Primary piRNA biogenesis: caught up in a Maelstrom

Radha Raman Pandey^{1,2} & Ramesh S Pillai^{1,2}

Precursors for most Piwi-interacting RNAs (piRNAs) are indistinguishable from other RNA polymerase II-transcribed long non-coding RNAs. So, it is currently unclear how they are recognized as substrates by the piRNA processing machinery that resides in cytoplasmic granules called nuage. In this issue, Castaneda *et al* (2014) reveal a role for the nuage component and nucleo-cytoplasmic shuttling protein Maelstrom in mouse piRNA biogenesis.

See also: J Castaneda *et al* (September 2014)

Germ cells are entrusted with the task of faithfully transmitting genetic information from one generation to the next. A major threat to germline genome integrity is the activity of mobile genetic elements called transposons, as they have the potential to cause mutations, usually leading to infertility. To counteract this threat, animal germlines have evolved a conserved small RNA-based transposon defense system composed of Piwi proteins and their associated piRNAs (Malone & Hannon, 2009). In their simplest form, piRNAs guide Piwi endonucleases to cleave transposon transcripts resulting in their degradation. More complex systems come into play when nuclear Piwi proteins mediate transcriptional silencing of target transposon loci by recruitment of H3K9me3 chromatin marks and/or DNA methylation as in *Drosophila* and mice, respectively. While piRNAs targeting transposable elements is a universal feature across the animal kingdom, the mammalian male germline expresses an abundant set of piRNAs that are depleted of transposon sequences. In mice, they begin to be expressed in meiotic

pachytene spermatocytes and later in haploid round spermatids and are deposited into Piwi proteins Mili and Miwi. These unique non-repeat so-called pachytene piRNAs have no obvious targets, and their function is currently unknown.

How piRNAs are made is a problem that continues to intrigue researchers. We know that 50–100 kilobases, long-defined noncoding transcription units called piRNA clusters, are sources of most piRNAs. These are transcribed by RNA polymerase II, after which the capped and polyadenylated precursor transcripts are believed to be exported to cytoplasmic granules called nuage ('cloud' in French) where piRNA biogenesis factors reside (Li *et al*, 2013). The precursor is then converted into tens of thousands of mature primary piRNAs via a poorly understood primary biogenesis pathway. Importantly, how the nuclear history of transcription from a cluster locus is linked to the cytoplasmic fate of piRNA production is not known. In this issue, Castaneda *et al* (2014) identify a role for the nuage component and nucleo-cytoplasmic shuttling protein Maelstrom (Findley *et al*, 2003) in mouse primary piRNA biogenesis.

Maelstrom (Mael) is a conserved factor essential for transposon silencing and fertility in both flies and mice, but its exact biochemical function remains a mystery (Lim & Kai, 2007; Soper *et al*, 2008). To examine this, Castaneda *et al* isolated Mael complexes from adult mouse testes and identified associated proteins by mass spectrometry. Miwi and tudor domain-containing protein 6 (Tdrd6) were dominant partners of this complex. This association is likely to be direct as it can be reproduced in transfected somatic human cells and is resistant to RNase treatment. By carrying out RNA sequencing after

immunoprecipitation (RIP-seq), the authors revealed an enrichment of pachytene piRNA precursors (~100 nt long reads) in the Mael complexes. Interestingly, mature piRNA sequences are depleted in the RIP-seq libraries, indicating that precursors present in the Mael complexes are undergoing fragmentation/processing into primary piRNAs. Indeed, mice lacking *mael* display a drastic reduction (10-fold) in piRNA levels, with pachytene piRNAs being specifically affected. Based on these studies, a model for mouse primary piRNA biogenesis can be proposed where the nucleo-cytoplasmic shuttling protein Mael binds pachytene piRNA precursors and delivers them to the nuage for processing.

What is the consequence of loss of pachytene piRNAs? Mutant mice display male-specific infertility, and the arrested round spermatids show acrosome and flagella formation defects. Ribosome profiling analysis in the *mael* mutant testes identified 880 mRNAs with reduced translation, many of which encode proteins needed for acrosome and flagellum formation. The precise reason for this translational inhibition is currently not known, but a direct role for Mael or sequence-specific implication of pachytene piRNAs in promoting translation is not among the suggested possibilities. Thus, the mystery surrounding pachytene piRNAs is only deepening.

As with other interesting studies, this work also opens up new questions that await answers. How can Mael distinguish pachytene piRNA precursors from other transcripts? Is there a coupling between transcription from the piRNA cluster promoter and fate of the precursor in the cytoplasm? How do other established primary piRNA biogenesis factors access precursors in the

¹ European Molecular Biology Laboratory, Grenoble Outstation, Grenoble, France

² Unit for Virus Host Cell Interactions, University Grenoble Alpes-EMBL-CNRS, Grenoble, France. E-mail: pillai@embl.fr

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Figure 1. Models for Maelstrom function in the animal germline.

(A) Maelstrom is involved in transcriptional silencing of transposons in fly ovaries. The protein acts downstream of Piwi-mediated deposition of H3K9me3 chromatin marks. (B) In mouse male germ cells, Maelstrom is required for primary piRNA biogenesis as it is shown to bind precursors of pachytene piRNAs (in this issue). (C) Since Maelstrom is a nucleo-cytoplasmic shuttling protein, it can bind RNA substrates (transposons in fly ovaries or piRNA precursors in mice) and deliver them to cytoplasmic nuage. The individual fate of these RNAs is different in the fly and mouse systems.

Mael complex? In fly ovaries, Mael is shown to be largely dispensable for piRNA biogenesis, but is implicated as an effector of nuclear Piwi-mediated transcriptional silencing of transposons (Sienski *et al*, 2012). Specifically, it was placed downstream of piRNA-guided deposition of H3K9me3 marks on target transposon loci. So, how can one reconcile the different roles in flies and mice? One possibility is that fly Mael binds nascent transposon transcripts arising from genomic loci undergoing transcriptional silencing and conducts them to cytoplasmic granules for degradation. It is known that components of the piRNA pathway and the mRNA decay machinery are co-localized in

cytoplasmic granules in both flies and mice (Aravin *et al*, 2009; Lim *et al*, 2009). In both situations, Mael functions to bind RNAs and chaperone them to the nuage.

How does Mael biochemically perform all these tasks? Mael is composed of an N-terminal HMG box and a highly conserved C-terminal MAEL domain that is predicted to take up an RNase-H-like fold (Zhang *et al*, 2008). It is likely that the HMG box serves to grip the RNA substrates. Indeed, a mutant lacking the domain fails to support transposon silencing and female fertility in flies (Sienski *et al*, 2012). Nuclease activity is not reported for Mael, but point mutations of conserved residues (EHHCHC) within the

MAEL domain abrogates its *in vivo* role in the fly ovaries (Sienski *et al*, 2012). Future structural and biochemical studies will be required to shed more light on what activities these domains provide.

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