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A milestone in small RNA biology—piRNA biogenesis from start to finish

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Credit: Institute of Molecular Biotechnology

PIWI-interacting RNAs, or piRNAs for short, are a class of 'small regulatory RNAs'—tiny pieces of nucleic acid just 22–30 nucleotides in length. They may be small, but with their associated Argonaute proteins, piRNAs have the power to 'silence' transposable elements, so called egoistic genes found in the genomes of plants, fungi, and animals. piRNA-guided silencing can act on chromatin to block transposon transcription, or by destroying transposon mRNAs in order to block their translation into proteins.

Although scientists understand quite well how piRNAs repress gene expression, until now, it has been much less clear how piRNAs are actually made. In a milestone research paper published in *Nature*, scientists from the Institute of Molecular Biotechnology in Austria (IMBA) have painstakingly unravelled the sequence of events that generate piRNAs with a defined length and sequence, a central requirement to define the target spectrum of the silencing system.

Mystery of piRNA biogenesis explained

Julius Brennecke, one of the paper's senior authors, explained:

"We already knew that piRNAs are formed from longer RNA species that are chopped up into pieces by Argonaute proteins or a protein called Zucchini. This forms the 5' ends of so-called pre-piRNAs, which are loaded into Argonaute proteins and subsequently trimmed and modified to yield mature piRNAs. As we had a fairly good understanding of the generation of piRNA 5' ends, our group focused on the 3' ends, a process that was not understood for nearly ten years."

Using the common fruit fly Drosophila melanogaster, a major genetic model organism, IMBA scientists Rippei Hayashi and Jakob Schnabl—both first authors of the article—revealed that piRNA 3' end formation in fact follows one of two parallel pathways.

"Once biogenesis is initiated, some piRNA 3' ends are actually generated by Zucchini, the endonuclease that is primarily known to generate piRNA 5' ends", said last author Stefan Ameres. "But Zucchini explains the biogenesis of only a subset of piRNAs. We then discovered that the exonuclease Nibbler is a second key-enzyme that can form piRNA 3' ends and realized that two genetically separated pathways act in parallel in the cell. This was a true deja vu as we also found Nibbler to mature some microRNAs, yet another class of small RNA molecules, during my postdoctoral work."

Two parallel pathways in tune

Beyond unravelling these pathways, their place of action, and their implications for downstream gene regulatory mechanisms, the team also made some interesting observations that might provide clues as to the evolution of small RNA biogenesis. "The nucleases we've identified in this study have homologs in animals ranging from sponges to human. Interestingly, some notable exceptions are apparent. Nematode worms, for example, have lost the Zucchini enzyme, and mosquitos from the Anopheles genus have lost Nibbler. Whether here other piRNA trimming mechanisms exist or whether in these species the two-pathway model is reduced to one, is unclear. Remarkably, upon simultaneous ablation of Zucchini and Nibbler in Drosophila, piRNAs can still be generated, in this case by closely spaced piRNA-guided Argonaute cleavage events. This Argonaute-only pathway might be the ancient piRNA generating system, onto which sophisticated nucleases like Zucchini and Nibbler were added later to enhance efficiency and accuracy of piRNA biogenesis," concludes Julius Brennecke.

More information: Rippei Hayashi et al. Genetic and mechanistic diversity of piRNA 3′-end formation, *Nature* (2016). DOI: 10.1038/nature20162

Provided by Institute of Molecular Biotechnology