

on the path to pathological TDP-43 aggregation.

DECLARATION OF INTERESTS

A.D.G. has been a consultant for Aquinah Pharmaceuticals, Prevail Therapeutics, and Third Rock Ventures.

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Set Phasers to Cleave: PIWI Cleavage Directs All piRNA Biogenesis

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In this issue of *Molecular Cell*, Gainetdinov et al. (2018) show that PIWI proteins direct both piRNA biogenesis and piRNA function in most animals.

PIWI-interacting RNAs (piRNAs) have been monikered the “dark matter of the genome,” and with reason. They are small RNAs that bind an Argonaute protein of the PIWI subclade (Cox et al., 1998), but everything else about them is quite distinct from other small regulatory RNAs such as microRNAs. piRNAs, along with PIWI proteins, are expressed in the germline of essentially all sexually reproducing animals. Many piRNAs target complementary retrotransposons for silencing via “classic” small-RNA-dependent Argonaute-directed cleavage. Their mysterious biogenesis drew a lot of attention right from the start: they were Dicer independent (Vagin et al., 2006) and many of them arose in an imprecise manner from genomic areas called piRNA clusters.

Studies over the past decade have revealed a complicated biogenesis route. One of the earliest observations was that piRNAs with complementary sequence overlap engage in an amplification loop called ping-pong that utilized PIWI cleavage (Brennecke et al., 2007) (Gunawardane et al., 2007). With notable prescience, Hannon and colleagues in 2007 proposed a model for piRNA production involving a single cleavage generating the 5' end (preferably at a uridine), followed by incorporation into a PIWI, followed by 3' end generation (Aravin et al., 2007). Studies over the next 10 years would validate and tweak this model and uncover enzymes responsible for piRNA processing, in addition to PIWI: the MITOPLD (Zuc in fruit flies) endonu-

lease; the 3'-5' exonuclease PNLDC1 (Nibbler in fruit flies) that trims 3' ends of piRNAs; and the MOV10L1 (Armitage in fruit flies) RNA helicase (Huang et al., 2017). Investigations of MOV10L1 revealed that endonucleolytic cuts of the long precursor transcript occur in a 5'-3' direction to generate intermediate piRNA precursor fragments to be bound by PIWI proteins for processing (Vourekas et al., 2015). Working independently, the Zamore, Brennecke, and Pillai labs discovered that these cuts were successive and achieved through the so-called phasing (or inchworming) mechanism, which is the consecutive, tail-to-head generation of piRNAs by Zuc-dependent cleavage of long precursors (Han et al., 2015) (Mohn et al., 2015) (Homolka



et al., 2015). The difference between fruit flies and mice is that in the former there is very little 3' end trimming. In these models, piRNAs produced downstream of a PIWI cleavage event are known as secondary while those produced from precursor transcripts that are not subjected to PIWI slicing are known as primary. Phased piRNA production was observed for mouse pachytene clusters (Han et al., 2015) (Mohn et al., 2015). However, what triggered phased piRNA production in the postnatal mouse remained unknown as it was thought that the pachytene cluster transcripts were processed as primary piRNAs independent of PIWI slicing. The two mouse PIWI proteins that bind pachytene piRNAs are known as Mili and Miwi.

In this issue of *Molecular Cell*, Gainetdinov et al. (2018) present compelling evidence that, in fact, piRNA-directed PIWI protein cleavage of pachytene piRNA precursor transcripts does occur and triggers phased piRNA production. The authors specifically retrieved 5' monophosphate-containing long (>150 nt) RNAs from sorted pachytene spermatocytes that map within the pachytene clusters to identify potential PIWI cleavage products. They found that many of these fragments share common 5' ends with mature piRNAs, which means that they are genuine piRNA precursor fragments. Notably, these fragments show increased occurrence of adenine at position 10, accompanied by significant 10 nt overlap with complementary Mili and Miwi loaded piRNAs, which means that they are the products of PIWI cleavage. Moreover, phasing initiating piRNAs are enriched for transposon sequences.

The above observations bring forth a unified model for piRNA biogenesis that describes piRNA-mediated, PIWI-dependent cleavage as a gatekeeper for the initiation of piRNA biogenesis, for both primary and secondary pathways. In essence, Piwi cleavage defines a piRNA precursor in action, by giving the cleaved RNA the “kiss of death” that marks it as a substrate for piRNA production. Another key aspect of the work by the Zamore lab is that the PIWI protein actively participates in the downstream cleavage site selection, thus molding the size of the

piRNA precursor intermediate fragment (Gainetdinov et al., 2018). It does so by positioning Zuc (which apparently prefers to cleave at Us), or by not allowing it to cut within a certain length of the PIWI-protected precursor piRNA. The most convincing evidence comes from the sequence analysis of the different sized precursor piRNA fragments loaded on Mili and Miwi: whenever allowed by neighboring U residues, the precursor piRNA will be cleaved smaller for Mili and longer for Miwi. The final size of the mature piRNA is similarly shaped: 3' end trimming is physically restricted by the PIWI protein footprint, resulting in characteristically different piRNA populations for Miwi (~30 nt) and Mili (~25–26 nt). We note that standard immunoprecipitation methodology retrieves primarily stably bound RNAs and may underestimate transient products with very short half-lives. It is thus possible that PIWI proteins quickly reject certain intermediate fragments, for example those that do not carry a 5' U.

Strikingly, Gainetdinov et al. (2018) show that PIWI slicing can initiate phasing in a wide range of animals that cover almost a billion years of evolutionary history. This suggests that the ability of PIWI and piRNAs to self-regulate piRNA production was an early adaptation that played an important role in animal evolution, presumably one that safeguarded genome stability during gametogenesis.

Like every significant advancement, the study by Gainetdinov et al. (2018) is raising important questions for future investigations. What is the degree of complementarity that is required for these cleavage events? What is the origin and mechanism of generation of the “seeding” piRNAs that trigger pachytene precursor processing? In fruit flies, any cytoplasmic RNA that enters into the Zuc-Armitage machinery is processed into piRNAs (Vrettos et al., 2017) (Huang et al., 2017), and PIWI slicing is not required for piRNA biogenesis in follicle cells of the ovary (Darricarrère et al., 2013). However, it is unknown how the 5' ends are defined in these conditions and whether similar processes function in mammals. Undoubtedly, this study by the Zamore lab has shone a bright new light in the dark matter of the genome,

advancing our understanding of how piRNAs are made.

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