAs are highly expressed in testis and guide one of the three PIWI proteins to regulate their targets. The purpose of the 3′ end 2′-*O*-methyl modification in piRNAs is unknown. It has been speculated that the modification increases stability and facilitates function of piRNAs, but the direct evidence is lacking. My dissertation addresses two unanswered questions about mouse piRNAs: (1) how are piRNAs produced and how conserved is the piRNA pathway in all animals, and (2) why are mouse piRNAs 2′-*O*-methylated at their 3′ ends?

How piRNAs are generated is still poorly characterized in several model organisms. Studies of these model organisms imply the mechanisms that produce piRNAs differ among animals, tissues and cell types. Here, we demonstrate that a single unified mechanism can explain piRNA production in most animals, from human to the non-bilateral animal hydra. Our analysis elucidated that, in male mouse and female fly germlines, PIWI proteins guided by the initiator piRNA slice long piRNA precursor transcripts, and this PIWI-guided slicing action starts the piRNA biogenesis. PIWI proteins also position the endonuclease to further fragment long piRNA precursor transcripts into a string of tail-to-head, phased trailing piRNAs in a stepwise manner. Our discovery shows the central role of PIWI proteins in the piRNA pathway: both initiating and sustaining the production of piRNAs.

For the second question, we discovered that pre-piRNA trimming and piRNA 2′-*O*-methylation protect piRNAs from separate decay mechanisms. We showed that in the absence of 2′-*O*-methylation, mouse piRNAs with extensive complementarity to long RNAs are destabilized and destroyed by a mechanism similar to target-directed microRNA degradation (TDMD). On the other hand, untrimmed pre-piRNAs are destroyed by a different mechanism, independent of their extensive complementarity to long RNAs. In the absence of both 2′-*O*-methylation and trimming, the piRNA pathway collapses which supports the idea of piRNA trimming and methylation collaborating to stabilize piRNAs. Our work suggests that 2′-*O*-methylation and trimming are important for maintaining the steady-state abundance of piRNAs which is necessary for their function in either transposon silencing or gene regulation.

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